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Poster

293. Microglia Development and Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 293.01/A1

Topic: B.12. Glial Mechanisms

Support: NIH Training Grant 5T32 GM008541 (MW)

PhRMA Foundation Pre-doctoral Fellowship in Pharmacology & Toxicology (MW)

Landreth Family Foundation and Nancy Lurie Marks Family Foundation (TI)

Title: Microglial replenishment modulates behavioral, neuroanatomical and neurophysiological impairments associated with autism spectrum disorders

Authors: *M. WOODBURY[1], A. VAN ENOO[2], C. HOLLAND[3], M. MEDALLA[3], T. GUILLAMON-VIVANCOS[3], P.-H. CHAO[4], M. BOTROS[2], L. ESTRADA[3], S. IKEZU[2], O. BUTOVSKY[6], W. E. JOHNSON[5], J. I. LUEBKE[3], T. IKEZU[4];
[1]Grad. Program in Neuroscience; Pharmacol. and Exptl. Therapeut., [2]Pharmacol. and Exptl. Therapeut., [3]Anat. & Neurobio., [4]Pharmacol. and Exptl. Therapeutics; Neurol., [5]Computat. Biomedicine, Boston Univ. Sch. of Med., Boston, MA; [6]Harvard Med. Sch., Brigham and Women's Hosp., Boston, MA

Abstract: Autism spectrum disorders (ASDs), a group of debilitating neurodevelopmental disorders, currently affect 1% of the world population. The number of children affected by an ASD has grown at an alarming rate, with a current U.S. diagnosis of 1 in 45 births. Despite this, no cure exists. Epidemiological studies report an association between maternal infection and development of ASD in children, regardless of the pathogenic species. ASD patients and genetic animal models show immune dysfunction and abnormalities in the numbers and morphology of microglia, the brain's innate immune cells that are critical for normal neurodevelopment. However, the precise changes in microglial homeostatic functions and their effects on neuropathophysiology remain unknown. We hypothesize that maternal immune activation (MIA) abnormally programs fetal microglia, thereby impairing their neurogenic and pruning functions and leading to detrimental effects on brain connectivity thought to be responsible for cognitive and behavioral deficits in ASD. We performed longitudinal behavioral, morphological and neurophysiological evaluations and microglial RNA-expression profiling studies of an MIA mouse model using the innate immunity ligand polyinosinic:polycytidylic acid. MIA offspring displayed core symptoms of ASD (social/communication deficits; repetitive and hyperactive behaviors). Microglia in MIA adult offspring displayed alterations in specific homeostatic genes, sustained impairment of pruning-related dendritic spine interactions, and increased distal branching complexity, which were associated with increased spine density and neuronal hyper-

excitability in prefrontal layer V cortical neurons. Postnatal pharmacological microglia replenishment using a specific colony stimulating factor -1 (CSF1)-receptor inhibitor corrected repetitive behavior and social interaction deficits, and reduced neuronal excitability in the MIA cohort. However, only a portion of genes was normalized in replenished microglia 18 days after depletion. These data describe specific microglial gene expression and morphological aberrations resulting from insults during embryonic development, and their restoration by postnatal microglia replenishment. This suggests that targeting specific microglial genes may be a viable therapeutic approach for pharmacological intervention in ASD.

Disclosures: **M. Woodbury:** None. **A. Van Enoo:** None. **C. Holland:** None. **M. Medalla:** None. **T. Guillamon-Vivancos:** None. **P. Chao:** None. **M. Botros:** None. **L. Estrada:** None. **S. Ikezu:** None. **O. Butovsky:** None. **W.E. Johnson:** None. **J.I. Luebke:** None. **T. Ikezu:** None.

Poster

293. Microglia Development and Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 293.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 MH101188

Title: A comparison of microglial distribution in embryonic primate and rodent using multiple immunofluorescence histochemistry

Authors: ***N. BARGER**[1], C. WEIDENTHALER[2], S. C. NOCTOR[2];
[1]Univ. of California, Davis - MIND Inst., Sacramento, CA; [2]Univ. of California, Davis, Davis, CA

Abstract: Microglia, the resident immune cells of the nervous system, have recently become recognized for their participation in normal developmental processes in addition to their traditional immune functions. We have previously shown that manipulating microglial numbers impacts embryonic neurodevelopmental programs in mammals, indicating that they regulate critical aspects of neurodevelopment. We have also found evidence of similar function in a broad array of mammals, suggesting that these functions are conserved. However, the distribution of microglia in the developing cortex is not uniform across species. We have noted that microglial distribution in macaque monkeys at mid-gestation is more heterogeneous than in rats at a similar stage of development. We will present data that augment our prior findings by systematically assessing microglial distribution throughout the rostrocaudal extent of the developing cortex in cross-sectional profiles of prenatal rhesus macaques and Sprague Dawley rats, targeting a longer

developmental period. We have developed a multiple immunofluorescence staining protocol that allows for the visualization of microglial distribution, with antibodies to Iba1, relative to markers commonly used to differentiate the ventricular from the subventricular zone, Pax6 and Tbr2, respectively. We labeled tissue from multiple rostrocaudal levels in prenatal macaque neocortex and in rats from embryonic days 15-21. Species differences in distribution of microglia are assessed using confocal and wide-field florescence microscopy. Addressing variation across species in this way can provide novel translational information important for developing experimental models of neurodevelopmental disorders.

Disclosures: **N. Barger:** None. **C. Weidenthaler:** None. **S.C. Noctor:** None.

Poster

293. Microglia Development and Function

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Topic: B.12. Glial Mechanisms

Support: The Alexander von Humboldt Foundation

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Title: Infiltrating monocytes promote brain inflammation, contribute to breakdown of the blood-brain barrier, and exacerbate neuronal damage after status epilepticus

Authors: ***N. H. VARVEL**[1], J. NEHER[2], A. BOSCH[2], W. WANG[1], R. RANSOHOFF[3], R. MILLER[4], R. DINGLEDINE[1];
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Abstract: The generalized seizures of status epilepticus (SE) induce a succession of molecular and cellular events producing cognitive deficits and can culminate in the development of epilepsy. Known early events include opening of the blood brain barrier and astrocytosis accompanied by activation of brain-resident microglia. Whereas circulating monocytes do not

infiltrate the healthy CNS, monocytes can enter the brain in response to injury and contribute to the neuroimmune response. We examined the cellular components of innate immune inflammation in the days following pilocarpine SE by discriminating microglia versus brain-infiltrating monocytes. CCR2+ monocytes invade brain tissue between one and three days after SE. The initial cellular sources of the chemokine CCL2, a ligand for CCR2, included perivascular macrophages and microglia. *Ccr2* knockout mice displayed reduced monocyte recruitment into brain and decreased hippocampal induction of the pro-inflammatory cytokine IL-1β after SE, but the induction of IL-6, TNFα, CCL2, and iNOS was not different between *Ccr2* sufficient and knockout animals. Surprisingly, the induction of the pro-inflammatory cytokine IL-1β was greater in FACS-isolated microglia (referenced to microglia in saline-treated control mice) than in brain-invading monocytes (referenced to blood monocytes from saline-treated control mice), when assessed four days after SE. Importantly, preventing monocyte recruitment accelerated weight regain, attenuated neuronal damage in the hippocampus, and reduced blood-brain barrier leakage as assessed four days after SE by serum-derived albumin in the cortex. Our findings identify brain-infiltrating monocytes as a myeloid cell subclass that contributes to neuroinflammation, deterioration of the blood-brain barrier, and morbidity after SE. Inhibiting brain invasion of CCR2+ monocytes could represent a viable method for alleviating several deleterious consequences of SE.

Disclosures: **N.H. Varvel:** None. **J. Neher:** None. **A. Bosch:** None. **W. Wang:** None. **R. Ransohoff:** None. **R. Miller:** None. **R. Dingledine:** None.

Poster

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Topic: B.12. Glial Mechanisms

Support: SFB TRR 43

Neurocure

MD fellowship of the Boehringer Ingelheim Fonds to E. Wogram

Title: Satellite microglia show spontaneous activity that is uncorrelated with activity of the attached neuron

Authors: ***E. WOGRAM**[1], S. WENDT[2], M. MATYASH[2], T. PIVNEVA[3], A. DRAGUHN[1], H. KETTENMANN[2];

[1]Inst. of Physiol. and Pathophysiology, Univ. of Heidelberg, Heidelberg, Germany; [2]Max-Delbrück-Center for Mol. Med., Berlin-Buch, Germany; [3]Gen. Physiol. of Nervous Syst. Dept., Bogomoletz Inst. of Physiol., Kiev, Ukraine

Abstract: Microglia are innate immune cells of the brain. We have studied a subpopulation of microglia, called satellite microglia. This cell type is defined by a close morphological soma-to-soma association with a neuron, indicative of a direct functional interaction. Indeed, ultrastructural analysis revealed closely attached plasma membranes of satellite microglia and neurons. However, we found no apparent morphological specializations of the contact and biocytin injection into satellite microglia showed no dye-coupling with the apposed neuron or any other cell. Likewise, evoked local field potentials or action potentials and postsynaptic potentials of the associated neuron did not lead to any transmembrane currents or non-capacitive changes in the membrane potential of the satellite microglia in the cortex and hippocampus. Both satellite and non-satellite microglia, however, showed spontaneous transient membrane depolarizations which were not correlated with neuronal activity. These events could be divided into fast-rising and slow-rising depolarisations, which showed different characteristics in satellite and non-satellite microglia. Fast-rising and slow-rising potentials differed with regard to voltage dependence. The frequency of these events was not affected by the application of tetrodotoxin, but the fast-rising event frequency decreased after application of GABA. We conclude that microglia show spontaneous electrical activity that is uncorrelated with the activity of adjacent neurons.

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Poster

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Topic: B.12. Glial Mechanisms

Support: DIM Cerveau&Pensée

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Université Sorbonne Paris Cité RIPPTSA

Title: Functional investigation of microglia involvement in the maturation of synapses during postnatal development of the somatosensory neocortex

Authors: *C.-A. M. MOSSER;
INSERM U1128, Paris, France

Abstract: Microglial cells, the only immune cells permanently residing in the central nervous system (CNS), have been classically studied in pathological contexts. However, recent evidence revealed that these cells also actively participate to the normal CNS development by regulating developmental apoptosis, neuronal survival, and by pruning inappropriate synapses. Moreover, we previously demonstrated that reciprocal interactions between neurons and microglia are essential for functional maturation of thalamocortical excitatory synapses. During the first postnatal week, microglial cells are recruited at sites of maturating synapses in the mouse somatosensory barrel cortex by a mechanism involving the neuronal chemokine fractalkine and its microglial receptor CX3CR1. Furthermore, CX3CR1 deficiency affects the functional maturation of postsynaptic glutamate receptors at excitatory thalamocortical synapses. Besides the increasingly investigated interactions between microglia and developing excitatory synapses, little is known on the role of these immune cells on the maturation of inhibitory synapses**.** We therefore aimed at investigating whether microglial cells influence the functional development of inhibitory synapses in the somatosensory cortex. Using patch-clamp recordings of layer IV neurons of the barrel cortex, we first compared the evolution of spontaneous (sIPSCs) and miniature (mIPSCs) inhibitory postsynaptic currents in wild type (WT) and CX3CR1 deficient (KO) mice from P5 to P12. We observed that the frequency of sIPSCs and mIPSCs increased similarly in WT and CX3CR1 KO mice during this developmental window. There was no difference either in the mean amplitude of sIPSCs and of mIPSCs between WT and CX3CR1 KO mice. However, the amplitude distribution of sIPSCs, but not that of mIPSCs, differed between the two genotypes. In addition, the decay time constant of mIPSCs was significantly slower in CX3CR1 KO than in WT mice and this difference was already observed as early as P5 when the first IPSCs could be recorded. These results suggest that CX3CR1 deficiency impairs the functional expression of synaptic $GABA_A$ receptors at very early stages of synaptogenesis. To further explore the roles of microglia in the maturation of cortical inhibitory synaptic networks, we currently investigate the consequences of depleting microglia at early postnatal days as well as those of activating these immune cells during embryonic development.

Disclosures: C.M. Mosser: None.

Poster

293. Microglia Development and Function

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Singapore Immunology Network Core Fund.

ERC CoG NImO

Title: *In utero* development of microglia: instrinsic programs and impact of microbiota

Authors: ***M. S. THION**[1], D. LOW[2], J. CHEN[2], P. SQUARZONI[1], P. GRISEL[1], A. A. AMOYO[3], M. POIDINGER[2], S. PETTERSSON[4,5], S. GAREL[1], F. GINHOUX[2]; [1]IBENS, INSERM U1024, CNRS UMR 8197, Paris, France; [2]Singapore Immunol. Network, Agency for Science, Technol. and Res., Singapore, Singapore; [3]Natl. Cancer Ctr., Singapore, Singapore; [4]Lee Kong Chian Sch. of Med. and Sch. of Biol. Sciences, Nanyang Technological Univ., Singapore, Singapore; [5]Dept. of Microbiology, Tumor and Cell Biology, Karolinska Inst., Stockholm, Sweden

Abstract: Microglia, the resident macrophages of the central nervous system, continuously sense their environment to control brain homeostasis in physiological conditions and are able to quickly adapt to inflammation and injury. These cells colonize the brain during early embryonic development and persist throughout life. While much is known about microglia physiology in the adult, we still have limited information on the developmental phases and factors that regulate their differentiation during brain development. Here, we used transcriptomic analyses to delineate specific stages of microglia differentiation during embryogenesis and early life. We identified and validated embryonic microglia markers that are up-regulated during this developmental time period and potentially mediate specific functions in brain wiring. In spite of showing specific profiles, embryonic microglia expressed the main components of its "sensome" very early on. Consistently, embryonic microglia was affected by the absence of microbiota in germ free (GF) animals. Indeed, while brain patterning and morphogenesis were not affected in embryonic GF animals, microglia density in the neocortex, the preoptic area and the striatum was increased with altered morphology. By comparing control and GF embryonic microglia, we highlighted pathways dysregulated by the absence of microbiota (*i.e.* nurture) as compared to those that are not changed in GF animals (*i.e.* nature). Our study reveals that from embryonic stage to later on, microglia follows discrete developmental steps coordinated by intrinsic developmental programs and microbiota-derived environmental signals.

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Poster

293. Microglia Development and Function

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Topic: B.12. Glial Mechanisms

Support: NIH Grant 4R01MH101183-04

Title: Sex differences in microglial and fast-spiking interneuron maturation in mice and in human disease

Authors: *R. HANAMSAGAR[1], M. ALTER[2], C. BLOCK[3], H. SULLIVAN[3], J. BOLTON[4], S. BILBO[1];
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Abstract: Many neurological disorders have a clear sex difference associated with their incidence or outcome such as autism, depression and schizophrenia. Several of these diseases also have a major immune-component associated with their pathophysiology, including over activation of microglia (MG). MG are increasingly implicated in normal brain development and are involved in functions such as cytokine release and synaptic pruning. Furthermore, MG modulate fast-spiking (FS) interneuron maturation and migration during normal development. FS interneurons, specifically those that express the maturation marker parvalbumin (PV), are critical for synaptic inhibition; any disruptions in the development or migration of this population are implicated in several neuropsychiatric disorders including autism. Hence, changes in MG maturation could potentially impact neuronal migration and connectivity, resulting in increased susceptibility to development of neurological disorders. However, little is known about how MG impact specific populations of neurons during development, in health or disease, or if there are sex differences in these processes. We performed whole transcriptome analysis on MG isolated from hippocampus of male and female mice at different developmental time points, calculated maturity indices (IDX) based on global gene expression patterns and found that not only does MG maturity increase with development, there is a striking sex difference in this IDX - adult female MG are more mature than male MG at baseline. When IDX was quantified for mouse fast-spiking (FS) interneurons using transcriptome datasets obtained from publically available literature, it was found that FS-interneuron maturation also increases with development. Interestingly, when the indices were applied to datasets obtained from human clinical studies such as Alzheimer's disease and Autism, it was found that in the diseased state, the relationship between MG maturity and FS-interneuron cell maturity displayed a steep inverse slope that was significantly different from the controls. This suggests that increased MG maturation, that takes place during diseased state, can result in exacerbated decrease in FS-interneuron maturity.

Similarly, systemic injection of lipopolysaccharide in mice accelerated transcriptional maturity in male but not female MG, and inverted the association with PV expression as seen in human disease. These data suggest that males may be more vulnerable to developmental disorders such as autism based on sex differences in microglial maturation and its impact on FS cell development in response to immune activation.

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Poster

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Topic: B.12. Glial Mechanisms

Support: Fundação para a Ciência e a Tecnologia Portugal: PTDC/BIM-MEC/4778/2014

Title: Microglia and astroglia phenotype in a neuron specific - A_{2A} receptor overexpression model with age-like alterations.

Authors: ***J. E. COELHO**[1], I. MARQUES-MORGADO[1], M. BADER[2], D. BLUM[3], L. V. LOPES[1];
[1]Faculdade de Medicina da Univ. de Lisboa, Inst. De Medicina Mol., Lisbon, Portugal; [2]Max-Delbrück-Center for Mol. Med., Berlin, Germany; [3]Alzheimer & Tauopathies, Inserm URM-S1172, Univ. de Lille, Lille, France

Abstract: Upregulation of A_{2A} receptors in hippocampal and cortical brain regions is a common feature following brain insult and in Alzheimer's disease (AD). Previous work in our lab has shown this effect is accompanied by memory and synaptic plasticity impairment and structural changes, namely dendritic retraction (Batalha et al., 2013, Mol. Psychiatry). Little is known on the mechanism involved in this $A_{2A}R$ upsurge, although various studies suggest that this upregulation is an early-aging phenotype and may be instrumental in driving neurodegeneration. To address this issue we have generated a transgenic rat model with a postnatal neuronal-specific overexpression of human $A_{2A}R$ under the control of the CaMKII promoter [Tg(CAMKII-$hA_{2A}R$)]. These animals display age-like alterations in hippocampal function, with cognitive, synaptic and molecular impairments that are reversed upon $A_{2A}R$ blockade. (Batalha et al., 2013, Mol. Psychiatry; Temido-Ferreira et al., 2015, SFN). The aim of the present work was to clarify and characterize the phenotype of microglia and astroglia in these animals to evaluate the involvement of different cellular subsets in the $A_{2A}R$ neuronal dysregulation. We assessed glial

density, reactivity and morphology in Tg(CaMKII-hA_{2A}R) as compared to WT littermates. Along with a significant increase of the microglial marker Iba1 in the hippocampus (n=7; $P<0.05$), we found significant morphological differences in microglial cells of CA1 area of the hippocampus of transgenic animals: a decrease in the area of cellular influence; process retraction and acquisition of a more elongated cellular shape (n=2-3; $P<0.05$). The total number of microglia cells did not differ between WT and Tg animals. A significant decrease in GFAP protein was found in the hippocampus of Tg animals (n=7; $P<0.05$), which did not correlate with morphological changes neither in the length nor branching of astrocytic processes in CA1 area. These data reveal that A_{2A}R overexpression in forebrain neurons is sufficient to drive significant changes in glial cell function, inducing a primed state of microglia - triggering morphological alterations that resemble early states of activation process - and an asthenic phenotype of astrocytes. This suggests that the pathological process of A_{2A}R dysregulation derives from a synergy of synaptic and glial dysfunction, mimicking features of hippocampal aging. **Funding**: FCT- PTDC/BIM-MEC/4778/2014; LVL is an Investigator FCT, JEC is an FCT fellow (SFRH/BPD/87647/2012).

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Poster

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Topic: B.12. Glial Mechanisms

Support: ISF Grant 206/12

ISF Grant 1284/10

Title: The role of microglia and their CX3CR1 signaling in olfactory bulb neurogenesis

Authors: *R. RESHEF[1], E. KUDRAYAVISTKAYA[2], H. SHANI[2], N. RIMMERMAN[1], A. MIZRAHI[2], R. YIRMIYA[1];
[1]Dept. of Psychology, The Hebrew Univ., Jerusalem, Israel; [2]Dept. of Neurobiology, The Hebrew Univ., Jerusalem, Israel

Abstract: Microglia are known to play important roles in brain development and plasticity, but their roles in development and maturation of synapses on adult-born neurons remains unknown. To elucidate the role of microglia in neurogenesis, we labeled newborn granule neurons (GNs)

migrating to the olfactory bulb (OB) in microglia-depleted mice (induced by CSF-1 receptor inhibition) and CX3CR1 deficient ($CX3CR1^{-/-}$) mice, by injecting a tdTomato-expressing lentivirus. 28 days later we imaged infected maturing OB GNs using 2-photon microscopy, followed by immunohistochemical examination of their spine density and size. Our data shows that microglia-depleted and $CX3CR1^{-/-}$ mice had a significant reduction in spine density. Moreover, $CX3CR1^{-/-}$ mice had smaller spine heads. Time-lapse data indicated that microglia depletion and CX3CR1 deficiency caused stabilization of synaptic turnover in maturing GNs, as fewer dendritic spines were formed and lost over 24h. These morphological changes were found to have functional significance as *in vivo* calcium imaging showed enhanced responses to odors of mitral cells, which are normally inhibited by GNs. RNA-Seq analysis indicated that the morphological changes in the two experimental models were associated with different molecular mechanisms, including the complement and cytokines transcriptional programs in microglial depletion, and MHC-I, wnt and interferon transcriptional programs in CX3CR1 deficiency. Our findings indicate that microglia are involved in normal development, maturation and plasticity of adult-born OB neurons, with functional implications to the OB output.

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Poster

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Program#/Poster#: 293.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R21MH104280 and R01MH106553

Title: Sex differences in microglia number and activation in the developing rat brain

Authors: *A. TURANO, J. LAWRENCE, J. SCHWARZ;
Univ. of Delaware, Newark, DE

Abstract: Microglia are the resident immune cells of the brain. During development, microglial progenitor cells migrate and infiltrate into the nervous system from the periphery. This period of migration and infiltration is critical to the maturation of the developing brain. Microglia in the developing brain are quite distinct from microglia in the adult brain as they have a round, amoeboid shape with short, thick processes and they produce elevated levels of cytokines and chemokines imperative for many processes of normal brain development. Activation of microglia in the developing brain, via neonatal infection or immune activation, can often lead to

long-term neuronal and cognitive dysfunction. Furthermore, microglial activation is associated with multiple neurodevelopmental disorders including autism, ADHD, schizophrenia, and cerebral palsy - disorders also known or suspected to have immune etiologies. All of these disorders exhibit a strong sex bias in males. We have previously seen that male rats have significantly more microglia in the developing hippocampus, cortex and amygdala than female rats on postnatal day 4 (P4) (Schwarz et al. J. Neurochem., 2012). It is possible that this difference is mediated by the surge of testosterone occurring in males during masculinization of the brain (Lenz et al. J. Neurosci., 2013). More so, females do not show the same vulnerability that males show to infection at P4 (Bilbo et al. J. Neuroimmune Pharmacol., 2011). Given these rodent data and well-known human epidemiological data, we hypothesize that male rat pups will be more vulnerable to an immune challenge during the critical period in which microglia are infiltrating the nervous system. To test this hypothesis, we treated male and female rat pups with a mild *E.coli* infection on P4 and observed the presence of activated microglia in the developing hippocampus. Also, using an *in vitro* method, we treated isolated sex-specific microglia cultures from the hippocampus of male and female rat pups with a mild lipopolysaccharide (LPS) challenge on P4 and examined whether immature microglial cells exhibit a sex-specific response to immune activation. Lastly, sex-specific microglia cultures from the hippocampus of male and female rat pups were treated with testosterone on P0 and P1 and then treated with LPS on P4. Cultures were analyzed for inflammatory gene expression to determine whether immature microglial cells exhibit a sex-specific response to immune activation and whether this is mediated by the early surge in testosterone that occurs around birth. Funding source: NIH R21MH104280 and R01MH106553.

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Poster

293. Microglia Development and Function

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Topic: A.01. Neurogenesis and Gliogenesis

Support: The Patterson Trust Clinical Research Award

NIH Grant K08-MH086812-06

T32-NS007421

Title: The distinct effects of prenatal stress on microglia morphologies in adult and embryonic brain and mediation by interleukin 6

Authors: *S. B. GUMUSOGLU[1,3], R. S. FINE[3], S. J. MURRAY[3], M. E. DAILEY[2], H. E. STEVENS[4,3];
[2]Dept. of Biol. Sci., [1]Univ. of Iowa, Iowa City, IA; [3]Yale Child Study Ctr., Yale Univ., New Haven, CT; [4]Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: Background: Microglia are widely understood as the brain's immune cells, responsible for pathogenic defense but also other functions including regulation of synapses and progenitor number. Like other cells in the brain, microglia function may be developmentally regulated. Microglia can also be functionally altered in circumstances of infection and/or stress. Prenatal stress (PS) occurs very early in development and can increase offspring risk for altered neurodevelopmental and psychiatric phenotypes. Microglia may play a dynamic role in the persistent effects of PS across neurodevelopment. Here, we sought to elucidate the effects of PS on microglia in the prenatal and postnatal brain. By targeting one potential mediator of PS effects, maternal interleukin 6 (IL-6), we found that distinct microglia changes in embryonic and adult brain after PS may in fact be linked. A better understanding of stress-induced microglial changes across neurodevelopment may elucidate mechanisms for prevention and intervention in psychiatric disorders.
Method: CD-1 mouse dams were either maintained as controls or restraint stressed three times daily. Some dams were also injected IP with anti-IL-6 neutralizing antibody or vehicle 15-20 minutes before restraint for the last week of gestation. Offspring cortices were analyzed for microglia density and morphology along a spectrum at embryonic day 14 (E14) or at postnatal day 90 (P90). A third cohort of control dams received IL-6 injections three times daily with offspring examined at E14. Microglia morphology in embryonic brain explants were also examined via live confocal microscopy.
Results: While adult microglia displayed more ramified features across the microglia morphological spectrum, embryonic microglia occupied only its most amoeboid end. PS offspring showed increased densities of ramified microglia at P90 but, surprisingly, also showed increased densities of multivacuolated, amoeboid-like microglia at E14. PS effects on microglia appeared to be in part mediated by IL-6:IL-6 provoked the same changes at E14, and anti-IL-6 prevented morphological changes at both E14 and P90. Live imaging microscopy confirmed the presence of a dynamic microglia morphological spectrum at E14, as microglia shift from more ramified (i.e. multiple spines) to less ramified, before becoming amoeboid or multivacuolated.
Conclusions: These findings suggest that PS exposure, perhaps through IL-6 mechanisms, disrupts the normal developmental trajectory of microglia in the brain. Furthermore, pre- and post-natal microglia may serve different roles, particularly in response to stress, as evidenced by distinct morphologies.

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Poster

293. Microglia Development and Function

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Program#/Poster#: 293.12/A12

Topic: B.12. Glial Mechanisms

Support: NSF Grant 1463098

NSF Grant 1547693

ARL contract W911NF-2-0010

Title: Use of multiple sub-threshold glutamate stimuli to monitor brain cell calcium dynamics in cultures with decreasing microgial and astrocyte content

Authors: *K. C. ST MARTHE[1], C. N. POOLE[1], M. GRAGSTON[2], N. NGUYEN[1], T. PIEHLER[3], L. PIEHLER[3], M. A. DECOSTER[1];
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Abstract: Recent work identifies astrocytes and microglia as active elements of the brain, maintaining numerous homeostatic functions. Disturbances in these functions result in development of neuropathologies, including effects in neuroinflammation, traumatic brain injury, and different stages of brain tumors. Astrocytes and microglia in the central nervous system (CNS) contribute to homeostasis for dynamic second messengers in the CNS, including intracellular calcium concentration ($[Ca^{2+}]_i$). We hypothesized that altering glial density in primary rat cortical brain cell cultures could shape network $[Ca^{2+}]_i$ responses after multiple stimuli with subthreshold (nM), concentrations of the neurotransmitter glutamate (GLU). We used antimitotic agents, 10 micromolar (uM) cytosine arabinoside, or 5-fluorodeoxyuridine (FdU) at 1-4 uM, to inhibit proliferating glia. After developing for 14-21 days in vitro to establish network connections and to modify glial content with antimitotics, cells were stimulated with 250, 500, and/or 750 nM GLU and $[Ca^{2+}]_i$ dynamics measured using digital imaging microscopy with the fluorescent Ca^{2+} indicator fluo3-AM. GLU stimuli were determined to be subthreshold and non-toxic, since once added, GLU was not washed from the culture well, and $[Ca^{2+}]_i$ returned back to baseline levels. For both AraC and FdU, responses to multiple GLU stimuli were consistent: cultures high in glia (no antimitotics), resulted in transient, non-synchronized $[Ca^{2+}]_i$ dynamics after GLU stimuli. In contrast, cultures depleted of glia showed synchronized, more sustained $[Ca^{2+}]_i$ elevations. Quantum dot- and 2 micron fluorescent bead- uptake was used to identify phagocytic microglia, the expression marker glial fibrillary acidic protein used for astrocytes, and microtubule-associated protein-2 used for neurons. Since Hui et al. (2016) have recently described the importance of FdU concentration in

mediating the effects of neuroinflammation by altering glial content in cultures, we varied the concentration of FdU in our studies using 1, 2, or 4 uM before GLU stimulus. Our results support the hypothesis of a tight concentration gradient for effective FdU treatment, as our most dynamic $[Ca^{2+}]_i$ results after GLU stimulus occurred after 1 uM FdU, which along with controls, had 8-fold higher bead uptake than 2 and 4 uM FdU conditions. Higher concentrations of FdU (2 or 4 uM) resulted in overall decreases in all cellular responses to GLU stimulus, although a variety of cell morphologies were intact. Thus $[Ca^{2+}]_i$ network dynamics can be shaped by controlling glial cell density, revealing information processing by the system in response to multiple GLU stimuli.

Disclosures: K.C. St Marthe: None. **C.N. Poole:** None. **M. Gragston:** None. **N. Nguyen:** None. **T. Piehler:** None. **L. Piehler:** None. **M.A. DeCoster:** None.

Poster

293. Microglia Development and Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 293.13/A13

Topic: B.12. Glial Mechanisms

Support: the Fundamental Research Funds for the Central Universities

Title: Role of the complement receptor C3aR in excitotoxicity-induced neuropathology

Authors: *H. LIAN[1], X. LI[2];
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Abstract: Excitotoxicity participates in the pathology development of many neurological diseases. Inflammation caused by glial activation is now recognized not only as a consequence but also a contributor to excitotoxicity-induced neuropathology. Complement system is an essential conservative inflammation-regulatory pathway activated in patient brains of many neurological diseases. As an important downstream receptor for complement activation, C3aR has been shown to regulate synaptic function and neuronal excitability. By using pharmacological and genetic manipulation of C3aR activity in vivo and in vitro, we'll dissect out how complement signaling through C3aR activation mediates pathophysiology caused by excitotoxicity.

Disclosures: H. Lian: None. **X. Li:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.01/B1

Topic: A.02. Postnatal Neurogenesis

Title: Role of anti-oxidant supplementation on developing cerebellum of rats exposed to sodium arsenite ($NaAsO_2$)

Authors: *P. DHAR[1], P. KUMAR[2], P. KAUSHAL[2];
[2]Anat., [1]All India Inst. of Med. Sci., New Delhi, India

Abstract: Background:
Millions of people across the globe are exposed to inorganic arsenic (*iAs*). Ground water contaminated with *iAs* and soil dust are the major environmental sources of exposure to *iAs*. Higher incidence of cancer has been reported in exposed populations. Gestational and early postnatal exposure to *iAs* in animal models has been associated with structural and functional alterations. Oxidative stress has been suggested as one of the important factors underlying *iAs* induced adverse effects.
Aim: To determine the role of anti-oxidant supplementation on rat cerebellum following sodium arsenite ($NaAsO_2$) exposure during early postnatal period.
Methods:
Pregnant Wistar rats (18-19 days gestation) were housed under controlled laboratory conditions. The day of delivery of pups was considered as postnatal day zero (PND 0). The pups from different litter groups were randomly assigned to control and experimental groups such as normal controls and vehicle controls (receiving distilled water, ethanol and (DMSO), *iAs* ($NaAsO_2$) alone treated, *iAs*+ALA and *iAs*+Curcumin treated. The test substances ($NaAsO_2$ alone and $NaAsO_2$ $NaAsO_2$along with ALA/Curcumin) were administered by intraperitoneal route from PND 1 to 21 to experimental groups where as the control groups were given no treatment or received only the vehicle. During this period behavioral tests were carried out and, the animals were sacrificed on PND22. The cerebellar tissue obtained from perfusion fixed animals (n=6/group) was processed for immunohistochemical localization of various proteins and fresh tissue was used for Western blot analysis.
Results:
The behavioral tests showed functional deficits with context to cerebellar functions in *iAs* alone treated group and a substantial recovery in ALA/Curcumin supplemented groups. Also, IHC expression of proteins associated with dendritic and axonal growth (MAP2 and Tau), synaptogenesis (Syp and PSD95) and myelination (MBP) showed downregulation in *iAs* alone treated groups where as co-administration of ALA or Curcumin along with *iAs* resulted in significant upregulation in expression of these proteins.

Conclusions:
Exposure to *iAs* during early postnatal period resulted in adverse effects on the developing cerebellum. However, substantial recovery observed in antioxidant-supplemented groups is suggestive of the potential of ALA and Curcumin in ameliorating *iAs* induced developmental neurotoxicity to a certain extent.

Disclosures: **P. Dhar:** A. Employment/Salary (full or part-time): AIIMS. 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; AIIMS, ICMR. **P. Kumar:** A. Employment/Salary (full or part-time): AIIMS. **P. Kaushal:** A. Employment/Salary (full or part-time): AIIMS.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.02/B2

Topic: A.02. Postnatal Neurogenesis

Support: National Institute on Aging Intramural Research Program

Title: Running enhances structural maturity of young adult-born dentate granule cells in mouse hippocampus

Authors: *S. T. LUBEJKO[1], N. SAH[1], C. VIVAR[2], H. VAN PRAAG[1];
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Abstract: The dentate gyrus (DG) of the mammalian hippocampus is one of two brain regions in which new neurons are continuously integrated throughout adult life. Voluntary running has been shown to increase hippocampal neurogenesis and performance on hippocampal-related behavioral tasks in rodent models. Additionally, running has been demonstrated to boost dendritic morphological maturity and structure in adult-born DG granule cells. Adult-born cells require weeks to differentiate, integrate, and mature in the granule cell layer of the DG; however, less is known about the structure and function of these neurons and the possible effects of running within the first week after cellular division. To determine the exercise effects on neurogenesis and the morphology of very young seven-day-old neurons in the adult DG, we stereotactically injected a retroviral vector expressing green fluorescent protein (GFP) in the DG of 5-week-old C57Bl/6 male mice housed in standard cages or with unlimited access to running

wheels. In addition, animals received three days of intraperitoneal bromodeoxyuridine (BrdU) injections for a systemic quantification of dividing cells. Quantification of $BrdU^+$ cells showed that running animals exhibited a significant increase in proliferation in the DG after seven days compared to controls. Furthermore, one-week-old neurons located in the dorsal aspect of the DG already display structural plasticity in response to running conditions. Retrovirus-infected cells from running animals showed increases in multiple aspects of fine morphology such as soma size and dendritic branching. Our results indicate that a short duration of voluntary exercise modifies the structure of one-week-old adult-born neurons, potentially allowing for more functional connections within their neural circuits.

Disclosures: **S.T. Lubejko:** None. **N. Sah:** None. **C. Vivar:** None. **H. van Praag:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.03/B3

Topic: A.02. Postnatal Neurogenesis

Support: Gallagher Foundation

Sigma Xi GIAR

Title: Developmental influence of stress and fluoxetine on the oxytocinergic system in the california mouse (Peromyscus californicus)

Authors: *J. CRUZ, S. PETERSON, E. A. BECKER;
St. Joseph's Univ., Philadelphia, PA

Abstract: A recent unprecedented increase in antidepressant use among pregnant and lactating mothers has caused concern among researchers and clinicians over the safety of this practice for developing offspring. Infants may be exposed to antidepressants, as selective serotonin reuptake inhibitors (SSRIs) cross the placental barrier and the ductal epithelium of mothers. Researchers, prescribing physicians and mothers alike must carefully weigh the potentially detrimental effects of perinatal stress on offspring development against the risks associated with early SSRI exposure. Previous research has indicated that SSRI exposure results in altered social behavior of the offspring later in life. Although mounting evidence suggests a significant impact on offspring development, the neural mechanisms through which SSRI exposure in development affects these social behaviors has not been fully characterized. Because oxytocin (OT) is sensitive to serotonergic insult early in development and is involved in social behaviors, it is a reasonable

candidate. We used the California mouse (*Peromyscus californicus*) to investigate the interaction between maternal stress and SSRI exposure on developing offspring. We exposed offspring prenatally to a stress condition (no stress/ stress) and a postnatal fluoxetine condition (control/fluoxetine) and examined oxytocin (OT) at a multiple sites of the brain (SON & PVN). At PND 120, offspring were sacrificed and brains were preserved for immunocytochemical analyses. Preliminary data has revealed a significant decrease in OT-ir staining in the stressed/fluoxetine groups compared to the stressed/no fluoxetine group and the no stress/fluoxetine group. Our preliminary data further implicates developmental fluoxetine as harmful to normal neural outcomes later in life.

Disclosures: J. Cruz: None. **S. Peterson:** None. **E.A. Becker:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.04/B4

Topic: A.02. Postnatal Neurogenesis

Support: NIH grant R01NS080844

IRSP grant, the University of Mississippi Medical Center

Title: Early postnatal lipopolysaccharide exposure leads to enhanced neurogenesis and impaired communicative functions in rats

Authors: *Y. PANG[1,2], X. DAI[2], A. ROLLER[2], K. CARTER[2], I. PAUL[2], A. BHATT[2], R. LIN[2], L.-W. FAN[2];
[1]Univ. of Mississippi Dept. of Med., Jackson, MS; [2]Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Perinatal infection is a well-identified risk factor for a number of neurodevelopmental disorders with neurobehavioral impairments, including the white matter injury (WMI) and Autism Spectrum Disorders (ASD). The underlying mechanisms by which early life inflammatory events cause aberrant neural, cytoarchitectural, and network organization, reflecting neurobehavioral dysfunction, remain elusive. This study is aimed to investigate how systemic lipopolysaccharide (LPS)-induced neuroinflammation affects microglia phenotypes and early neural developmental events in rats. We show here that LPS exposure at early postnatal age leads to a robust microglia activation which is characterized with mixed microglial proinflammatory (M1) and anti-inflammatory (M2)-like phenotypes. More specifically, we found that microglial M1 markers iNOS and MHC-II were induced at relatively low levels in a

regionally restrict manner, whereas M2 markers CD206 and TGFβ were upregulated in a sub-set of activated microglia in multiple white and gray matter structures. This M2-biased microglia polarization was associated with a markedly decrease in natural occurring apoptosis, but increase in cell proliferation in the subventricular zone (SVZ) and the dentate gyrus (DG) of hippocampus. LPS exposure also leads to a significant increase in oligodendrocyte lineage population without causing discernible hypermyelination. Moreover, LPS-exposed rats exhibited significant impairment in communicative and cognitive functions. These findings suggest a possible role of a M2-like microglial activation in abnormal neural development underlying ASD-like behavioral impairments in the current animal model.

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Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.05/B5

Topic: A.02. Postnatal Neurogenesis

Support: USUHS IN HOUSE GRANT R070304115

Title: Regional distribution and cellular colocalization of KCC2 in ferret neocortex

Authors: ***F. T. DJANKPA**[1], M. CHATTERJEE[3], S. L. JULIANO[2];
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Abstract: KCC2 plays several important roles in the development of the cerebral cortex that range from neuronal migration to maturation and differentiation. KCC2 is an ion channel protein also known as a potassium/chloride co-transporter. This protein modulates neuronal chloride homeostasis by influencing the switch of GABA polarity from depolarizing in young neurons to hyperpolarizing in mature neurons. Although the developmental regulation and regional distribution of KCC2 has been described in rodents, little information describes the distribution and regional localization of KCC2 in ferrets, which are the smallest mammals with a gyrencephalic brain. In addition, very few reports in any species describe the developmental cellular distribution of KCC2 in the cerebral cortex. As an ion channel protein, we do not know whether KCC2 is expressed in a large cohort of neurons or in a selective phenotype. We describe here the expression pattern of KCC2 during development, the regional distribution, subcellular

localization and cellular colocalization with neuronal markers in ferret cortex. KCC2 is strongly expressed in the subplate at P0 and P7, which reorganizes to a subtle laminar pattern at P14 as the neocortical layers develop. In the adult, however, KCC2 has a homogenous distribution throughout the cortical thickness. In younger brains (P0, P7), KCC2 immunoreactivity occurs in the cytoplasm of neurons, while by P14, KCC2 appears in a pattern apparently surrounding cells or in their processes. Subcellular localization by separating cytoplasmic and membrane fractions shows that KCC2 remains in the membrane fraction of neocortical samples. We also found that KCC2 colocalizes with multiple neuronal markers including TUJ1 and MAP2 and the presumptive inhibitory markers parvalbumin and calretinin in both young and mature neocortex. We also know from previous studies that administration of the toxin methylazoxymethanol (MAM) to pregnant ferrets results in an upregulation of KCC2 in the cerebral cortex of the offspring. This increase is reflected in a number of functional and structural consequences (e.g. Abbah and Juliano 2015). We also see histologic alterations in the distribution of KCC2 after MAM treatment that reflects the increased KCC2 levels and altered laminar organization. In an organotypic culture model, cells migrating away from the ganglionic eminence show reduced speed when treated with agents that increase KCC2. Our findings indicate that the action of KCC2 in ferret cortex is likely to change focus as the animal develops and may influence the activities of both excitatory and inhibitory neurons.

Disclosures: F.T. Djankpa: None. **M. Chatterjee:** None. **S.L. Juliano:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.06/B6

Topic: A.02. Postnatal Neurogenesis

Title: Role of metabolic and nutritional status in programming cognition by early-life stress: potential for peripheral interventions using fatty acids

Authors: *K.-Y. YAM[1], E. NANINCK[1], L. SCHIPPER[2], S. LA FLEUR[3], A. GREFHORST[4], A. OOSTING[2], E. VAN DER BEEK[2], P. LUCASSEN[1], A. KOROSI[1];
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Abstract: Early-life stress (ES) is associated with cognitive deficits in adulthood. Mechanisms underlying such programming remain elusive and the role of metabolism and nutrition are

largely ignored in this context. Importantly, leptin, the metabolic hormone secreted from white adipose tissues (WATs), and essential fatty acids (FAs) play key roles in brain development. Imbalances in circulating leptin as well as in FAs early in life are also associated with cognitive impairments in later life. We therefore studied if; i) ES affects metabolic and nutritional status, both centrally and peripherally, ii) early dietary intervention with a reduced linoleic acid (LA) to α-linoleic acid (ALA) ratio prevents these ES-induced cognitive impairments, and if iii) modulations of hippocampal neurogenesis might mediate effects of this diet.We used a chronic ES mouse model in which C57/BL6j dams are housed with limited nesting/bedding material from postnatal day (P) P2-P9, resulting in cognitive decline in adulthood. Dietary intervention consisted of subjecting mice to a diet equal in total FAs, but either containing a low (1) or high (15) LA/ALA ratio between P2-P46. Cognitive performance was assessed using object recognition, object location and morris water maze tasks and neuronal survival determined by BrdU+ immunocytochemistry. Chronic ES resulted in; i) lasting reductions in WAT weight, circulating leptin levels and WAT *Lep* mRNA expression, together indicating an ES-induced programming of WAT function. In addition, ES did not affect hippocampal leptin receptor (*Lepr*) mRNA expression, but interestingly, upregulated *Lepr* expression in the choroid plexus, indicating a compensation for the peripheral changes. Moreover, ES leads to increased omega-6-to-3 FA ratio in the hippocampus, while it was decreased in livers of ES mice early in life. In addition, ii) low LA/ALA diet early in life prevented the ES-induced cognitive impairments in all three behavioral tasks. And iii) this diet prevented the reduction of adult born neuron survival caused by ES, suggesting that the beneficial effects of the diet may, at least partly, be mediated by preserving the neurogenic capacity in the ES-exposed offspring. Further characterization of the lasting effects of ES and FAs diet on nutritional status in peripheral and central organs are currently under investigation. These results highlight for the first time the relevance of body-to-brain signaling by leptin, and the potential of dietary intervention with LA/ALA in programming the brain by ES. This creates exciting innovative and non-invasive opportunities to prevent the ES-induced cognitive impairments.

Disclosures: **K. Yam:** None. **E. Naninck:** None. **L. Schipper:** None. **S. la Fleur:** None. **A. Grefhorst:** None. **A. Oosting:** None. **E. van der Beek:** None. **P. Lucassen:** None. **A. Korosi:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.07/B7

Topic: A.02. Postnatal Neurogenesis

Support: FAPESP 2014/22313-3

FAPESP 14/50457-0

Title: Maternal melatonin deprivation during pregnancy and lactation impairs spatial reference and working memory in adult rats

Authors: *L. C. TEIXEIRA[1], A. V. MACHADO-NILS[2], F. G. AMARAL[1], G. F. XAVIER[2], J. CIPOLLA NETO[1];
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Abstract: Maternal melatonin plays a role in providing photoperiodic information to the fetus and by that influencing the regulation and timing of internal rhythms of the offspring and their preparation for extrauterine life. Maternal melatonin deprivation during prenatal and early postnatal has negative health consequences for the offspring that continue into adulthood (e.g. altered energy metabolism). There is a paucity of data examining the effects of maternal melatonin suppression on cognitive function of offspring. The present study examined the spatial memory of adult offspring born to pinealectomizeddams. Female Wistar rats were submitted to pinealectomy (PINX) or SHAM surgery (CTL). The PINX rats were divided into two groups and received either melatonin (PINX+MEL) or vehicle (PINX). After 4 weeks the rats were allowed to mate and received the treatment until lactation's end. Adult male offspringwere trained in water maze. Probe test was performed 24 hr after training.Two days after probe test, working memory training was conducted.Preliminary data show that maternal melatonin deprivation during pregnancy and lactation disrupts both spatial reference and working memory; these results may be related to changes in neurogenesis in the hippocampus. Importantly, these impairments were reversed by maternal melatonin replacement.

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Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.08/B8

Topic: A.02. Postnatal Neurogenesis

Support: RFUMS-DePaul Alliance

Title: Hippocampal neuronal loss and impaired neurogenesis following repeated closed-head concussive impacts

Authors: *D. A. PETERSON[1], S. G. CHIREN[1], E. REISENBIGLER[1], N. JAMNIA[2], N. KAPECKI[3], G. DEJOSEPH[2], J. H. URBAN[2], R. A. MARR[3], G. E. STUTZMANN[3], D. A. KOZLOWSKI[4];
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Abstract: Head impact and/or acceleration-deceleration can produce traumatic brain injury (TBI) that can be categorized as severe or mild, with concussions categorized typically as mild TBI. There is emerging evidence that the accumulation of concussive injuries, frequently encountered in sports or combat, contributes to the development of chronic traumatic encephalopathy (CTE). To assess the sequence of degenerative and regenerative responses following repeated concussions, we utilized a closed-head model of controlled cortical impact to deliver injury to rats. All procedures were approved by IACUC. Young adult Long Evans rats received a sham procedure (anesthetic) or the addition of impact on the surface of the head by a modified Leica CCI device at a location overlying the sensorimotor cortex. Experimental groups received a single impact or three successive impacts separated by 48 hour intervals. After 30 days, animals were deeply anesthetized, transcardially perfused, and the brains were collected and sectioned at 40 μm. There was no evidence of skull fracture and no macroscopic deformation of the cerebral cortex underlying the impacted skull. Closed head impact produced neuronal disorganization in the underlying cerebral cortex and the volume of cerebral cortex and corpus callosum was reduced in experimental groups. Hippocampal volume was also reduced and we investigated further the effect of impact on this more distant structure. Repeated concussive impact produced no noticeable alteration in Iba1+ microglial or Olig2+ oligodendrocyte progenitor cell populations in the hippocampus. However, repeat concussion produced astrocytic (GFAP+) hypertrophy throughout the hippocampus that was pronounced at the hippocampal fissure. The population of DCX+ neuroblasts was reduced in the dentate gyrus following repeat concussions with more severe loss in the buried blade of the dentate gyrus. Repeated concussion also resulted in neuronal (NeuN+) loss in Area CA1. Despite the distance of the hippocampus from impact, repeated mild impact results in an injury response, reduced neurogenesis, and neuronal loss in the hippocampus. Thus repeated concussion may contribute to long-term disruption of hippocampal circuitry.

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Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.09/B9

Topic: A.02. Postnatal Neurogenesis

Support: NSERC discovery grant

CIHR operating grant

Title: Early-age running enhances activity of adult-born dentate granule neurons following learning

Authors: O. SHEVTSOVA[1], Y. TAN[1], C. M. MERKLEY[1], G. WINOCUR[2], *M. WOJTOWICZ[1];
[1]Dept Physiol, Univ. Toronto, Toronto, ON, Canada; [2]Baycrest Inst., Toronto, ON, Canada

Abstract: We have shown previously that early-age running enhances survival of adult-born hippocampal neurons later in life. This effect may be a contributing factor to cognitive reserve (Merkley et al., 2014, Front. Neurosci.). In this study we are addressing the hypothesis that enhanced neuronal survival is a significant factor in improving functionality of dentate granule neurons in learning tasks. One group of young, 1 month old rats was given free access to running wheels for 6 weeks and another group was housed in standard laboratory cages. After this period the running animals were returned to standard cages. After 4 months all rats were trained on a contextual fear conditioning task and, 2 weeks later, tested for memory of the contextual fear response. The testing was done on the same context as well as similar and very different contexts to provide information on the quality of memories. A group of non-tested animals was used as controls. Neurogenesis was measured with standard immunohistochemical procedures using endogenous markers Ki67, DCX and NeuN. These static measures of neurogenesis did not provide any evidence for differences between early runners and non-runners. Cell activity was measured using the expression of c-fos at 90 minutes following the memory test. Neuronal survival was measured at 5 weeks following injections of a mitotic marker CldU. The overall expression of activity marker c-fos was enhanced 4-5 fold in the dentate gyrus of the tested vs. non-tested animals suggesting significant involvement of dentate gyrus in learning. However, there was no effect on overall c-fos expression due to early running. In contrast, the % expression of c-fos in CldU-labeled cells was significantly increased in early runners vs. non-runners. These preliminary results show enhanced functional status of adult-born dentate granule neurons in comparison to developmentally-born neurons and their preferential activity in animals that were exposed to early-age running. With respect to previously published studies this work provides further support for involvement of adult-born neurons in neurogenic and functional

cognitive reserve.
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Poster

294. Environmental Influences on Adult Neurogenesis

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.10/B10

Topic: A.02. Postnatal Neurogenesis

Support: Intramural Research Program at NIEHS/NIH

Title: Adult neurogenesis in the dentate gyrus is regulated by α7 nAChR activation.

Authors: *S. L. OTTO, J. L. YAKEL;
Neurobio. Lab. - Ion Channel Physiol. Group, Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

Abstract: Hippocampal adult neurogenesis is a life-long process whereby neural stem cells located in the subgranular zone of the mammalian dentate gyrus divide, differentiate, mature and integrate in the local circuitry. Our overall objective is to determine how cholinergic input affects the generation of new granule cells, and whether these changes impact learning and memory. A hallmark of neurodegenerative disorders is impaired adult neurogenesis. Alzheimer's disease shows both loss of cholinergic innervation and impairment of neurogenesis. The dentate gyrus receives cholinergic input from the basal forebrain, a source of signaling that may impact adult neurogenesis. Cholinergic receptors could contribute a counterbalanced set of signals to modulate neurogenesis. Here we focus on the α7 nicotinic acetylcholine receptors (nAChRs), considered important in maturation of granular cells in the dentate gyrus and expressed on cells in the subgranular zone. α7 nAChRs are highly expressed in neonates and then decline until reaching adult levels. Though environmental factors as diverse as exercise and solitude have been shown to have an effect on adult neurogenesis, extant literature conflicts as to possible cholinergic effects. Little is known of the possible mechanism(s) through which cholinergic signaling may act. To better understand adult neurogenesis in the dentate gyrus, we use a nestinCrERT2 mouse that permits the labeling of nestin^{+} neural stem cells. Using this model we show that expression of nestin^{+} neural stem cells in global α7 nAChR knock-out (KO) mice is significantly decreased. In addition, in organotypic cell culture, adding MLA (a selective antagonist of the α7 nAChR) caused a decline in the quantity of nestin^{+} cells. To further study

the mechanism involved we are quantifying nestin^{+} cells from organotypic cultures treated with PNU-120596, an α7 nAChR positive allosteric modulator, and choline. One possible explanation of the decline in nestin^{+} cells is that maturation of adult born granule cells is delayed, resulting in overall loss of neurons as they fail to integrate in a timely fashion. This may result in a compensatory increased neurogenesis, decreasing the neural stem cell pool. To investigate this we are using EdU to birthdate adult born granule cells as well as quantifying immature granule cells present in α7 nAChR KO or wild-type mice. We are also investigating whether pattern separation, a function of adult neurogenesis, is affected in α7 nAChR KO mice with reduced nestin stem cell expression. Ongoing research is using an AChRα7 fl/fl mouse to determine which cells are most responsible for the loss of nestin^{+} cells seen in a global α7 nAChR KO.

Disclosures: **S.L. Otto:** None. **J.L. Yakel:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.11/B11

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant K99ES022992 to CKT

Dart Neuroscience LLC to HTC

Hahn Family Foundation to HTC

NIH Grant EY011261 to HTC

Title: Reversible developmental stasis in response to nutrient availability in Xenopus laevis CNS.

Authors: *C. K. THOMPSON, C. R. MCKEOWN, H. T. CLINE;
The Scripps Res. Inst., La Jolla, CA

Abstract: Many organisms are confronted with intermittent nutrient availability, but the mechanisms used to cope with such fluctuations during development are not well understood. This is particularly true of the brain, the development and function of which is energy intensive. Here we examine the effects of nutrient restriction and availability on development of the visual system in Xenopus laevis tadpoles. During the first five-seven days of development, Xenopus tadpoles draw their nutrients from internal yolk stores in the gut, however upon depletion of these nutrients animals must forage for food in their environment. By altering access to external

nutrients starting when yolk is depleted, we have defined a period of reversible stasis during tadpole development. We demonstrate that nutrient restriction (NR) results a decrease in overall growth of the animals, a failure to progress through developmental stages, and a decrease in volume of the optic tectum. During NR, neural progenitors virtually cease neuronal proliferation, but tadpoles swim and behave normally. Delayed feeding following NR increases neural progenitor cell proliferation in the tectum by more than 10 fold relative to NR tadpoles, with the number of proliferating cells equaling that of fed counterparts after a week of supplemental feeding. The delayed feeding paradigm also rescued the NR-induced body length and tectal volume deficits, and partially rescued the delayed progression through developmental stages. We determined that tadpoles can recover from developmental stasis if food is provided at any point within the first 8 days of NR. Food availability restores neural progenitor cell proliferation in the tectum and rescues NR-induced developmental deficits. After 8 days of NR, however, access to food fails to increase cell proliferation. These results show that development of the brain in tadpoles is acutely sensitive to fluctuations in nutrient availability and that NR induces stasis of developmental processes, from which animals can recover if food becomes available within a critical window.

Disclosures: C.K. Thompson: None. **C.R. McKeown:** None. **H.T. Cline:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.12/B12

Topic: A.02. Postnatal Neurogenesis

Support: Mater Foundation

Title: Loss of the sulfate transporter Slc13a4 alters behavior and neurogenesis in adult mice

Authors: *Z. ZHANG[1], M. PIPER[1], P. DAWSON[2], D. SIMMONS[1];
[1]SBMS, the Univ. of Queensland, Brisbane, Australia; [2]Mater Res. Inst., Brisbane, Australia

Abstract: Sulfate conjugation (sulfonation) of molecules is essential for a diverse array of physiological processes. In the brain, sulfonation is a critical step in modulating the functions of extracellular matrix molecules, which in turn regulate adult neurogenesis. SLC13A4 is a sodium-dependent sulfate transporter which is primarily expressed both in the placenta, and by the choroid plexus of the brain. Our previous work has shown that deletion of *Slc13a4* causes severe fetal abnormalities and death in mice. Interestingly, we also found that $Slc13a4^{+/-}$ female mice lost a significant number of pups after birth compared with $Slc13a4^{+/+}$ females, regardless of pup

genotype, suggesting that *Slc13a4*$^{+/-}$female mice may have deficits in maternal behavior. Indeed, pregnant *Slc13a4*$^{+/-}$ mothers took significantly longer to retrieve their pups in a test of maternal care behaviors. Behavioral tests, including open field, elevated plus maze, odor discrimination and home cage metabolic tests were then performed on non-pregnant female mice to investigate whether the loss of *Slc13a4* alleles causes abnormal behavior more generally. *Slc13a4*$^{+/-}$ and *Slc13a4*$^{-/-}$ mice were found to exhibit increased exploring behavior in a novel environment compared with *Slc13a4*$^{+/+}$ mice. Analysis of *Slc13a4* expression in choroid plexus during pregnancy identified a significant peak at gestational day 8.5, which is consistent with the peak in adult neurogenesis in the maternal subventricular zone (SVZ) during pregnancy. This coincidence suggested that *Slc13a4* might participate in the regulation of neurogenesis. Therefore BrdU injections were performed to track adult neurogenesis in non-pregnant *Slc13a4*$^{+/+}$, *Slc13a4*$^{+/-}$ and *Slc13a4*$^{-/-}$ mice. We found that both *Slc13a4*$^{+/-}$ and *Slc13a4*$^{-/-}$ mice displayed significantly increased cell proliferation in the neural stem cell niches. Intriguingly, no differences were found in BrdU labeled cell numbers within the olfactory bulb or molecular layer of dentate gyrus 28 days after final injection, indicating the extra cells born in the niches of *Slc13a4* mutant mice do not appear to integrate into their appropriate networks. Therefore, our recent work indicates that altered sulfate transport within the choroid plexus has important implications for both behavior and the regulation of adult neurogenesis.

Disclosures: **Z. Zhang:** None. **M. Piper:** None. **P. Dawson:** None. **D. Simmons:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 294.13/B13

Topic: A.02. Postnatal Neurogenesis

Support: NIAAA F31 AA023459

NIAAA R01 AA016959

Title: Neurons born during reactive neurogenesis activate to Morris water maze similarly between binge alcohol exposed and control rats

Authors: *C. R. GEIL, K. NIXON;
Univ. of Kentucky, Lexington, KY

Abstract: Excessive alcohol intake in alcohol use disorders results in neurodegeneration in corticolimbic regions including the hippocampus. In the hippocampus, deficits can recover with

abstinence, potentially due, in part, to reactive adult neurogenesis. Alcohol intoxication inhibits neural progenitor cell (NPC) proliferation and adult neurogenesis, effects that appear to contribute to neuronal loss. However, after 7 days of abstinence (T7) following a 4 day alcohol binge, striking increases in NPC proliferation result in increases in adult neurogenesis. Here, we investigate whether these new neurons are functionally activated (c-Fos) following a hippocampal-dependent task. Thus, we used a 4 day binge to model an AUD, bromodeoxyuridine (BrdU) to label proliferating cells on T7, and the Morris water maze (MWM) to activate hippocampal cells. Briefly, 21 adult male Sprague-Dawley rats were fed an ethanol (25% w/v) or isocaloric control diet via gavage every 8h for 4d with the ethanol dose titrated based on the rat's intoxication score (ethanol dose: 9.7 ± 0.2 g/kg/d, blood ethanol concentration: 401 ± 14 mg/dl). On T7, rats were injected with BrdU (100 mg/kg, i.p.) every 8h for 24h (3 injections). After 6 weeks (7 weeks post-binge) animals underwent 4d of MWM and 90 min following the last trial (i.e. 52d post-binge; T52), animals were transcardially perfused, brains were removed, post-fixed, and sectioned at 40 μm. Every 12th section was processed for fluorescent BrdU/c-Fos/NeuN immunohistochemistry to label cells that divided on T7, were recently activated, and mature neurons, respectively. In the dentate gyrus at T52, ethanol rats had a 2.9-fold increase in the number of BrdU+/NeuN+ cells compared to controls ($p=0.006$). Thus, more cells were labeled with BrdU on T7 and survived to T52. Next, the number of c-Fos+/NeuN+ cells were similar between ethanol and controls, as was the percentage of BrdU+ cells that were triple labeled for BrdU/c-Fos/NeuN. Therefore, a similar percentage of newborn neurons were activated following ethanol as in controls. Surprisingly, ethanol rats appeared to learn faster than controls in the MWM, the task used to induce c-Fos expression. Two-way repeated measures ANOVA revealed significant Diet x Day interactions for time to locate the platform, distance traveled to locate the platform, and swim speed ($p<0.05$). Post-hoc tests revealed that ethanol animals located the platform more quickly than controls on the first 2 days of testing ($p<0.05$). Therefore, increases in neurogenesis may contribute to improved performance in the MWM, but newborn cells were functionally activated at the same rate in ethanol-exposed and control rats.

Disclosures: **C.R. Geil:** None. **K. Nixon:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NASA grant NNX13AB66G (PKS)

National Space Biomedical Research Institute fellowship NCC 9-58 (LRV)

Title: Effect of thyroxine on neurogenesis following galactic cosmic radiation

Authors: *L. R. VOSE, O. MIRY, K. R. GOPAUL, G. SUBAH, P. K. STANTON;
Cell Biol. and Anat., New York Med. Col., Valhalla, NY

Abstract: Simulated galactic cosmic radiation (GCR) impacts learning, memory, synaptic plasticity, neuronal physiology, and adult neurogenesis. Neurons, especially neural stem cells, are highly susceptible to damage, which may cause both subtle alterations in the function of mature neurons, and impair the ability of the brain to replace these cells. Thyroid hormone (TH) is required for normal neurogenesis, and hypothyroid humans and rodents exhibit decreased cognitive function and depression-like behavior which is rescued by TH supplementation. Additionally, high dose radiation to treat cancer causes hypothyroidism with negative cognitive effects. Hippocampal neurogenesis can be upregulated after neural insults such as cerebral ischemia. It is not known if there are compensatory mechanisms to upregulate neurogenesis after GCR exposure, or if these mechanisms can be tapped therapeutically to protect neurogenesis and cognition. Multiple studies have shown that rodent cognitive abilities are impaired 3 mo after GCR. Reduced neurogenesis is seen 6-9 mo post-GCR, and surviving hippocampal neurons show altered neurotransmitter release and receptor subunit composition 3-6 mo post-GCR. Previous data from our laboratory has found that, while impaired at earlier time points post-GCR, synaptic plasticity and spatial learning are enhanced 20 mo post-GCR. These data suggest both that GCR effects persist for the remainder of an animals' life span, and that the mammalian brain may have compensatory capabilities with the capacity to respond to GCR and GCR-induced impairments in neurogenesis and cognition by upregulating these processes. We hypothesize that TH supplementation could be a novel and effective therapeutic strategy to protect neurogenesis from GCR-induced impairment with an FDA approved compound (thyroxine). We treated C57BL/6 mice with TH for 1 week before and after GCR (^{28}Si, 0.5 or 1 Gy). Three months post-GCR, newborn hippocampal neurons were labeled with doublecortin (DCX). Preliminary data did not show changes in DCX+ cell number in irradiated mice, but did show marked differences in dendrite arborization. TH treatment appeared to have more effect on dendritic arborization than the number of DCX+ cells. Understanding the interactions between GCR and neurogenesis, their effects on cognition, mood, and executive function, and identifying neuroprotective strategies, will all be critical for rational assessment of long-term CNS risk and development of countermeasures for in-mission risks from GCR in long-duration space travel.

Disclosures: L.R. Vose: None. **O. Miry:** None. **K.R. Gopaul:** None. **G. Subah:** None. **P.K. Stanton:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Program#/Poster#: 294.15/B15

Topic: A.02. Postnatal Neurogenesis

Support: TIFR Intramural Grant

Title: DREADD-mediated activation of adult hippocampal progenitors: effects on neurogenesis and behavior

Authors: *M. MAHESHWARI[1], S. SHAH[1], S. PATI[1], A. RAWAT[1], J. CHELLIAH[2], V. A. VAIDYA[1];
[1]Tata Inst. of Fundamental Res., Mumbai, India; [2]Jawaharlal Nehru centre for Advanced Scientific Res., Bangalore, India

Abstract: Adult-born granule cells represent a unique form of hippocampal plasticity wherein new neurons are generated throughout the adult life of an organism. It is a multi-step process which involves proliferation of progenitors, differentiation, survival and integration into mature neurons of the hippocampal niche. A period of few weeks after birth of progenitors is marked by lowered threshold to stimulus and heightened excitability. About half of the dividing cells generated in the niche are targeted for cell death, and a lack of environmental experience or neuronal activity during this critical period is thought to play a key role in determining cell survival. While several studies provide a correlative link between the nature of neuronal activity within the neurogenic niche and progenitor turnover and survival, thus far this has not been directly examined. Further, the impact of changes in neuronal activity or calcium signaling within the neurogenic niche do not delineate effects that are cell-autonomous or niche-mediated. To directly address whether alteration in Gq-mediated signaling and downstream calcium-driven responses can modulate progenitor turnover, survival, and mood-related behavioural function we used pharmacogenetic strategies to regulate Gq-signaling in hippocampal progenitors. Using bigenic mouse models expressing Gq-DREADD (Designer Receptors Exclusively Activated by Designer Drugs) under the control of the Nestin-drivers we examined the consequences of DREADD-mediated activation on different aspects of neurogenesis and behavior. Following chronic activation, mice exhibited a significant increase in - number of proliferative cells, total number of immature neurons and complexity of dendritic morphology. In addition, we observed a time-dependent emergence of anxiolytic behaviour at 4-8 weeks following activation of hippocampal progenitors. These effects were age-dependent, observed in both juvenile and adult animals, but not in aged mice. Experiments are currently underway to characterize the specific stages of hippocampal neurogenesis that are sensitive to DREADD-mediated activation and to examine the consequences on hippocampal function using electrophysiological studies. Taken

together, these results provide evidence that Gq-signaling driven activation can drive robust proliferative effects in hippocampal progenitors and eventual behavioural modulation in an age-dependent manner. Also, it supports the notion that newborn neurons may exert specific effects on hippocampal networks and behaviour at a critical junctures during their maturation and integration into hippocampal neurocircuitry.

Disclosures: **M. Maheshwari:** None. **S. Shah:** None. **S. Pati:** None. **A. Rawat:** None. **J. Chelliah:** None. **V.A. Vaidya:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 294.16/B16

Topic: A.02. Postnatal Neurogenesis

Title: Oxytocin stimulates hippocampal neurogenesis via oxytocin receptor expressed in CA3 pyramidal neurons in adult mice

Authors: *Y. LIN[1], C. CHEN[2], K. HSU[2];
[1]Inst. of Basic Med., Tainan, Taiwan; [2]Dept. of pharmacology, Tainan city, Taiwan

Abstract: Acute and repeated peripheral oxytocin (OXT) administration have been shown to increase hippocampal neurogenesis; however, the mechanisms underlying the action of OXT is still unclear. Here we show that OXT control adult neurogenesis through non-cell-autonomous mechanisms by OXT receptors (OXTR) expressed in CA3 pyramidal neurons. Using oxytocin receptor-Venus reporter mice, we found that OXTR is not expressed in the neural progenitor cells in the subgranular zone of the adult dentate gyrus, but is enriched in subpopulations of hippocampal CA2 and CA3 pyramidal neurons. Consistent with a function of OXT signaling in adult neurogenesis, conditional deletion of OXTR ($OXTR^{-/-}$) from excitatory neurons led to impaired differentiation, survival and maturation of newly generated dentate granule cells (DGCs). Retrograde neuronal tracing combined with immunocytochemistry revealed that the OXT neurons in the paraventricular nucleus project directly to the CA3 region of the hippocampus. In addition, bath application of OXT elicited a membrane depolarization and increased action potential firing in OXTR-expressing CA3 pyramidal neurons in slices from wild-type mice, which were completely abolished by OXTR deletion. Using adenoviral-mediated expression of engineered $G_{i/o}$-coupled human M4 (hM4Di) receptors, we observed that activation of hM4Di receptors with clozapine-*N*-oxide (CNO) reduced CA3 pyramidal neuronal activity and resulted in fewer newly DGCs in Floxed OXTR mice. Conversely, CNO-induced activation of G_q-coupled human M3 (hM3Dq) receptors restored adult neurogenesis in the DG of $OXTR^{-/-}$

mice. These results suggest that CA3 may regulate adult DG neurogenesis under basal conditions and OXT controls adult hippocampal neurogenesis via OXTR expressed in CA3 pyramidal neurons.

Disclosures: **Y. Lin:** None. **C. Chen:** None. **K. Hsu:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NSF-IOS 1121345

NSF-IOS 1456918

Staley Fellowship, Wellesley College

Title: Adult neurogenesis in a crustacean brain: Serotonin levels influence the integration of adoptively transferred immune cells into a neurogenic niche

Authors: J. L. BENTON, *B. S. BELTZ;
Neurosci. Program, Wellesley Col., Wellesley, MA

Abstract: Neurogenesis continues in the brains of adult decapod crustaceans and is regulated by serotonin levels. However, the 1st-generation neural precursors (neural stem cells), which reside in a neurogenic niche, are not self-renewing in crayfish (*Procambarus clarkii*) and must be replenished. Our previous studies indicate that the source of these neural precursors is the innate immune system. Semi-granular hemocytes, which are central players in innate defense mechanisms in crustaceans, have the capacity to become neural precursors that generate adult-born neurons. Following adoptive transfer of ethynyl-2'-deoxyuridine (EdU)-labeled hemocytes, these cells populate the neurogenic niche. After a 7-week survival time, EdU-labeled cells are located in brain clusters 9 and 10 where adult-born neurons differentiate, and express appropriate neurotransmitters. Recent experiments have shown that serotonin levels alter the outcomes of adoptive transfers. When EdU-labeled blood cells are transferred to recipient crayfish that are then exposed to serotonin (10^{-9} M), the proportion of EdU-labeled niche cells from the semi-granular cell lineage increases compared with control levels. In addition, the total numbers of cells that compose the niche rise. It also is known that serotonin stimulates the expression of astakine, a crustacean cytokine, which promotes the release of semi-granular cells. Further studies will therefore ask whether serotonin can influence the number of circulating semi-

granular hemocytes by regulating astakine levels, and also whether serotonin's effects on adoptive transfers and neurogenesis are mediated by this cytokine.

Disclosures: J.L. Benton: None. **B.S. Beltz:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant MH091844

NIH Grant MH105675

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Title: Transient inhibition of neural stem cell proliferation during early life decreases adult dentate gyrus neurogenesis

Authors: *M. YOUSSEF, G. KIRSHENBAUM, V. KRISH, T. BRINER, E. D. LEONARDO, A. DRANOVSKY;
Columbia Univ. Med. Ctr., New York, NY

Abstract: Early life stress has been shown to increase vulnerability to psychiatric illness in adulthood. The hippocampal dentate gyrus (DG) is altered by stress and plays a role in stress regulation. The DG is also one of the two brain regions in which neural stem cells give rise to neurons throughout an animal's life, a process that is negatively regulated by stress. In this study, we sought to determine whether inhibition of neurogenesis during critical developmental periods is sufficient to permanently decrease DG neurogenesis. We used a pharmacogenetic approach to transiently target dividing neural stem cells for elimination by administering the drug valganciclovir to GFAP-Tk mice during periods sensitive to stress. We then assessed the Nestin neural stem cell lineage in adulthood. We found that suppression of cell proliferation during the early postnatal, but not the periadolescent period, led to fewer neurons from the Nestin lineage in adulthood, suggesting that the early postnatal intervention leads to a permanent reduction in neurogenesis. This study highlights the early postnatal period as a sensitive period during which perturbations in stem cell proliferation are sufficient to dictate the homeostatic set point for adult neurogenesis. It is intriguing to speculate that infantile neurogenesis serves as a cellular target for the enduring effects of stress.

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Poster

294. Environmental Influences on Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: South African National Research Foundation

Title: Effect of voluntary exercise on adult hippocampal neurogenesis in maternally separated rats

Authors: ***V. A. RUSSELL**[1], N. HARDCASTLE[2], L. MARAIS[2], D. LANG[2];
[2]Human Biol., [1]Univ. Cape Town, Cape Town, South Africa

Abstract: Maternal separation (MS) produces depression-like behaviour in animals. The underlying mechanisms are unknown. Evidence suggests that neurogenesis may be a key factor. Voluntary wheel running is a form of exercise that increases neurogenesis and decreases depression-like behaviour in a rat model. However, the role of neurogenesis in exercise-induced antidepressant effects is unknown. Ki-67, an endogenous marker of cell proliferation, was used to study the effect of exercise on neurogenesis in the MS rat model of depression. Briefly, on postnatal day (PND) 2, litters were culled to eight pups (with a minimum of two females and two males per litter to normalize dam behaviour towards pups) and randomly assigned to MS (n = 8) or non-MS (n = 10) groups. MS pups were separated from their dams for 3 h per day from PND2 to PND14. Non-MS pups were left with their dams. On PND21, pups were weaned and housed in groups of 4-5 rats per cage. From PND 54 - 74, rats were housed in single cages with attached running wheels. Exercised rats were housed in cages with free-running wheels and could exercise at will, while non-exercised rats were housed in cages with locked wheels. On PND 74, rats were perfused with 4% paraformaldehyde. The brain was removed from the skull, placed in 4 % paraformaldehyde for three hours and then dehydrated in 30 % sucrose solution for 3 - 5 days until the brain sank. Thereafter brains were sectioned with no more than two days between sinking, freezing and sectioning. Coronal sections containing the dorsal hippocampus (40 μm sections from approximately 6.96 to 5.52 mm anterior to the inter-aural line) and the ventral hippocampus (50 μm sections from 3.84 to 2.76 mm anterior to the inter-aural line) were collected. Every 5th section was submerged in 3 % Triton X-100 in phosphate-buffered saline for 1 hour prior to immunohistochemistry. Ki-67 and DCX were used to determine the number of mitotically active cells that were destined to become neurons. Voluntary exercise produced the

expected increase in neurogenesis in non-MS rats, evidenced by increased Ki-67/DCX cell counts relative to non-MS rats housed in cages with locked wheels. Consistent with depression being associated with impaired neurogenesis, MS rats that were allowed free access to running wheels had fewer Ki-67/DCX-labelled cells in the dentate gyrus of both dorsal and ventral hippocampus when compared to non-MS rats that were allowed to exercise voluntarily. This finding suggests that MS alters neurogenesis in adult life and attenuates the effect of exercise on neurogenesis in the hippocampus.

Disclosures: **V.A. Russell:** None. **N. Hardcastle:** None. **L. Marais:** None. **D. Lang:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.20/B20

Topic: A.01. Neurogenesis and Gliogenesis

Title: Retinoic acid regulates neural stem & progenitor cell proliferation in the adult hippocampus

Authors: ***S. MISHRA**, J. SIEGENTHALER;
Univ. of Colorado ,Anschutz Med. Campus, Aurora, CO

Abstract: In the adult hippocampus, stem and progenitor cells (NSPCs) proliferate and differentiate to make new neurons which then integrate into functional neural circuits. Inhibition of NSPCs proliferation is associated with several neurodegenerative disorders. However, the mechanisms regulating NSPC proliferation are unclear.We are interested in understanding the role of meningeal derived signal Retinoic acid (RA) in regulating NSPC proliferation. To address this, we inhibited endogenous RA synthesis in adult mice using the drug disulfiram and examined NSPC proliferation in the hippocampus. After 3 days of treatment, disulfiram treated mice showed a decrease in total numbers of NSPCs. This decrease was not due to increased apoptosis as assessed by TUNEL assay. Instead we found decreased NSPC proliferation, as demonstrated by decreased number of total Edu+ cells in the dentate gyrus of disulfiram treated mice. To test if altered NSPC proliferation is due to direct effect of RA on NSPCs, adult hippocampal NSPCs were cultured in presence of RA and RA with pan-Retinoic acid Receptor (RAR) antagonist, which inhibits RA signaling. RA increased NSPC proliferation, an effect that was blocked with the pan-RAR antagonist. To test a possible mechanism of regulation of proliferation by RA, we determined if RA regulates vascular endothelial growth factor (VEGF) signaling in the adult hippocampus. VEGFs and their tyrosine kinase receptors (VEGFRs) are expressed by NSPCs in the adult hippocampus and are established, positive regulators of NSPC

proliferation. We find that hippocampal cell lysates from disulfiram treated mice showed substantially reduced VEGFA ligand protein levels as compared to control. To examine if upregulation of VEGF-A is a direct effect of RA on NSPCs, mouse adult hippocampal NSPCs were cultured in presence of RA and RA with pan-Retinoic acid Receptor (RAR) antagonist. RA increased VEGF-A protein levels and this expression was reduced when RA signaling was inhibited. We next investigated if the effect of RA on progenitor proliferation is mediated via increased VEGF-A synthesis by co-treating cells with RA and the VEGFR2 antagonist. When VEGF signaling was inhibited in presence of RA the proliferation index was equivalent to cells treated with VEGFR2 antagonist alone. This data suggests RA regulates progenitor proliferation through VEGFA/VEGFR2 signaling. Taken together, these results support the hypothesis that RA, a meningeal derived signal, activates proliferation via VEGFA/VEGFR2 signaling in NSPCs of the adult hippocampus.

Disclosures: S. Mishra: None. **J. Siegenthaler:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Program#/Poster#: 294.21/B21

Topic: A.02. Postnatal Neurogenesis

Support: Kuwait University Grant No. YM01/15

Title: Prenatal diabetes affects the postnatal hippocampal neurogenesis, learning and memory: Role of neurotrophic factors

Authors: *N. A. AL-BAHOUH, M. S. RAO;
Dept. of Anat., Kuwait Univ., Jabriya, Kuwait

Abstract: Diabetes mellitus is a chronic metabolic endocrine disorder and it is characterized by abnormally high glucose levels in blood. Diabetes mellitus is associated with central nervous system alterations. Gestational diabetes mellitus is the impairment of glucose tolerance that can affect pregnant women who have high blood glucose levels during pregnancy. Previous studies have shown that gestational diabetes produces a number of complications involving CNS including a significant decrease in hippocampal neurogenesis, significant reduction in the pyramidal cell density in the CA1 and CA3 sub regions of the hippocampus in the rats born to the diabetic mother rats (Gestational diabetic rats). Objective of the experiment is to study the effects of prenatal streptozotocin induced diabetes on young postnatal rat learning and memory, hippocampal cell proliferation, dentate gyrus neurogenesis, levels of brain derived neurotropic

factor (BDNF) and vascular endothelial growth factor (VEGF) in the hippocampus. Pregnant rats were injected with streptozotocin (STZ, 60mg/kg) on gestational day10. Diabetes was confirmed in them by measuring the blood glucose level 48 hrs after STZ injection. Pups born to the STZ-injected mother were divided into two age groups [40 days (STZ-40) and 60 days (STZ-60)] Pus born to the normal control mother were also divided into two age groups [40days (NC-40) and 60days (NC-60)]. Learning and memory was tested in these rats on 31^{st}-37^{th} (NC-40, STZ-40) and 51st -57^{th} (NC-60, STZ-60) postnatal day. Rats in all groups were sacrificed after learning and memory test, brain was dissected and processed for immunostaining, western blot and ELIZA analysis. Results showed that both STZ-40 and STZ-60 had significantly poor memory retention, decreased cell proliferation, and decreased neurogenesis, less number of doublecortin positive neurons in the crest, supra-pyramidal blade and infra-pyramidal blade regions of dentate gyrus compared to NC-40 and NC-60 groups. Further BDNF and VEGF were found to be significantly decreased in hippocampal tissue in STZ-40 and STZ-60 groups compared to control groups. We conclude from these results that gestational diabetes leads to an adverse effects on off-springs. Gestational diabetes can lead to necrotic changes, including a significant decrease in stem cell proliferation and neurogenesis in the dentate gyrus of the hippocampus of the offspring. It can cause a significant impairment in memory retention and poor memory recalling.

Disclosures: **N.A. Al-Bahouh:** None. **M.S. Rao:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: Technical assistance by M.Sc. Concepción Valencia

CONACyT Grant 131031

Title: Emergence of doublecortin positive cells from astrocytes in the substantia nigra in response to transplantation of cells derived from pluripotent stem cells

Authors: D. M. ARZATE[1], M. GUERRA-CRESPO[2], *L. COVARRUBIAS[1];
[1]Inst. de Biotecnologia, UNAM, Cuernavaca, Morelos, Mexico; [2]Mol. Neuropathology, Inst. de Fisiología Celular, Ciudad de México, Mexico

Abstract: Neurogenesis in the substantia nigra (SN) has been a controversial issue. Using embryoid body (EB) cells as an indicator of a neurogenic environment in the adult brain, we

found that SN allows, possibly involving an induction mechanism, efficient EB cell differentiation into neurons, suggesting that the SN may contain a silent neurogenic niche. Accordingly, we observed many host doublecourtin positive (DCX+) cells near the transplant. The aim of this study was to characterize these putative neuroblasts. Some host dividing neural precursor cells (NPC) Sox2+ were detected between 1-6 days postransplantation (dpt). In agreement with the activation of NPCs due to transplantation of EB cells, host neurosphere-like aggregates were generated from host cells in the presence of Egf and Fgf2; these aggregates showed neural multipotency as neurons and astrocytes derived from them *in vitro*. The contralateral to the transplanted SN showed very few dividing cells, none Sox2+ or Nestin+ cells, and neurospheres could not be derived from its cells. However, continuous BrdU administration after transplantation showed that only a fraction (~8%) of the host DCX+ progeny at 15 dpt derived from dividing cells and few BrdU+, some NeuN+, cells survived up to 30 dpt. Interestingly, in addition to detecting an increased proportion of host GFAP+ expressing Nestin around the transplant (~50%), we detected 30-50% of DCX+ or PSA-NCAM+ cells expressing glial markers such as GFAP and S100β. In order to confirm that a large proportion of host putative neuroblasts originated from astrocytes, we used a lineage tracing strategy in which we transplanted EB cells to brains of GFAP-CreER;R26-lox-mTomato-pA-lox-mEGFP-pA double transgenic mice that two weeks earlier were treated with tamoxifen for broad CreER activation in astrocytes. Consistent with an astrocytic origin of DCX+ cells, we found many DCX+/EGFP+ cells surrounding the transplant. Preliminary data indicate that Fgf2 and VEGF, two proteins produced by EBs, are able to promote neurosphere formation and the emergence of DCX+ cells in the absence of EB cells. Together our data suggest that the adult SN has the ability to generate new neurons mainly from astrocytes by a dedifferentiation/transdifferentiation mechanism.

Disclosures: D.M. Arzate: None. **M. Guerra-Crespo:** None. **L. Covarrubias:** None.

Poster

294. Environmental Influences on Adult Neurogenesis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 294.23/B23

Topic: A.02. Postnatal Neurogenesis

Support: The RBC Retirement Research Fellowship

The OTRV Seed Grant

Brain & Behavior Research Foundation

School of Public Health & Health Systems, University of Waterloo

Title: Age-related changes on hippocampal neurogenesis and mnemonic discrimination of similar objects and locations

Authors: S. ABDEL MALEK, S. SHARMA, R. DE, Y. E. WEN, A. N. S. CHOWDHURY, *E. SATVAT;
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Abstract: Adult hippocampal neurogenesis appears to be critical for pattern separation. Aging is associated with suppressed adult hippocampal neurogenesis but exercise and environmental enrichment stimulate it. The aim of our study is to investigate whether age-related suppressed adult hippocampal neurogenesis is associated with deficits in tasks designed to assess pattern separation and whether exercise or an enriched environment can reverse such deficits. We started with a water maze training that showed learning deficit in middle aged rats (8 months) in comparison to young rats (2 months). At this age however, there was no difference between the two groups in the probe test and the reversal training. The middle aged rats were then randomized into exercise, environmental enrichment or standard housing conditions. Young rats stayed in standard housing condition. All the rats received 50 mg/kg BrdU injections per day for the first 6 days. After 3 months, rats underwent a second water maze training followed by a reversal training and a significant difference between the old and the young group was found only during reversal training. Three months later, rat's ability to discriminate between very different objects as well as their ability to discriminate between highly similar objects were examined. With 5-hour delay interval, only the young group and the old-enriched group were able to comparably discriminate the two very different objects. With the same delay interval and highly similar objects none of the groups were able to discriminate familiar object from highly similar yet novel object. Next, rats underwent a spontaneous location recognition task. Regardless of the degree of separation, none of the groups discriminated between the novel and the familiar locations at either 1- or 5-hour delay intervals between the sample phase and the choice phase. Finally, when old and young rats were 18 and 12 months of age, respectively, a delayed nonmatching to place radial arm water maze task was performed to evaluate spatial pattern separation. Older rats showed impairment in the task only when the separation between the sample and choice arms were small. Interestingly, both exercise and environmental enrichment protected older rats from this deficit. Brain tissues were collected and are being analyzed for functional neurogenesis.

Disclosures: S. Abdel Malek: None. **S. Sharma:** None. **R. De:** None. **Y.E. Wen:** None. **A.N.S. Chowdhury:** None. **E. Satvat:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.01/B24

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

Support: 1MH107182

Title: Ankyrin-G interacts with X-linked intellectual disability-associated deubiquitinase Usp9X to regulate spine maintenance

Authors: *S. YOON, K. MYCZEK, P. PENZES;
Northwestern Univ., Chicago, IL

Abstract: Recent genome-wide association studies and whole-exome sequencing have shown that ANK3 (encoding Ankyrin-G, AnkG) is a risk gene for multiple neuropsychiatric disorders, such as bipolar disorder, autism spectrum disorder, intellectual disability and schizophrenia. Here, we identified a novel AnkG interacting partner, Usp9X (ubiquitin specific peptidase 9, X-linked), using the yeast two-hybrid system. Usp9X mutations are associated with X-linked intellectual disability (XLID) in humans, and Usp9X protein is known to regulate neurite outgrowth and synaptic growth during the early stages of development. We observed that the expression of AnkG and Usp9X highly overlaps in the cerebral cortex and hippocampus. At the cellular level, both proteins co-expresses mostly on the soma and dendritic area, and interact in co-immunoprecipitates from brain homogenates. Co-immunoprecipitation with deletion mutants of AnkG and Usp9X demonstrates that Usp9X binds to the 24 ankyrin repeats and regulatory domains of AnkG. Knockdown of Usp9X in cultured cortical neurons decreases the intensity of AnkG in the soma and dendrites and impairs spine maintenance. These data implicate Usp9X in spine morphogenesis through regulating AnkG protein levels and suggest that deubiquitination is a potential mechanism of synapse maintenance, which, when abnormal, could contribute to pathogenesis.

Disclosures: **S. Yoon:** None. **K. Myczek:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.02/B25

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

Support: NIH grant MH107182

NIH grant MH071316

Title: CNTNAP2 paracrine signaling by ectodomain shedding

Authors: *M. D. MARTIN-DE-SAAVEDRA[1], O. VAREA[1], R. GAO[1], B. P. SPIELMAN[1], K. J. KOPEIKINA[1], K. MYCZEK[1], E. A. HALL[2], J. N. SAVAS[2], P. PENZES[1];
[1]Physiol., [2]Neurol., Northwestern Univ., Chicago, IL

Abstract: The *CNTNAP2* gene is a risk factor for a complex range of phenotypes whose shared common features are intellectual disability, seizures, language impairments and autism spectrum disorder. *CNTNAP2* encodes the CNTNAP2 or Caspr2 transmembrane protein which belongs to the neurexin superfamily. CNTNAP2 KO mice showed repetitive/restrictive behaviors, impaired communication and decreased social interaction. However, little is known about how CNTNAP2 contributes to the physiopathology of neurodevelopmental disorders. We and others have recently shown that CNTNAP2 is present at spiny synapses in excitatory cortical neurons, and is required for correct trafficking of GluA1 subunits of AMPA receptors and spine maintenance. We therefore set out to investigate its functions at synapses using a wide range of imaging techniques (including confocal and super resolution imaging), biochemistry and mass spectroscopy. We found that CNTNAP2 undergoes activity-dependent ectodomain shedding mediated by matrix metalloproteases. Using structured illumination microscopy we determined the subcellular sites of Cntnap2 ectodomain shedding. Incubation with Cntnap2 ectodomain promotes morphological and functional changes in cortical neurons. Proteomics analysis has identified novel candidate binding partners of CNTNAP2 ectodomain. Taken together, our findings can provide new insight into the role of CNTNAP2 at synapses and in the pathogenesis of neurodevelopmental disorders.

Disclosures: **M.D. Martin-de-Saavedra:** None. **O. Varea:** None. **R. Gao:** None. **B.P. Spielman:** None. **K.J. Kopeikina:** None. **K. Myczek:** None. **E.A. Hall:** None. **J.N. Savas:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.03/B26

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

Support: 5F30MH096457-03

5R01MH09721604

Title: Regulation of cortical gabaergic interneuron function by the mental disorder susceptibility molecule cntnap2

Authors: *R. GAO, A. MELENDEZ, S. YOON, M. D. SAAVEDRA, M. FORREST, P. PENZES;
Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL

Abstract: While complex neuropsychiatric disorders have equally complicated genetic etiologies, recent research revealed common disruptions of individual genes across multiple diseases, suggesting similar pathological mechanisms. For example, several genetic studies have established contactin-associated protein-like 2 (CNTNAP2) as a risk gene in mental disorders such as autism spectrum disorders, schizophrenia, epilepsy, and intellectual disability. Studying the function of CNTNAP2, therefore, can lead to unprecedented insight into shared susceptibility pathways of mental diseases. CNTNAP2 is a member of the neurexin superfamily, is highly expressed in the brain, and clusters potassium channels in the axon's juxtaparanodes. However, its function outside of the axon is less explored. Recent studies of *CNTNAP2* knock-out mice revealed not only behavioral abnormalities and epileptic seizures, but also reductions of cortical interneurons and abnormal neuronal synchrony (Peñagarikano et al., 2011). In addition, CNTNAP2 knockdown in primary neuronal culture resulted in the reduction of both excitatory and inhibitory transmission (Anderson et al., 2012).Taken together, these data implicate CNTNAP2 in controlling E/I balance and behavior, possibly through cortical interneuron function. Indeed, epilepsy - an E/I imbalance disorder - is a common core phenotype of many human subjects with *CNTNAP2* disruption (Rodenas-Cuadrado et al., 2014). However, mechanistic studies verifying this hypothesis have not yet been established. To this end, we used a wide array of techniques including yeast-2-hybrid screening, confocal and high resolution imaging, biochemistry, primary neuronal culture, and *in vivo* models, to dissect CNTNAP2's role in cortical interneuron function and to uncover novel interaction candidates involved with this process.

Disclosures: R. Gao: None. **A. Melendez:** None. **S. Yoon:** None. **M.D. Saavedra:** None. **M. Forrest:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.04/C1

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

Support: 1MH107182

Title: A synaptic role of FKBP5, a genetic risk factor for stress-related psychiatric disorders.

Authors: *K. MYCZEK[1], I. OZSAN[1], H. YAMAZAKI[2], M. MARTIN-DE-SAAVEDRA[1], P. PENZES[1];
[1]Northwestern Univ., Chicago, IL; [2]Gunma Univ., Maebashi, Japan

Abstract: The development of psychiatric disorders, such as depression, post-traumatic stress-disorder, and bipolar disorder has been shown to be associated with alterations in neuronal structure and function, particularly in the cerebral cortex. While recent large scale clinical genomics studies have identified genetic risk factors for psychiatric disorders, the functional analysis of these risk genes and their encoded proteins is the next major challenge. Among these, FKBP5, encoding FK506 binding protein, is a prominent genetic risk factor for stress-related mood and anxiety disorders, including depression and post-traumatic stress-disorder. However, the mechanisms by which FKBP5 contributes to disease pathogenesis are not well understood. While a role for FKBP5 as a glucocorticoid receptor co-chaperone has been studied, additional mechanisms in the brain remain under investigated. We hypothesized that FKBP5 may also function at synapses, sites relevant for the pathogenesis of psychiatric disorders. By utilizing an *in vitro* model of stress and primary rodent cortical cultures we have found independent effects of stress and FKBP5 on neuronal morphology. Furthermore, our super-resolution microscopy and live cell imaging techniques have revealed a unique synaptic role of FKBP5. Further studies investigating the molecular pathways involved in FKBP5-mediated synaptic processes can provide insight into disease pathogenesis and may lead to novel therapeutic strategies.

Disclosures: K. Myczek: None. **I. Ozsan:** None. **H. Yamazaki:** None. **M. Martin-de-Saavedra:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

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Program#/Poster#: 295.05/C2

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

Support: NIH Grant 1MH107182

NIH Grant MH071316

NIH Grant 5R01MH097216-04

Title: Kalirin proteins display differential localization in dendritic spines and regulate spine morphology and trafficking of NR2B-containing glutamate receptors

Authors: T. A. RUSSELL, K. R. SMITH, K. J. KOPEIKINA, *P. PENZES;
Dept Physio, Nothwestern Univ. Feinberg Sch. Med., Chicago, IL

Abstract: Kalirin proteins are guanine nucleotide exchange factors that are essential for dendritic spine maintenance and function in forebrain pyramidal neurons, and have been shown to be linked to psychiatric disorders including schizophrenia. The three most abundant kalirin isoforms, kalirin-7, -9, and -12, have various combinations of protein-protein interaction and enzymatic domains, which have been shown to afford them distinct localizations and functions in developing cultured cortical neurons. However, he subcellular localizations of individual kalirin isoforms in mature neurons have not been ascertained. Here we used structured illumination microscopy to examine the nanoscale level distribution of kalirin proteins in dendritic spine compartments, and correlated the presence and levels of immunoreactive nanodomains with spine morphology. The majority of spines analyzed contained kalirin-7 and/or kalirin-12, but not kalirin-9, and the presence and levels of all three isoforms were associated with larger spines. We then chose to characterize the relative distributions of individual kalirin isoforms and the NMDA receptor subunit NR2B, since kalirin-7 and kalirin-12 have been shown to interact with NR2B, *KALRN* knockout mice have dysregulated NR2B expression during development, and like *Kalrn*, the gene encoding this subunit has been linked to psychiatric disorders. Interestingly, the presence of kalirin-12 in the spine neck appeared to preclude the presence of NR2B in spines, and was associated with smaller spines than those without kalirin-12 in the neck. Overexpression and shRNA- mediated knockdown of kalirin-12 confirmed the role of this isoform in regulating trafficking of NR2B- containing NMDA receptors to spine heads. Taken together, these data establish a heretofore unknown mechanism by which kalirin proteins regulate spine structural plasticity and glutamate receptor signaling.

Disclosures: **T.A. Russell:** None. **K.R. Smith:** None. **K.J. Kopeikina:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.06/C3

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

Support: NiH Grant 1MH107182

Title: Role of 190 kDa Ankyrin-G palmitoylation in spine and dendrite maintenance

Authors: *N. H. PIGUEL[1], K. R. SMITH[2], P. PENZES[1];
[1]Northwestern Univ., Chicago, IL; [2]Univ. of Bristol, Bristol, United Kingdom

Abstract: Common and rare variants in the ANK3 gene, encoding the ankyrin-G protein, have been associated with bipolar disorder, schizophrenia and autism. Ankyrin-G has been studied extensively in the axon initial segment, where 480 kDa and 270 kDa isoforms stabilized and compartmentalized several proteins such as sodium channels. Furthermore, those ankyrin-G isoforms can bind EB1 and EB3 proteins and seem to have a unique role in the axon involving EB3 and tubulin organization. Recent evidence implicates glutamatergic synapses as key pathogenic sites in psychiatric disorders. Our previous work showed 190 kDa ankyrin-G can form nanodomains in dendritic spines and specifically regulated AMPA stability and activity-dependent spine enlargement. Here, we provide evidence than ankyrin190 kDa is present in a complex with the NMDA receptors, another major actor in dendritic spines implicated in psychiatric disorders, and can stabilize this receptor through a palmitoylation site. We also illustrate that ankyrin-G can affect tubulin dynamics in dendrites and that these functions are critical for dendrite and spine maintenance.

Disclosures: **N.H. Piguel:** None. **K.R. Smith:** None. **P. Penzes:** None.

Poster

295. Synapse Maturation and Remodeling

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

Support: Research Grant Council of Hong Kong; General Research Fund (GRF) 16100814

Research Grant Council of Hong Kong; Early Career Scheme (ECS) 27119715

Title: The epilepsy and intellectual disability-related gene tbc1d24 encodes a novel synaptic protein that regulates dendritic spine morphogenesis in neuron

Authors: L. LIN[1], Q. LYU[1], E. FEI[2], N. Y. IP[2], *K.-O. LAI[1];
[1]Sch. of Biomed. Sci., The Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: The majority of excitatory synapses are located on dendritic spines of the postsynaptic neuron. Spine formation and turnover is considered as an important mechanism underlying brain development as well as learning and memory. Several missense mutations of the human *tbc1d24* gene have been associated with epilepsy and intellectual disability. However, the physiological role of the TBC1D24 protein remains largely unexplored. Here we report an essential role of TBC1D24 in regulating the density and morphology of dendritic spines in hippocampal neurons. We found that TBC1D24 protein was enriched in the synaptic plasma membrane fraction of adult mouse brains. Immunocytochemistry further revealed that TBC1D24 was present in close proximity to dendritic spines and the postsynaptic scaffold protein PSD-95. Notably, the expression of TBC1D24 in hippocampal neurons was bi-directionally regulated in response to elevation and blockade of neuronal activity. Using short-hairpin RNA (shRNA) to knock down its expression in mature hippocampal neurons, we demonstrated that the maintenance of dendritic spines critically depends on TBC1D24. Moreover, the small GTPase ARF6 was identified as the downstream mediator of TBC1D24 in the regulation of spine morphogenesis. These findings suggest that TBC1D24 is involved in activity-dependent spine morphogenesis in the postsynaptic neuron, and defects in spine development might contribute to the pathophysiology of intellectual disability in individuals harboring the loss-of-function gene mutations.

Disclosures: **L. Lin:** None. **Q. Lyu:** None. **E. Fei:** None. **N.Y. Ip:** None. **K. Lai:** None.

Poster

295. Synapse Maturation and Remodeling

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

Support: Research Grant Council of Hong Kong [General Research Fund (GRF) 16100814]

Research Grant Council of Hong Kong [Early Career Scheme (ECS) 27119715]

Title: A potential role of NMDA receptor-dependent expression of Striatin-4 in dendritic spine maturation

Authors: *L.-Y. LO, L. LIN, Q. LYU, K.-O. LAI;
Sch. of Biomed. Sci., The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Most excitatory synapses are located in dendritic spines of the postsynaptic neuron. Spines exist with various shapes: the immatures spines, such as the short and stubby spines or the long and thin filopodia, do not possess a distinct spine head. In contrast, the mature spines appear as mushroom-shaped with large heads, or thin spines with elongated necks and small heads. Spine maturation requires local dendritic synthesis of new proteins in response to ongoing synaptic activity. Dysregulated dendritic mRNA trafficking and local protein synthesis can lead to altered spine morphology in neurodevelopmental disorders such as Fragile-X syndrome and autism. Nonetheless, the molecular mechanism underlying activity-dependent spine maturation is not fully understood. Striatin-4 (also called Zinedin) was identified in recent transcriptomic studies as one of the mRNA transcripts present in hippocampal neuropil and a putative cargo of the RNA-binding protein FMRP. Stiratin-4 belongs to the striatin family which serves as scaffold protein for signal transduction. Interestingly, some of the striatin-interacting proteins, such as mammalian STE20-like protein kinase 3 (MST3) and cortactin-binding protein 2 (CTTNBP2), are encoded by autism risk genes. Despite previous studies demonstrating Striatin-4 enrichment in dendritic spines, the function of Striatin-4 in neuron remains unknown. Here we found that Striatin-4 mRNA and protein expression in cortical and hippocampal neurons was regulated by neuronal activity and NMDA receptors. Notably, Striatin-4 was preferentially expressed in mature dendritic spines, and its down-regulation by NMDA receptor antagonist APV was accompanied with a switch of mature spines to stubby spines and filopodia. Knockdown of striatin-4 in hippocampal neurons by shRNA led to the loss of mature spines and increased proportions of stubby spines and filopodia, therefore mimicked the spine phenotypes after NMDA receptor blockade. Taken together, these findings suggest that NMDA receptor-dependent synthesis of striatin-4 is crucial for the maturation of dendritic spines in hippocampal neurons.

Disclosures: **L. Lo:** None. **L. Lin:** None. **Q. Lyu:** None. **K. Lai:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.09/C6

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

Title: Role of IQSEC3 in inhibitory synapse formation

Authors: H. KANG[1,2], D. PARK[1,2], S. JEON[3], J. KO[3], *J. UM[1,2];
[2]BK21 PLUS Project for Med. Sci., [1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of;
[3]Dept. of Biochem., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Gephyrin is a central scaffold protein that mediates development, function and plasticity of mammalian inhibitory synapses by interacting with various inhibitory synaptic proteins. Here, we show that IQSEC3, a guanine nucleotide exchange factor (GEF) for ARF6, directly interacts with gephyrin, an interaction that is critical for the inhibitory synapse localization of IQSEC3. Overexpression of IQSEC3 increases inhibitory, but not excitatory, synapse density in a GEF activity-dependent manner. Conversely, knockdown (KD) of IQSEC3 decreases size of gephyrin cluster without altering gephyin puncta density. Collectively, these data reveal that IQSEC3 acts together with gephyrin to regulate inhibitory synapse development.

Disclosures: H. Kang: None. **D. Park:** None. **S. Jeon:** None. **J. Ko:** None. **J. Um:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

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Program#/Poster#: 295.10/C7

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

Support: MRC Grant MR/M501670/1

MRC Grant MR/K004603/1

MRC Grant MR/N003896/1

MRC Grant G0901Z99

Title: Spines require normal DISC1 function during development of their parent dendritic branch

Authors: *A. M. DE HAAN, N. R. HARDINGHAM, K. FOX;
Cardiff Univ., Cardiff, United Kingdom

Abstract: Disrupted-In-Schizophrenia 1 (DISC1) is known to play an important role in brain development and is implicated in mental disorders such as schizophrenia and autism. We investigated effects of disrupting DISC1 using a transgenic mouse with a dominant negative

fragment of the DISC1 c-terminal (DISC1cc), which can be activated via tamoxifen injection. DISC1cc is then active for 6-48 hours only, allowing a narrow time window of effect to be studied.

Activation of the mutant protein at postnatal day 7 (P7) results in schizophrenia-related phenotypes (Li et al., 2007) and a loss of LTP and experience dependent plasticity in layer 2/3 (L2/3) of adult barrel cortex (Greenhill et al., 2015). Disruption at P28 has no effect on experience dependent plasticity, suggesting a critical period for the development of plasticity itself (Greenhill et al., 2015).

Disruption at P7 was found to specifically affect 2nd and 3rd order basal dendrites, which corresponds to the age at which these dendritic segments largely develop. These dendrites showed lower spine density and had fewer mushroom spines compared either to 4th and 5th order dendrites (which develop later) in the same animals, or to 2nd and 3rd order dendrites in wild-type controls.

However, it remains unknown whether these effects are due to 2nd and 3rd order dendrites developing at P7, or whether these orders are uniquely vulnerable to any disruption of DISC1 around that time. To investigate this, we delayed the tamoxifen injection (and thus the disruption of DISC1) until P9.

When injected with tamoxifen at P9, 2nd and 3rd order dendrites showed spine density and morphology similar to that in wild-type controls. However, 4th order dendrites showed lower spine density and 5th order dendrites had fewer mushroom spines. The effects of DISC1 disruption starting at P9 were more variable than at P7, which might be due to more variance in distance to the soma and thus a more variable age of development. However, these data clearly show that DISC1 disruption only affects spine density and morphology at a point in time when the parent dendrite is forming.

Greenhill, S.D., Juczewski, K., de Haan, A.M., Seaton, G., Fox, K., & Hardingham, N.R. (2015). Adult cortical plasticity depends on an early postnatal critical period. *Science*, *349*(6246), 424-427.

Li, W., Zhou, Y., Jentsch, J.D., Brown, R.A.M., Tian, X., Ehninger, D., Hennah, W., Peltonen, L., Lönnqvist, J., Huttunen, M.O., Kaprio, J., Trachtenberg, J.T., Silva, A.J., & Cannon, T.D. (2007). Specific developmental disruption of disrupted-in-schizophrenia-1 function results in schizophrenia-related phenotypes in mice. *PNAS, 104*(46), 18280-18285.

Disclosures: A.M. De Haan: None. **N.R. Hardingham:** None. **K. Fox:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.11/C8

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

Support: NIH Intramural

Title: Dissecting the components of excitatory synapse maturation on hippocampal inhibitory interneurons

Authors: *G. AKGUL[1], K. A. PELKEY[2], C. J. MCBAIN[2];
[2]NICHD, [1]Natl. Inst. of Hlth., Bethesda, MD

Abstract: Inhibitory interneurons of the hippocampus and neocortex are derived from two lineages within the embryonic medial- and caudal ganglionic eminences (MGE and CGE,). Their MGE or CGE origin determines many features including their glutamate receptor subunit expression profiles. However, despite the early specification of synaptic receptor subtype some aspects of neuronal plasticity are sustained through adulthood. MGE-derived inhibitory interneurons that express calcium permeable GluA2-lacking AMPARs exhibit a form of NMDAR plasticity that converts GluN2B- to GluN2A-containing NMDARs during development. This switch can also be triggered prematurely with high frequency excitatory input by a mechanism dependent on an elevation of Ca^{2+} occurring through calcium permeable AMPARs, but not NMDARs. This mechanism of NMDAR plasticity is absent from CGE-derived interneurons that typically express GluA2 containing AMPARs and GluN2B-containing NMDARs throughout life. How much of this synaptic plasticity is genetically dictated and how much of it is an adaptation to environmental cues? To answer this question we selectively eliminated GluA2 subunit expression in CGE derived interneurons thus converting Ca^{2+}-impermeable AMPARs to Ca^{2+}-permeable AMPARs. GluA2 loss in CGE-derived interneurons was verified with AMPARs IV curves that showed strong inward rectification and synaptic currents that were sensitive to philanthotoxin block. Despite this shift to an MGE-like AMPAR profile, the NMDA/AMPA ratio of the evoked responses in GluA2-KO CGE derived interneurons remained the same as WT. However GluA2 loss of function triggered a reduction in the NMDAR decay time constant in neonatal animals indicating an increase in GluN2A subunit expression, suggesting a premature GluN2B to GluN2A subunit switch. Of interest, the frequency of spontaneous excitatory synaptic activity in CGE-KO interneurons was 50% lower than that observed in WT interneurons. However, despite this reorganization of glutamatergic synapses on GluA2-KO interneurons, sIPSCs had similar properties as WT interneurons in terms of frequency, amplitude and decay kinetics ruling out a possible cross talk between glutamatergic and GABAergic syanpses. These data suggest that Ca^{2+} entry via AMPARs are critical determinants of both synaptic NMDAR and AMPAR phenotype of hippocampal interneurons.

Disclosures: G. Akgul: None. **K.A. Pelkey:** None. **C.J. McBain:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

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

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the Health Medical Research Fund (HMRF14SC06)

Title: The pseudokinase CaMKv is required for the activity dependent maintenance of dendritic spines

Authors: *Z. LIANG[1,2,3], Y. ZHAN[1,2,3], Y. SHEN[1,2,3], C. C. WONG[4], J. YATES[4], F. PLATTNER[5], K.-O. LAI[1,2,3], N. Y. IP[1,2,3];
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Abstract: The stabilization of dendritic spines depends on afferent synaptic inputs and requires changes in actin cytoskeleton dynamics and protein synthesis. However, the underlying molecular mechanism remains unclear. Here, we identified calmodulin kinase-like vesicle-associated (CaMKv), a pseudokinase of the CaMK family with unknown function, as a synaptic protein crucial for dendritic spine maintenance. CaMKv mRNA localizes at dendritic spines, and its protein synthesis is regulated by neuronal activity. CaMKv function is inhibited upon phosphorylation by cyclin-dependent kinase 5 (Cdk5) at Thr-345. Furthermore, CaMKv knockdown in hippocampal CA1 pyramidal neurons *in vivo* impairs synaptic transmission and plasticity, resulting in hyperactivity and spatial memory impairment in mice. These findings collectively indicate that the precise regulation of CaMKv through activity-dependent synthesis and post-translational phosphorylation is critical for dendritic spine maintenance. Thus, this

study reveals an unusual signaling pathway involving a pseudokinase that regulates synaptic transmission and brain function.

Disclosures: **Z. Liang:** None. **Y. Zhan:** None. **Y. Shen:** None. **C.C. Wong:** None. **J. Yates:** None. **F. Plattner:** None. **K. Lai:** None. **N.Y. Ip:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.13/C10

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

Support: Postgraduate Scholarships Act of the Land of Baden-Wuerttemberg (LGFG)

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Innovative Medicines Initiative (IMI) Joint Undertaking under grant agreement n° 115300

Title: Knockout of RICH2, a SHANK3 interacting protein, leads to enlarged dendritic spines via RAC1 dependent Actin remodeling and causes neophobia in mice

Authors: *T. SAROWAR[1,2], S. GRABRUCKER[3], T. BOECKERS[3], A. GRABRUCKER[1,2]; [1]Dept. of Neurol., Inst. of Anat. & Cell Biol., Ulm, Germany; [2]WG Mol. Analysis of Synaptopathies, Neurol. Department., Neurocenter of Ulm Univ., Ulm, Germany; [3]Inst. for Anat. and Cell Biology, Ulm Univ., Ulm, Germany

Abstract: The post-synaptic densities (PSD) in the dendritic spines are functional units of signal transduction, structural stability, neuronal morphogenesis and synaptic activity. Many neurological and psychiatric disorders have been characterized in terms of the alteration of signaling, and structural and functional proteins in the PSD. There, activity dependent signaling molecules such as small GTPases interact with each other. The disruption of their interactions may lead to abnormal spine morphology and function, and, ultimately, behavioral phenotypes. The PSD protein Shank3 is very well characterized in terms of its central role in synaptopathies such as Autism Spectrum Disorders (ASD). RICH2 was identified as an interaction partner of Shank3 at excitatory glutamatergic synapses. RICH2 is a brain specific PSD protein comprising of three domains - an N-BAR domain, a RhoGAP domain and a C-terminal PDZ domain. We have generated a mouse model which lacks all the isoforms of RICH2. The disruption of the

Shank3-Rich2 interaction results in alteration in synaptic protein composition, receptors and signaling molecules. In Golgi analyses, alterations in the morphology of dendritic spines were detected that can be correlated to changes in the number of PSDs in specific brain regions. The changes are caused by increased activity of Rac1 and Cdc42, two key components of small Rho GTPases, leading to alteration in actin dynamics in spines. Interestingly, on behavioral level, besides increased repetitive behaviors, a pronounced neophobia and impaired motor learning have been observed in RICH2 knock-out mice. This mouse model points towards the importance of fine tuning among scaffolding molecules like Shank3, Rho GTPases and actin at the PSD mediated by the Shank3 interacting protein Rich2 that might be especially affected in the amygdala given the phobia observed in RICH2 knock-out mice.

Disclosures: **T. Sarowar:** None. **S. Grabrucker:** None. **T. Boeckers:** None. **A. Grabrucker:** None.

Poster

295. Synapse Maturation and Remodeling

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Title: Axonal input to nearby Purkinje cells in early postnatal mouse cerebellum analyzed with serial section electron microscopy

Authors: ***A. M. WILSON**[1], R. SCHALEK[1], A. SUISSA-PELEG[1], T. JONES[1], S. KNOWLES-BARLEY[2], J. LICHTMAN[1];
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Abstract: In both the peripheral and central nervous systems of developing mammals, axons prune branches to innervate fewer postsynaptic target cells, causing these cells to become innervated by fewer but stronger inputs. This synaptic reorganization is regulated by synaptic activity. This phenomenon has been mainly studied with optical techniques in parts of the peripheral nervous system, but the small sizes and dense arrangements of cells and synapses

have undermined comparable optical approaches in the central nervous system. In order to overcome the barriers to exploration of synaptic rewiring in the central nervous system, we used serial section scanning electron microscopy to produce 3D volumes of high-resolution images from cerebella of early postnatal mice. The resolution of electron microscopy suffices to identify virtually all synapses in a tissue sample. We used a recent adaptation of this technique (ATUM) in which long series of thin sections are collected on tape and automatically imaged with scanning electron microscopy. We focused on developing cerebellum because it undergoes large scale changes in connectivity and is intrinsically less complicated than cerebral cortex. We have reconstructed neonatal Purkinje cells and for one cell all of its climbing fiber, parallel fiber and inhibitory inputs. In addition we identified other Purkinje cells that shared the same climbing fiber inputs. We did this kind of analysis at several different developmental ages. We have found that parallel fibers form progressively more synapses onto Purkinje cells during the first week of postnatal life. We have also found that multiple climbing fiber axons innervate the same developing Purkinje cell. These climbing fiber axons extend over large distances in the Purkinje layer both in the sagittal and coronal dimensions. Their terminal arbors branch frequently in the Purkinje layer and form small branches and varicosities, in contrast with other axon types. Developing climbing fibers innervate a subset of closely positioned Purkinje cells. We are now inquiring whether these subsets are specific or random.

Disclosures: **A.M. Wilson:** None. **R. Schalek:** None. **A. Suissa-Peleg:** None. **T. Jones:** None. **S. Knowles-Barley:** None. **J. Lichtman:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.15/C12

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

Support: NIMH Silvio Conte Center (P50MH094271)

Title: Serial section super-resolution (STORM) imaging of the perineuronal net and synaptic maturation during critical period development

Authors: ***Y. SIGAL**[1], L. BOGART[1], H. BAE[1], X. ZHUANG[1,2], T. K. HENSCH[1,3]; [1]Harvard Univ., Cambridge, MA; [2]Howard Hughes Med. Inst., Cambridge, MA; [3]Boston Chirdrens Hosp., Boston, MA

Abstract: Perineuronal nets (PNNs) are specialized extracellular matrix structures which tightly enwrap a subset of parvalbumin-positive (PV+) inhibitory neurons in the mature neocortex.

Potential PNN functions include synapse stabilization, molecular signaling and protection against oxidative stress characteristic of mental illnesses. Notably, PV+ circuit maturation is pivotal for establishing critical periods of brain development during which sensitivity of synaptic connectivity to environmental stimuli is heightened. Yet, many details underlying these structural rearrangements remain unknown. Here, we applied our previously developed super-resolution STORM imaging platform for volumetric imaging and automated analysis of neuronal and synaptic structures to the studies of the PNN. Using this technique, we visualized and quantified chondroitin-sulfate proteoglycan (CSPG) labeling and synaptic 'holes' within the PNN and analyzed morphological changes across three conditions. First, during and after the normal critical period (postnatal days 30 and 90, respectively), we observed that PNNs in layers 4/5 of primary visual cortex increased in intensity and local CSPG density concomitant with a condensed lattice around the cell body and a smaller, more uniform hole size within the PNN mesh. Over this same period, synaptotagmin-2 positive (PV) boutons increased in intensity and spatial organization on the PV+ somata, as well as in interactions with the PNN proper, consistent with a role of the PNN in stabilizing inhibitory contacts. Second, we explored critical period delay by the complete absence of visual experience from birth. Postnatal day 90 dark-reared mice exhibited immature organizational aspects comparable to young light-reared animals. Third, we examined a complementary disease model of precocious critical period onset. Mice lacking MECP2 - a model of Rett Syndrome - displayed a more mature PNN profile at postnatal day 30 as compared to age-matched wildtype littermates. This was notably accompanied by a relative increase in excitatory contacts. Taken together, STORM imaging provides novel insights into the molecular and circuit level changes underlying critical period development in visual cortex, as well as the imbalanced synaptic distribution of excitatory and inhibitory contacts associated with their altered trajectory by deprivation or autism risk genes.

Disclosures: **Y. Sigal:** None. **L. Bogart:** None. **H. Bae:** None. **X. Zhuang:** None. **T.K. Hensch:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

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Program#/Poster#: 295.16/C13

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

Support: CNMPB

Title: Cooperative action of neuroligins and BDNF mediates presynaptic maturation

Authors: *A. PETKOVA[1], N. GÖDECKE[2], M. KORTE[2], T. DRESBACH[1];
[1]Inst. for Anat. and Embryology, Synaptogenesis Group, Univ. Med. Ctr. Goettingen, Goettingen, Germany; [2]Cell. Neurobio., Tech. Univ. Braunschweig, Braunschweig, Germany

Abstract: Synaptic maturation is a process that allows synapses to acquire their full functionality, and failures in synaptic maturation are thought to contribute to psychiatric disorders such as autism. Neuroligins are postsynaptic cell-adhesion molecules essential for postsynaptic and presynaptic maturation, but the transsynaptic pathways by which Neuroligins act to regulate presynaptic maturation are incompletely understood.
Here, we show that presynaptic maturation relies on the cooperative action of Neuroligins and brain-derived neurotrophic factor (BDNF). Applying BDNF to neuronal cultures mimicked the maturation-promoting effect of the overexpressed Neuroligin isoforms NL1 and NL2. Reducing the levels of BDNF by applying a BDNF scavenger (TrkB-Fc) or by Cre-induced depletion of BDNF blocked the action of NL1 and NL2. In particular, inhibiting endogenous BDNF signaling reduced the positive effects of NL1 on presynaptic maturation and of NL2 on synapse formation. Applying BDNF to cultures from NL1-knockout mice rescued defective presynaptic maturation both in early (DIV6) and late (DIV15) culture stages, indicating that BDNF acts downstream of NL1-mediated cell adhesion.
Our data introduce BDNF as a novel and essential component in a transsynaptic pathway linking NL-mediated cell adhesion, neurotrophin action and presynaptic maturation.

Disclosures: **A. Petkova:** None. **N. Gödecke:** None. **M. Korte:** None. **T. Dresbach:** None.

Poster

295. Synapse Maturation and Remodeling

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

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NIH Grant MH64570

Title: Synapse tagging by C1q

Authors: *J. W. HAMMOND, H. A. GELBARD;
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Abstract: The classical complement system has been shown to play a role in synapse elimination during development and disease. However, little is known about how complement

activation at synaptic terminals is regulated or how synapses are tagged by the initiating complement member, C1q. In order to address the question of C1q synapse tagging, we added C1q to *in vitro* hippocampal cultures and looked for changes in binding due to changes in neuronal activity or neuronal stress. We also sought to identify binding partners of C1q at synapses. We noted that addition of C1q, surprisingly, resulted in an increased number of synapses. This is reminiscent of other members of the C1q superfamily expressed in the nervous system (C1qL1-4 and Cbln1-4) that have been shown to act as trans-synaptic organizers recruiting select presynaptic and postsynaptic receptors in order to strengthen and maintain synapses between particular neurons. We hypothesize that C1q may bind synaptic proteins and facilitate synapse organization in a similar manner. We show that C1q binds the Bai family of adhesion G-protein coupled receptors. Bai1 and 3 in neurons have been shown to play a key role in synapse development and plasticity. On the other hand, Bai1 is also a phagocytosis receptor used by microglia and macrophages. Thus, this C1q-Bai interaction could have important implications for the role of C1q in both synapse tagging and phagocytic pruning of synapses by glia cells during development and disease.

Disclosures: J.W. Hammond: None. **H.A. Gelbard:** None.

Poster

295. Synapse Maturation and Remodeling

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

Support: CAS XDB02010000

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PSSCS 16XD1404800

Title: An inducing role for the pre-synaptic cadherin/catenin/p140Cap complex in functional synapse formation in the neocortex

Authors: M.-Y. LI, W.-Y. MIAO, S.-J. HE, *X. YU;
Inst. of Neurosci., Shanghai City, China

Abstract: The formation of functional synapses requires coordinated assembly of pre-synaptic transmitter release machinery and post-synaptic trafficking of functional receptors and scaffolds.

Here, we show that cadherin/catenin cell adhesion complexes are necessary and sufficient for functional synapse formation and spinogenesis in the developing mouse somatosensory cortex. Importantly, pre-synaptic expression of stabilized β-catenin in either layer 4 (L4) excitatory neurons or L2/3 pyramidal neurons significantly up-regulated excitatory synaptic transmission and dendritic spine density in L2/3 pyramidal neurons, while its sparse post-synaptic expression in L2/3 neurons had no such effects. In addition to increasing synapse density, pre-synaptic β-catenin expression also enhanced release probability of glutamatergic synapses; both effects required β-catenin interacting protein p140Cap specifically in the presynaptic locus. Together, our results demonstrate that cadherin/catenin complexes contribute significantly to functional synapse formation through anterograde signaling in the neocortex, providing important molecular evidence for an inducing role of pre-synaptic components in the formation of excitatory synapses in the neocortex.

Disclosures: **M. Li:** None. **W. Miao:** None. **S. He:** None. **X. Yu:** None.

Poster

295. Synapse Maturation and Remodeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 295.19/DP01 (Dynamic Poster)

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

Title: Caught in the act: live imaging of microglia-synapse interactions by lightsheet microscopy

Authors: *L. WEINHARD[1], G. DI BARTOLOMEI[1], U. NENISKYTE[1], G. BOLASCO[1], A. VADISIUTE[1], P. MACHADO[2], Y. SCHWAB[2], C. GROSS[1];
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Abstract: Several recent studies have demonstrated that microglia contribute to synaptic maturation during postnatal brain development. *In vivo* time-lapse imaging in the mouse brain has shown that microglia occasionally contact synapses and experiments in fixed brain tissue identified synaptic material inside microglia, suggesting that engulfment of synapses by microglia may contribute to synapse elimination and circuit maturation during development. However, until now no evidence exists to support the selective phagocytosis of synaptic material by microglia under non-pathological conditions. Here we have used high-resolution time-lapse multicolor fluorescent lightsheet microscopy to image microglia-synapse interactions in organotypic hippocampal cultures. This approach allowed us to systematically identify, follow, and quantify transient contacts between microglia processes and dendritic spines. In parallel, we carried out volume correlative light-electron microscopy (CLEM) to produce 3D images of microglia-synapse interaction ultrastructure.

Disclosures: **L. Weinhard:** None. **G. di Bartolomei:** None. **U. Neniskyte:** None. **G. Bolasco:** None. **A. Vadisiute:** None. **P. Machado:** None. **Y. Schwab:** None. **C. Gross:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant R21 NS087225 02

FRAXA

Title: Beta arrestin 2 couples $mGlu_5$ to fmrp-regulated protein synthesis and is a novel target for the treatment of fragile x syndrome

Authors: ***R. K. SENTER**[1], L. J. STOPPEL[1], B. D. AUERBACH[2], A. R. PREZA[1], R. J. LEFKOWITZ[3], M. F. BEAR[1];
[1]Picower Inst. of Learning and Memory, MIT, Cambridge, MA; [2]The Ctr. for Hearing and Deafness, Dept. of Communicative Disorders and Sci., The State Univ. of New York at Buffalo, Buffalo, NY; [3]Dept. of Medicine, Howard Hughes Med. Inst., Duke Univ. Med. Ctr., Durham, NC

Abstract: Fragile X Syndrome (FX) is caused by silencing the FMR1 gene, and consequent loss of the encoded protein FMRP. Considerable evidence suggests that dysregulated synaptic protein synthesis downstream of metabotropic glutamate receptor 5 ($mGlu_5$) activation is a major contributor to disease pathogenesis. Therapies targeting this dysregulation, however, have yet to succeed in clinical trials, possibly due to our incomplete understanding of the signaling pathways that couple $mGlu_5$ activation to neuronal mRNA translation. Here, we demonstrate that β-arrestin2 is a critical link between $mGlu_5$ activation, FMRP-regulated translation, and synaptic plasticity. Heterozygous deletion of β-arrestin2 severely blunts $mGlu_5$-stimulated protein synthesis, ERK activation, and $mGlu_5$-LTD while preserving canonical Gq signaling. Interestingly, genetic reduction of β-arrestin2 in *Fmr1*-null mice is sufficient to correct many recognized deficits, including exaggerated protein synthesis and $mGlu_5$-LTD as well as many cognitive and behavioral impairments. Importantly, this reduction in β-arrestin2 does not induce the same psychotomimetic side effects associated with full $mGlu_5$ inhibitors, indicating that targeting β-arrestin2-mediated signaling may be a more selective approach to the treatment of FX.

Disclosures: **R.K. Senter:** None. **L.J. Stoppel:** None. **B.D. Auerbach:** None. **A.R. Preza:** None. **R.J. Lefkowitz:** None. **M.F. Bear:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: NEOMED Bridge Funding

Title: Neuromodulation of synaptic transmission by group I mGluRs in MNTB neurons in a mouse model of fragile X syndrome

Authors: *Y. LU;
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Abstract: Fragile X syndrome (FXS) is the leading single-gene cause for mental retardation. Among other deficits, FXS patients experience compromised sensory processing. Previous research has shown that in a fragile X mental retardation gene 1 (FMR1) knock out mouse model (Fmr1 KO), auditory cortical neurons exhibit abnormal auditory responses and impaired neural plasticity. In the medial nucleus of trapezoid body (MNTB, a critical nucleus in the brainstem sound localization circuit), the presynaptic terminal (calyx) become larger, cell size decreases, and Kv channels change their distribution. The loss of FMR protein function also results in exaggerated activity of group I metabotropic glutamate receptors (mGluR I). Altered neuromodulation may underlie compromised auditory processing. Here, we investigated mGluR I neuromodulation of synaptic transmission in MNTB neurons in Fmr1 KO and wild type (WT) mice, using whole-cell recordings from brainstem slices obtained from P12-P22 mice. The basal level of excitatory transmission in the KO was slightly reduced and compromised, whereas the basal level of inhibitory transmission was strengthened. MNTB neurons of the WT showed primarily all-or-none evoked EPSCs (eEPSCs), while most KO neurons showed graded input-output functions. Proportionally more KO neurons exhibited synaptic facilitation in a paired-pulse paradigm. In about half of the recorded neurons, activation of mGluR I by 3,5-DHPG (200 μM) suppressed eEPSCs, and the suppression in the KO was moderately stronger than in the WT. 3,5-DHPG also produced an inward current at the holding potential of -60 mV. Surprisingly, 3,5-DHPG increased spontaneous EPSC (sEPSC) frequency in both WT and KO neurons. Similarly, 3,5-DHPG enhanced sIPSCs, without affecting eIPSCs. The differential modulation of spontaneous versus evoked transmitter release suggests that the vesicle pools

responsible for these two different modes of release, as well as the mechanisms underlying mGluR modulation of the two release machineries, are different.

Disclosures: Y. Lu: None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

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Japan Foundation for Neuroscience and Mental Health Grant

Title: Maturation of fast-spiking neurons in the cortex is delayed in Fragile X mice

Authors: *T. NOMURA[1], A. CONTRACTOR[2];
[1]Physiol., [2]Northwestern Univ., Chicago, IL

Abstract: Fragile X Syndrome (FXS) is a prototypical neurodevelopmental disorder that causes intellectual disability and autism. The mouse model of FXS (*Fmr1* KO) recapitulates aspects of the human disorder including alteration in sensory processing. There is a delay in the development of synapses and a shift in the timing of the critical period for plasticity in the somatosensory cortex (barrel cortex) of *Fmr1* KO mice and sensory evoked responses in the cortex are exaggerated. There are known alterations in GABA signaling that have been described in the cortex of FXS mice, however the extent of these alterations and how they contribute to synaptic development are not fully characterized. In the visual cortex there is strong evidence that fast-spiking (FS) interneurons are central to establishing cortical critical periods. Here we proposed to map the functional development of FS interneurons in the somatosensory cortex during the course of the critical period in *Fmr1* KO mice. We crossed *Fmr1* KO mice with a strain expressing eGFP in parvalbumin containing interneurons to facilitate the targeted patch clamp recording of FS interneurons in a slice. As a measure of FS interneuron maturation, we examined the degree of spike adaptation, quantified as spike ratio (the initial to final spike frequency during a train of action potentials). Comparison of the firing characteristics of FS interneurons in *Fmr1* WT and *Fmr1* KO neonatal mice demonstrated a reduction in spike ratio in *Fmr1* KO mice during the critical period in the barrel cortex. In *Fmr1* WT mice at postnatal day (P) 9, the spike ratio was 0.69 ± 0.01 ($n = 37$), whereas in *Fmr1* KO mice the measured spike

ratio was 0.62 ± 0.01 (n = 25) ($p < 0.01$). These data demonstrate that at P9 FS interneurons spiking shows greater adaptation and suggests that FS interneuron maturation is delayed in the somatosensory cortex of *Fmr1* KO mice. It is well established that the trophic factor BDNF has a crucial role in neuronal maturation. Therefore we determined if the administration of a TrkB receptor agonist (LM22A-4) to neonates could reverse the delays in FS neuron maturation. Daily administration of a TrkB receptor agonist normalized functional measures of FS interneuron maturation in *Fmr1* KO mice. The spike ratio at P9 was 0.61 ± 0.02 (n = 36) and 0.69 ± 0.01 (n = 39) in vehicle and LM22A-4 treated group in *Fmr1* KO mice ($p < 0.01$) demonstrating that there was a normalization of this measure in KO mice treated with a TrkB agonist. Administration of LM22A-4 had no effect on the spike ratio in WT mice. These results demonstrate that there is a delay in FS interneuron maturation in the cortex of *Fmr1* KO mice that is due to a deficit in BDNF - TrkB signaling.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Support: FRAXA Research Foundation (U.S.A.) grant 2013

Telethon Foundation (Italy) grant GGP13145

Title: Activation of 5-HT_7 receptors for serotonin rescues hippocampal synaptic plasticity in a mouse model of Fragile X Syndrome through a cyclic AMP-mediated mechanism involving Cyclin-dependent Kinase 5 and protein synthesis.

Authors: *L. CIRANNA[1], L. COSTA[2], L. M. SARDONE[1], M. SPATUZZA[3], C. M. BONACCORSO[4], S. D'ANTONI[3], M. LEOPOLDO[5], E. LACIVITA[5], M. V. CATANIA[3,4]; [1]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 Neurobiol., IRCCS Oasi Maria Santissima, Troina (EN), Italy; [5]Dept. of Pharm., Univ. of Bari, Bari, Italy

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability, frequently associated with epilepsy and autism. *Fmr1* KO mice, a model of FXS, display excessive metabotropic glutamate receptor-mediated long-term depression (mGluR-

LTD), altered dendritic spine morphology, learning deficit and autistic behavior. We have shown that 5-HT_7 receptor (5-HT7R) agonists reverse mGluR-LTD in wt and *Fmr1* KO mice (Costa et al., Biol. Psych. 2012, 72:924-933). We have preliminary data showing that 5-HT7R agonists also rescue dendritic spine morphology, learning and behavior in *Fmr1*KO mice, suggesting that they might become a novel therapeutic strategy for FXS.
To identify the mechanisms underlying 5-HT7R-mediated effects, we used patch clamp on hippocampal slices from wild-type (wt) and *Fmr1* KO mice to test the effects of 5-HT7R agonists on mGluR-LTD in the presence of specific blockers of intracellular messengers. LP-211, a selective 5-HT_7 R agonist, reversed mGluR-LTD in the CA3-CA1 synapse in wt and *Fmr1* KO slices. The effect of LP-211 was mimicked by forskolin and was abolished by the adenylate cyclase inhibitor SQ22536. The effect of LP-211 persisted in the presence of cAMPS-Rp or PKA inhibitor peptide fragment 6-22 (PKA inhibitors) or PD98059 (a MEK/ERK inhibitor), but was reduced in the presence of lithium (a PI3K/GSK3 inhibitor) and was completely abolished in the presence of SB216763 (a GSK3 inhibitor), roscovitine (a Cdk5 inhibitor) or anisomycin (a protein translation inhibitor). Taken together our data show that 5-HT_7 receptor activation reverses mGluR-LTD acting through a cAMP-dependent mechanism involving Cdk5 and GSK3 kinases and protein synthesis; we are currently investigating at which level these pathways interact.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Support: Fonds Wetenschappelijke Onderzoeks (FWO) Grant G.0503.12

ERC Starting Grant 335561

Title: Reduced lateral inhibition impairs olfactory computations and behaviors in a *Drosophila* model of Fragile X Syndrome

Authors: *L. M. FRANCO MÉNDEZ[1,2], Z. OKRAY[1], B. A. HASSAN[1,3], E. YAKSI[2,4]; [1]VIB Ctr. for The Biol. Of Dis., [2]Neuroelectronics Res. Flanders (NERF), KU Leuven, Leuven, Belgium; [3]Inst. du Cerveau et de la Moelle Epinière (ICM), Hôpital Pitié-Salpétrière, Paris,

France; [4]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, NTNU, Trondheim, Norway

Abstract: Fragile X syndrome (FXS) patients present neuronal alterations that lead to severe intellectual disability, but the underlying neuronal circuit mechanisms are poorly understood. An exciting hypothesis postulates that reduced GABAergic inhibition of excitatory neurons is a key component in the pathophysiology of FXS. Here, we directly test this idea. First, we show that a FXS *Drosophila* model exhibits strongly impaired olfactory behaviors. In line with this, olfactory representations are less odor-specific due to broader response tuning of excitatory projection neurons. We find that impaired inhibitory interactions underlie reduced specificity in olfactory representations. Finally, we show that defective lateral inhibition across projection neurons is caused by weaker inhibition from GABAergic interneurons. We provide direct evidence that deficient inhibition impairs sensory computations and behavior in an *in vivo* model of FXS. Together with evidence of impaired inhibition in autism and Rett syndrome, these findings suggest a potentially general mechanism for intellectual disability.

Disclosures: L.M. Franco Méndez: None. **Z. Okray:** None. **B.A. Hassan:** None. **E. Yaksi:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: The Chinese Ministry of Science and Technology grants (2014CB942803)

Title: PQBP1 mutations promote FMRP degradation

Authors: *X.-Y. ZHANG[1,2], Y.-Q. SHEN[2], X. LIU[2], Z. ZHANG[2];
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Abstract: Renpenning syndrome is a group of X-linked intellectual disability (XLID) syndromes caused by mutations in human polyglutamine-binding protein 1 (*PQBP1*) gene. Little is known about the molecular pathogenesis of the various mutations that cause the notable variability in patients. In this study, we examined the synaptic and cellular functions of the most common mutations found the patients: c.461_462delAG, c.459-462delAGAG, and c.463_464dupAG in an AG hexamer in PQBP1 exon 4. All three mutations result in the frame-shifts and premature termination. In contrast to current loss-of-function pathogenic hypothesis, we discovered that the

frame-shifted sequences of PQBP1 c.459_462delAGAG and c.463_464dupAG mutations encode a new C-terminal epitope that preferentially binds non-phosphorylated fragile X mental retardation protein (FMRP) and promotes its ubiquitinmediated degradation. Consequently, the PQBP1 mutant-induced reduction of FMRP relieves its inhibition on translation and leads to the up-regulation of its targets such as MAP1B. Elevated MAP1B disrupts the local cytoskeleton structure and results in a remarkable synaptic over-growth in the neuromuscular junction (NMJ) of PQBP1 c.463_464dupAG transgenic flies, which can be rescued by exogenously expressing dFMRP. Our evidence strongly supports a gain-of-function pathogenic mechanism of the prevailing mutations of Renpenning syndrome PQBP1 c.459_462delAGAG and c.463_464dupAG. In addition, we reveal that the pathology of Renpenning syndrome is directly linked to Fragile X syndrome. These findings will reshape our thinking of therapeutic strategies for X-linked intellectual disability.

Disclosures: **X. Zhang:** None. **Y. Shen:** None. **X. Liu:** None. **Z. Zhang:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 296.07/C22

Topic: A.07. Developmental Disorders

Support: 2014/14/E/NZ3/00375 Sonata BIS for MDz

Title: Synaptic translation of neuroligins 1, 2 and 3 is regulated by FMRP

Authors: ***M. DZIEMBOWSKA**, J. PODSIADŁOWSKA, K. JĄCZYŃSKA, J. MIŁEK, B. KUŹNIEWSKA;
Ctr. of New Technologies, Univ. of Warsaw, Warszawa, Poland

Abstract: Neuroligins (NLGNs) are postsynaptic cell adhesion proteins which bind to their presynaptic partner neurexin across the synaptic cleft. Neuroligins play a key role in the formation, maturation and maintenance of synapses. Here, we sought to determine whether the synaptic translation of NLGN mRNA is regulated by fragile X protein. The mRNA for three studied neuroligins NLGN1, NLGN2 and NLGN3 was immunoprecipitated with anti-FMRP antibody suggesting that FMRP regulates synaptic translation of neuroligins. We have also observed a rapid, activity-dependent polyadenylation of NLG1 mRNA. The profile of polyribosomes isolated from Fmr1 KO synaptoneurosomes confirms its elevated translation in the basal state and the lack of response to stimulation. Moreover our results indicate that excessive neuroligin synthesis at the synapse of Fmr1KO mice leads to their higher abundance

on the synapse that was detected in synaptoneurosomes by the biotynylation assay. We have also observed that in synaptoneurosomes, glutamate-induced signaling can stimulate cleavage of neuroligin1 both in WT and Fmr1 KO mice. The correct level of synaptic NLGNs may be responsible for proper synapse formation and stability of the synaptic connections which are deregulated in fragile X syndrome. Characterizing NLGN1, NLGN2 and NLGN3 mRNAs as FMRP substrates will contribute to understanding the synaptic defects of FXS.

Disclosures: M. Dziembowska: None. **J. Podsiadłowska:** None. **K. Jączyńska:** None. **J. Miłek:** None. **B. Kuźniewska:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: FRAXA Fellowship

Brain Canada Research Grant

The Azrieli Foundation Grant

Title: Astrocyte purinergic signaling significantly altered in fragile x syndrome model

Authors: *A. L. SCOTT, A. CHEN, L. DOERING;
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Abstract: Neural communication and the intricate choreography of signals required for the formation and preservation of neural connections is heavily dependent on reciprocal neuronal and glial interactions. Astrocytes are key participants in neurodevelopmental processes and defects to astrocyte signaling are implicated in disorders such as Fragile X Syndrome (FXS). In FXS, the loss of the Fragile X mental retardation protein (FMRP) expression from astrocytes is associated with delayed dendrite maturation and improper synapse formation. These findings emphasize the importance of astrocyte-derived signals to neuronal connections and illustrate the devastating consequences an imbalance to these signals can cause. During development astrocytes release a wide range of gliotransmitters; however, the use of ATP is one of the predominant means of communication between astrocytes and neurons within the CNS. ATP is a fast, excitatory neurotransmitter known to act on astrocytes, modulate glio-neuronal transmission, and in this way regulate synaptic function. Given the integral role of ATP and its various metabolites to the regulation of synaptic development and function, we compared the

physiological responses of astrocytes isolated from either post-natal wild-type ($FMRP^{+/+}$) mice or from transgenic *fmr 1* knockout ($FMRP^{-/-}$) mice to exogenous purinergic stimulation. The quantitative analysis of intracellular calcium levels revealed a significantly greater flux of intracellular calcium in $FMRP^{-/-}$ astrocytes in response to ATP (and UTP) than observed in $FMRP^{+/+}$ astrocytes. Interestingly, blockade of several purinergic receptors with suramin returned abnormal astrocytic calcium flux to wild-type levels. In addition, immunocytochemical and protein analysis demonstrated greater expression of purinergic receptors, P2Y2 and P2Y4 in particular, in $FMRP^{-/-}$ astrocytes over the wild-type counterparts in astrocytes alone. The differential expression may underlie the enhanced sensitivity of $FMRP^{-/-}$ astrocytes and lead to atypical neural communication during development in FXS. Future analysis of the effects on astrocyte-neuron purinergic signaling in the $FMRP^{-/-}$ model will help elucidate the role these signals play in synapse function and the potential therapeutic relevance to FXS.

Disclosures: **A.L. Scott:** None. **A. Chen:** None. **L. Doering:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: MH60163

Title: Fragile X circuits show differential developmental delays of spontaneous and evoked network activity but normal homeostatic plasticity

Authors: *H. MOTANIS, D. BUONOMANO;
Dept. of Neurobio. and Pshychology, and Integrative center for learning, UCLA, Los Angeles, CA

Abstract: Since the generation of the first mouse model of Fragile X (FX) syndrome a broad range of neurophysiological phenotypes have been reported. However, it remains unclear which phenotypes are casually related to the cognitive deficits associated with FX and which are an indirect consequence of abnormal development or experience. FX syndrome is characterized by developmental delays, and recently we have demonstrated an *in vitro* developmental delay of spontaneous Up state activity (Motanis et al., 2015)—suggesting that this neural phenotype is a direct consequence of the FX mutation as opposed to an indirect product of compensation or abnormal development. Here we use this *in vitro* approach to characterize developmental delays of network-level activity and to determine whether FX circuits adapt normally to chronic

external inputs. At 11-15 days *in vitro* (DIV) evoked EPSP strength was not different between WT and FX cortical circuits, however evoked network activity was significantly reduced in FX circuits ($p<0.01$). At 25-30 DIV WT circuits exhibited a developmental change in synaptic strength as evidenced by an increase in the asymptote of the input-output curves, EPSPs in FX circuits, however, were significantly weaker ($p<0.005$). By 35-40 DIV there were no differences in EPSPs strength or evoked network activity between WT and FX circuit—indicating that evoked activity is developmentally delayed in FX circuits. Next we explored network-level plasticity, by examining activity-dependent modulation of evoked activity. We used chronic optogenetic stimulation (COS) to emulate an increase in externally driven activity and induce homeostatic plasticity of network activity. WT and FX slices were stimulated for two days. COS resulted in significant reduction of evoked EPSP strength ($p<10^{-7}$), with no genotype difference. These results indicate that FX circuits exhibit normal homeostatic plasticity and hint at the possibility that some previously described neural phenotypes observed in FX may be compensatory. We are currently examining whether FX circuits exhibit deficits in a form of "*in vitro* learning". Specifically, it has been shown that slices exposed to patterned stimulation, in the form of different intervals between electrical and optical stimulation, learn the interval used during training (Goel et al., 2016). In WT experiments in which one electrical pathway (CS+) was paired with optical stimulation and the other was not (CS-), there was an increase in network activity evoked by the CS+ pathway compared to the unpaired electrical pathway ($p=0.02$), and are currently determining whether there is a difference in the form of plasticity between WT and FX circuits.

Disclosures: H. Motanis: None. **D. Buonomano:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH/OD DP5OD009134

NIH/NICHD U54HD083092

Autism Speaks

Title: Testing the mGluR5 theory of FXS in the laboratory rat

Authors: S. VEERARAGAVAN, L. YUVA, R. PAYLOR, *R. C. SAMACO;
Mol. and Human Genet., Baylor Col. of Medicine/Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX

Abstract: The group I metabotropic glutamate receptor 5 (mGluR5) encoded by *GRM5* mediates aspects of synaptic plasticity and behavior, and studies in human have linked disruptions in mGluR5 signaling with several neuropsychiatric conditions including schizophrenia and attention-deficit/hyperactivity disorder. In addition, excessive mGluR5 signaling has been proposed as a primary contributor to features of Fragile X syndrome (FXS) as evident in a series of mouse model studies; however, recent clinical trials targeting this pathway in individuals with FXS have not been successful to date. Among the several reasons put forth to explain the lack of success of these clinical trials, we hypothesized that perhaps the reliance on findings solely from the laboratory mouse may be one contributing factor and previously found that behavioral phenotypes are not entirely overlapping in male rats and mice lacking *Fmr1*. Therefore, in preparation for genetic and pharmacologic studies to re-evaluate the findings that decreased dosage of mGluR5 in a second mammalian rodent species may improve *Fmr1*-related phenotypes, we conducted two independent replication studies characterizing the behavioral features of *Fmr1* rats, as well as evaluated the behavioral consequences of mGluR5 deficiency in parallel studies of *Grm5* rat and mouse models. Ongoing work suggests that deficits in play behavior are reproducible across cohorts of animals and across individual testers. Furthermore, heterozygous deficiency of mGluR5 in rats and mice tested at the same ages do not result in behavioral impairments as previously observed. Interestingly, *Grm5* rats and mice with complete loss of mGluR5 do not show identical behavioral impairments with the exception of hyperactivity. Taken together, these findings provide the framework for future studies in the rat that will evaluate the hypothesis that targeting the mGluR5 signaling may improve phenotypic outcome.

Disclosures: S. Veeraragavan: None. **L. Yuva:** None. **R. Paylor:** None. **R.C. Samaco:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH RO1 MH094839

FRAXA Research Foundation

Title: Prefrontal cortex dysfunction in Fragile X Syndrome: Single-unit responses of excitatory and inhibitory neurons correlated with behavior.

Authors: *J. J. SIEGEL, R. A. CHITWOOD, W. TAYLOR, J. M. DING, R. GRAY, D. JOHNSTON;
Ctr. for Learning & Memory, Univ. of Texas at Austin, Austin, TX

Abstract: Fragile X Syndrome (FX) is an autism spectrum disorder and the most common heritable cause of mental disability, resulting from an inability to produce the protein FMRP. FX patients show a number of cognitive and emotional impairments, including working memory deficits due to prefrontal cortex (PFC) dysfunction. Identifying behavioral tasks in the FX mouse model that capture the impairments observed in the human patient population has been challenging. We have developed a task for use in mice that directly engages the same neural mechanism in the PFC believed to support working memory (persistent spiking), known traditionally as trace eyeblink conditioning (TEC). We show here that FX mice are profoundly impaired in TEC (Wilcoxon Test, $U=319.5$, $p<0.0005$; Day 12 median response rate; FX: n=25, 1.89%; C57: n=16, 62.62%). Furthermore, knockout of FMRP in the PFC alone was sufficient to observe deficits (n= 15 and 16, $U=157.5$, $p=0.06$). The observed impairment appears to be one of the most striking behavioral deficits reported for FX mice, and offers a new tool to investigate PFC dysfunction in FX. Despite the profound deficit, a modest proportion of FX mice (~one-third) were able to overcome the dysfunction with additional training and ultimately express learning. To determine how a minority of FX mice were able to compensate for dysfunction and eventually learn, we recorded single-unit activity in the PFC of FX mice during TEC and compared neural responses between FX nonlearners to the atypical FX "learner". No differences were observed in the average firing rates or spike widths of putative excitatory or inhibitory PFC cells between FX and wild-type mice. The excitatory cells of FX mice showed persistent spiking to conditional stimuli, similar to that observed in wild-type mice. Putative inhibitory cells in FX mice, however, showed remarkably strong persistent spiking in response to conditional stimuli relative to that observed in wild-type mice (bootstrap test, $p<0.001$ each 50 ms trial time bin). The excitatory cells of wild-type mice showed increased persistent spiking in association with learning. For the FX mice that were able to express learning, excitatory cells did not increase in association with learning. Instead, learning in FX mice was associated with reliable decreases in the responses of inhibitory cells, which was not observed in the more typical FX non-learner. The data suggest that a training-associated decrease in inhibition during the conditional stimulus may be a compensatory mechanism to allow learning in FX mice, perhaps by restoring the excitatory/inhibitory balance thought to underlie cortical dysfunction in FX and other autistic disorders.

Disclosures: **J.J. Siegel:** None. **R.A. Chitwood:** None. **W. Taylor:** None. **J.M. Ding:** None. **R. Gray:** None. **D. Johnston:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: DoD Grant W81XWH-12-1-0320

Title: Inhibitory function in the piriform cortex of the Fragile X Syndrome mouse model

Authors: *A. WIDMER, J. LARSON;
Psychiatry, Univ. of Illinois at Chicago Dept. of Psychiatry, Chicago, IL

Abstract: Fragile X Syndrome (FXS), the major inherited cause of intellectual disability and a leading contributor to autism, is a complicated disorder characterized by altered synaptic function, network hyperexcitability, and cognitive impairment. Though the genetic underpinning of the disease is relatively well understood, the consequences of the silencing of the *Fmr1* gene are varied. Reports of altered function in both excitatory and inhibitory synaptic activity have been published in recent years, but there is still no clear cause of the excitatory/inhibitory imbalance observed in the FXS brain. The current research utilizes complementary techniques in order to characterize the inhibitory circuitry in a select region, the primary olfactory cortex. By combining different methodologies, one can comprehensively map the distribution and quantities of select gamma-aminobutyric acid subtype A (GABA-A) receptor species and correlate these data with differences in electrophysiological function. The GABA-A receptor is a heteromeric receptor protein, and the subunit makeup of the receptor can alter both its distribution within the neural tissue and its function. Of particular interest in FXS is the inhibitory conductance mediated by tonically active GABA-A receptors. Changes in the expression of these specific receptors may underpin many of the observed pathologies associated with FXS, such as disordered information processing, epilepsy, and abnormal sensorimotor integration. This work provides a comprehensive measure of the contribution of both phasic and tonic GABA-A receptors to the inhibitory environment of the primary olfactory cortex. Single-cell analysis of GABAergic events provides an excellent measure of miniature inhibitory postsynaptic currents, revealing parity between FXS and wild-type inhibitory synapses. Parameters such as peak amplitude, rise and decay constants, and temporal spacing are similar between the two genotypes tested, suggesting no derangement of synaptic inhibition in this region in the FXS model. Specific agonism and antagonism of extrasynaptic receptor subunits is planned to provide further data about the tonic current mediated by these receptor species. Additionally, parallel investigations using molecular biological techniques will be conducted to quantify the levels of subunit mRNA transcripts and protein expression in the cortical principal cells. Through the use

of these varied research approaches, we will gain a multifaceted understanding of the inhibitory mechanisms in FXS within a single cortical brain region.

Disclosures: A. Widmer: None. **J. Larson:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: Indiana University School of Medicine Biomedical Research Enhancement Grant

Title: Calcium-binding protein regulation in a *Drosophila* model of fragile x syndrome

Authors: *C. R. TESSIER[1], C. G. SWINFORD[2];
[1]Indiana Univ. Sch. of Medicine-South Bend, South Bend, IN; [2]Univ. of Notre Dame, Notre Dame, IN

Abstract: Intracellular calcium levels are tightly regulated by a host of proteins which function to transport, sequester, and store calcium ions under different physiological circumstances. Defects in calcium homeostasis are known to have profound consequences ranging from improper circuit development to aberrant cognitive behaviors. Fragile X Syndrome (FXS) is an autism spectrum disorder that arises from improper neuronal development and is hallmarked by increased synaptic transmission and augmented calcium transients. The dysregulation of calcium homeostasis in FXS is further evidenced by the misexpression of many calcium-binding proteins in the *Drosophila* model of the disease. The *Drosophila* calcium-binding protein Cbp53E is the single homolog of a small vertebrate calcium-binding protein family consisting of calbindin, calretinin, and secretagogin. These proteins function principally as calcium buffers during acute changes in intracellular calcium levels. Cbp53E expression is reduced in the *Drosophila* model of FXS and loss of function of Cbp53E leads to increased neuronal growth which is consistent with the increased neuronal elaboration seen in FXS. Interestingly, overexpression of Cbp53E is able to rescue the synaptic overgrowth seen in the central nervous system of the *Drosophila* FXS model. These findings suggest that restoring proper neuronal calcium signaling may restore molecular and behavioral defects of FXS animals as well. Given the fundamental necessity for proper calcium regulation in neurons, manipulating calcium-binding proteins may serve as a useful therapeutic strategy for FXS and other diseases.

Disclosures: C.R. Tessier: None. **C.G. Swinford:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH grant

Department of Defense Grant

Title: Fragile X-associated tremor/ataxia syndrome: linking calcium dysregulation and DNA damage responses

Authors: *G. A. ROBIN[1], J. R. LÓPEZ[1], S. HULSIZER[1], P. J. HAGERMAN[2,3], I. N. PESSAH[1,3];
[1]Mol. Biosci., UC Davis, Davis, CA; [2]Dept. of Biochem. and Mol. Med., UC Davis,, Davis, CA; [3]Med. Investigations of Neurodevelopmental Disorders (M.I.N.D) Inst., Sacramento, CA

Abstract: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset, neurodegenerative disorder that affects carriers of the premutation CGG-repeat allele (preCGG; 55-200 repeats) of the fragile X mental retardation 1 (*FMR1*) gene. Early abnormal growth and Ca^{2+} dynamics in *FMR1* premutation preCGG knock-in mice and human iPSC-derived preCGG neurons precede neurodegeneration and FXTAS. Whether intranuclear inclusions containing DNA damage response (DDR) proteins, pathologic hallmark of FXTAS, causally link Ca^{2+} dysregulation with abnormal neuronal growth and survival is unknown. We hypothesize a role for CdK5 and ATM in FXTAS pathogenesis, two key signaling kinases involved in both DDR and synaptic signaling. FMRP expression in postnatal day 0 (P_0), 6-week- and 6-month-old preCGG mouse brain is reduced by 60-, 50- and 30% compared to *wt,* respectively. In primary preCGG hippocampal neurons, FMRP reduction is more pronounced in soma (20% reduction, $p<0.0001$) compared to neurites (5% reduction, $p=0.0177$). Resting cytoplasmic calcium concentration ($[Ca^{2+}]_i$) in preCGG hippocampal neurons is chronically elevated 3-fold compared to *wt* at 7 DIV, 14 DIV and 21DIV, and can be reversed by acute exposure to 10μM dantrolene. Elevated $[Ca^{2+}]_i$-dependent calpain activity (20-40% increase) and p25/p35 (20-45% increase) at 6-weeks and 6-months in preCGG cortex indicate abnormal CdK5 regulation. CdK5 substrate ATM is upregulated by 1.5-2-fold and P-Ser^{1981}-ATM is increased by 1.5-fold ($p<0.01$-0.007) compared to *wt* at P_0 and 6 months in preCGG brain. Finally, the ratio Bax:Bcl-2 is higher by 30% in 6-months-old cortical and hippocampal tissue in preCGG mice compared to *wt*, that might indicate a greater vulnerability to apoptotic activation. We propose that chronic Ca^{2+} dysregulation amplifies Cdk5/ATM signaling pathway activity possibly linking impaired DDR and neurodegeneration leading to FXTAS with early abnormal $[Ca^{2+}]_i$ and synaptic activity.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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NIMH grant 1R36MH108362-01

Title: The actin depolymerizing factor cofilin is critical to spine abnormalities and autism relevant behaviors in a mouse model of fragile x syndrome

Authors: *A. PYRONNEAU, M. PORCH, R. ZUKIN;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disabilities and a leading cause of autism. The neuroanatomical hallmark of Fragile X is an overabundance of immature dendritic spines, a factor thought to underlie impaired cognition. Although aberrant spine morphology and density in Fragile X has been an area of intense interest for nearly two decades, the mechanisms that underlie this defect are as yet unclear. Here we show that the actin depolymerizing factor cofilin, a downstream target of the Rho GTPase Rac1 and major determinant of dendritic spine structure, is dysregulated in Fragile X and is causally related to these spine abnormalities. Cofilin phosphorylation was elevated (indicative of inactivation) in the somatosensory cortex of young *Fmr1* KO mice. Consistent with this, the filamentous to globular (F/G) actin ratio, a functional readout of cofilin inactivation, was elevated. We further show that phosphorylation/activation of Lim kinase-1 and phosphorylation/inactivation of the phosphatase Slingshot were also elevated. This is significant in that Slingshot and LimK are downstream targets of Rac1/PAK1 and upstream regulators of cofilin. Inhibition of PAK1 with a small molecule inhibitor rescues cofilin signaling in Fragile X mice, indicating a causal relation between PAK1 and cofilin signaling. Viral delivery of a constitutively active cofilin mutant (cofilin-S3A) into the somatosensory cortex of *Fmr1* KO mice rescued the immature dendritic spine phenotype and elevated spine density. Moreover, PAK inhibition rescued sensory hypersensitivity and impaired whisker-dependent texture discrimination in Fragile X mice. These findings demonstrate a causal relation between elevated Rac1/cofilin signaling, spine defects and autism relevant behaviors in Fragile X and uncover a

previously unappreciated role for unchecked cofilin activity in the aberrant spine morphology and spine density associated with this devastating human condition.

Disclosures: **A. Pyronneau:** None. **M. Porch:** None. **R. Zukin:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Support: Wellcome Trust & Royal Society Grant 104116/Z/14/Z

MRC Grant MR/M006336/1

Title: Enhancement of NMDA receptor signalling for the treatment of fragile x syndrome

Authors: ***S. BARNES**[1], S. G. N. GRANT[2], N. KOMIYAMA[3], E. K. OSTERWEIL[1];
[2]Ctr. for Clin. Brain Sci., [3]Ctr. for Neuroregeneration, [1]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Fragile X syndrome (FXS) is a leading monogenic cause of intellectual disability and autism spectrum disorder. Over the past decade investigations in the mouse model of FXS have revealed that one of the key pathological deficits in the FXS brain is an increase in cerebral protein synthesis [1]-[3]. Interestingly, pharmacological strategies that reduce protein synthesis, such as inhibitors of $mGlu_5$ or ERK1/2, alleviate many of the additional synaptic and behavioural abnormalities in the *Fmr1* knockout (KO) mouse [2]-[4]. Despite these advances no treatment strategy has successfully completed clinical trails indicating the urgency to identify new therapeutic targets that regulate protein synthesis in the FXS brain.
Using a metabolic labelling assay in hippocampal slices obtained from *Fmr1* KO and WT mice, we have identified the ionotropic NMDA receptor (NMDAR) as a key regulator of protein synthesis. From these studies we have found that inhibition of the NMDAR leads to an increase in hippocampal protein synthesis whilst exacerbating elevated protein synthesis in *Fmr1* KO mice. In contrast, positive modulators of the NMDAR restore protein synthesis to WT levels, which is consistent with previous studies that have demonstrated NMDAR co-agonists restore deficient LTP in the dentate gyrus and the deficits in contextual fear conditioning [5]. Interestingly, experiments using a unique mutant mouse reveal that protein synthesis downstream of the NMDAR is altered when the C-terminal domain (CTD) of the GluN2A subunit is swapped with that of the GluN2B subunit ($GluN2A^{2B(CTD)}$). This finding indicates that the GluN2B

subunit of the NMDAR may act tonically to control protein synthesis by coupling to intracellular signalling pathways via the C-terminus. The intriguing possibility is that modulation of intracellular signalling cascades that couple to GluN2B may be a novel strategy for normalizing protein synthesis in the mouse model of FXS.
[1] Qin et al., "Postadolescent changes in regional cerebral protein synthesis: an in vivo study in the FMR1 null mouse," *J. neurosci*, 2005.
[2] G. Dölen et al., "Correction of Fragile X Syndrome in Mice," *Neuron,* 2007.
[3] Osterweil et al., "Hypersensitivity to mGluR5 and ERK1/2 Leads to Excessive Protein Synthesis in the Hippocampus of a Mouse Model of Fragile X Syndrome," *J. neurosci*, 2010.
[4] Osterweil et al. "Lovastatin corrects excess protein synthesis and prevents epileptogenesis in a mouse model of fragile X syndrome.," *Neuron*, 2013.
[5] Bostrom et al., "Rescue of NMDAR-Dependent Synaptic Plasticity in Fmr1 Knock-Out Mice.," *Cereb. Cortex*, 2015.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: Wellcome Trust Sir Henry Dale Fellowship 104116/Z/14/Z

Tuberous Sclerosis Association 2014-F04

Medical Research Council MRC MR/M006336/1

Title: Cell type specific analysis of mRNA translation in fragile X syndrome

Authors: *S. R. THOMSON[1], S. S. SEO[1], O. DANDO[1], S. A. BARNES[1], P. C. KIND[1], M. F. BEAR[2], E. K. OSTERWEIL[1];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]MIT, Cambridge, MA

Abstract: Fragile X Syndrome (FXS) is a neurodevelopmental disorder with a high incidence of autism, epilepsy, and intellectual disability (ID). Many of the neurological disturbances associated with FXS are believed to be the results of excessive cerebral protein synthesis. This includes the exaggeration of long-term synaptic depression (LTD) downstream of metabotropic glutamate receptor 5 (mGluR5). Previous evidence suggests that reduction of protein synthesis

can alleviate several phenotypes in the *Fmr1*$^{-/y}$ mouse model (*Fmr1*$^{-/y}$), however the mRNAs that are aberrantly translated have not been identified. This information is key for understanding the underlying pathways that contribute to FXS symptoms, and it may be an important strategy for uncovering new targets for therapeutic intervention.
Previous attempts to identify mistranslated proteins in FXS have been limited by both insufficient sensitivity to low yield proteins, and a lack of cell type specificity. To overcome these issues, we have used Translating Ribosome Affinity Purification (TRAP) to isolate translating mRNAs from specific subpopulations of neurons in the FXS mouse model (*Fmr1*$^{-/y}$). We have combined TRAP with RNA sequencing (RNAseq) to produce an unbiased list of differentially translated mRNAs in hippocampal CA1 pyramidal neurons, which express the exaggerated LTD phenotype in the *Fmr1*$^{-/y}$ mouse.
Surprisingly, our analysis revealed an overexpression of mRNAs encoding specific subtypes of muscarinic acetylcholine receptor (mAChR) in *Fmr1*$^{-/y}$ CA1 pyramidal neurons. Further qPCR and immunofluorescence analyses of isolated CA1 neurons confirm these findings. Ongoing investigations in the hippocampus of the *Fmr1*$^{-/y}$ mouse have revealed perturbed cholinergic function, which is consistent with a change in mAChR expression. Together, these results show that cell type specific analysis of mRNA translation reveals novel information about the impact of excessive protein synthesis on neuronal function in the FXS brain.

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Poster

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Topic: A.07. Developmental Disorders

Support: NIH/NICHD HD067225

Title: Altered surface expression of delta subunit-containing $GABA_A$ receptors in a mouse model of Fragile X syndrome

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Abstract: While numerous changes in the GABA system have been identified in models of Fragile X Syndrome (FXS), alterations in subunits of the $GABA_A$ receptor ($GABA_AR$) that mediate tonic inhibition are particularly intriguing. Considering the key role of tonic inhibition in regulating neuronal excitability, reduced tonic inhibition could contribute to FXS-associated disorders such as hyperactivity, altered anxiety and increased seizure susceptibility. Our studies have focused on altered expression and function of the δ subunit of the $GABA_AR$, a major subunit involved in tonic inhibition, in Fragile X mental retardation protein 1 (Fmr1) knockout (KO) mice. We found a small but consistent decrease in immunolabeling of the δ subunit in Fmr1 KO mice compared to wildtype (WT) littermates in the molecular layer of the dentate gyrus. However, electrophysiological studies in dentate granule cells (DGCs) revealed a marked, nearly four-fold, decrease in tonic inhibition in the Fmr1 KO mice, as well as reduced effects of two δ subunit-selective pharmacological agents, THIP and DS2, supporting the suggestion that δ subunit-containing $GABA_ARs$ are compromised in the Fmr1 KO mice. The discrepancy between the large deficits in GABA-mediated tonic inhibition in the DGCs in the Fmr1 KO mice and the modest reductions in immunolabeling of the δ subunit in the dentate gyrus led us to examine the surface expression of the δ subunit. To measure cell surface protein levels, cross-linking experiments followed by Western blot analysis were performed, and these demonstrated a small but non-significant decrease in total δ subunit protein in the Fmr1 KO mouse. However, a six-fold decrease in surface expression of the δ subunit was found in the KO mice. We then used postembedding immunogold labeling to determine the subcellular localization of the δ subunit on dendrites of DGCs, where the δ subunit is normally located at perisynaptic and extrasynaptic sites. All symmetric synapses with clear postsynaptic densities were photographed with an electron microscope. The presence and location of immunogold labeling was determined at each synapse. In WT mice, immunogold particles were localized along the plasma membrane at perisynaptic locations at approximately 79.0% of the synapses. In contrast, only 21.8% of GABAergic synapses exhibited surface labeling at perisynaptic sites in the Fmr1 KO mice. Together these findings suggest that, in the dentate gyrus, altered surface expression, rather than a decrease in δ subunit expression alone, may be limiting tonic inhibition in this model of FXS. Thus finding ways of increasing surface expression of the δ subunit could be a novel new approach to treatment in FXS.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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NIH R25 MH060490

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Title: Insulin signaling misregulation underlies circadian and cognitive deficits in a *Drosophila* Fragile X Model

Authors: *S. M. MCBRIDE[1], R. E. MONYAK[2], D. EMERSON[1], B. SCHOENFELD[1], X. ZHENG[3], D. CHAMBERS[5], C. ROSENFELT[5], P. HINCHEY[6], C. CHOI[7], T. MCDONALD[6], F. BOLDUC[5], A. SEHGAL[4], T. A. JONGENS[2];
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Abstract: Fragile X syndrome (FXS) is an undertreated neurodevelopmental disorder characterized by low IQ and a wide range of other symptoms including disordered sleep and autism. Although FXS is the most prevalent inherited cause of intellectual disability, its mechanistic underpinnings are not well understood. Using Drosophila as a model of FXS, we showed that select expression of dfmr1 in the insulin-producing cells (IPCs) of the brain was sufficient to restore normal circadian behavior and to rescue the memory deficits in the fragile X mutant fly. Examination of the insulin signaling (IS) pathway revealed elevated levels of Drosophila insulin like peptide 2 (Dilp2) in the IPCs and elevated IS in the dfmr1 mutant brain. Consistent with a causal role for elevated IS in dfmr1 mutant phenotypes, the expression of dfmr1 specifically in the IPCs reduced IS, and genetic reduction of the insulin pathway also led to amelioration of circadian and memory defects. Furthermore, we showed that treatment with the FDA-approved drug metformin also rescued memory. Finally, we showed that reduction of IS is required at different time points to rescue circadian behavior and memory. Our results indicate that insulin misregulation underlies the circadian and cognitive phenotypes displayed by the Drosophila fragile X model, and thus reveal a metabolic pathway that can be targeted by new and already approved drugs to treat fragile X patients.

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Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: 2T32MH067564.

Title: Maturation of adult-born dentate granule cells in fragile x mice.

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Abstract: Fragile X syndrome (FXS) is the most common form of inherited mental retardation and the most common known cause of autism. Loss of FMRP in mice (*Fmr1* KO) leads to alterations in synaptic and circuit maturation in the hippocampus, which correlates with alterations in hippocampal dependent behaviors. Previous studies have demonstrated that the rate of proliferation of progenitor cells is increased in the dentate gyrus of *Fmr1* KO mice. Loss of Fmrp specifically in adult progenitors disrupts the acquisition of a hippocampal dependent conditioning task. While these studies have demonstrated a role for Fmrp in adult neurogenesis, it is not known whether the functional synaptic maturation and circuit integration of adult-born dentate granule cells (DGCs) is altered in the *Fmr1* KO. In this study we used retroviral labeling to express a fluorescent protein to birth date newborn DGCs in 6-8 week old *Fmr1* KO mice. This allowed us to use targeted patch clamp recording to measure synaptic inputs to these neurons at precise time points based on the number of days post injection (dpi) we recorded from labeled DGCs. Adult born DGCs are initially innervated by tonic GABA before establishing GABAergic and then glutamatergic synapses. To determine whether the temporal profile of these events is altered we first measured the tonic GABA mediated currents in *Fmr1* KO and littermate controls at 7, 14, and 21 dpi. Contrary to what has been previously reported we found that tonic GABA current was not prominent in either *Fmr1* KO (present in 0/6 cells at 7 dpi, 1/5 at 14 dpi, and 0/6 at 21 dpi) or control mice (only in 0/5 at 7 dpi, 1/8 at 14 dpi, and 2/6 at 21 dpi), whereas we could record a large amplitude tonic current in all mature DGCs we recorded from (19.2 ± 2.2 pA, n = 21 in *Fmr1* KO and 21.4 ± 2.2 pA, n = 24 in controls). We next recorded spontaneous $GABA_A$ mediated synaptic events from labeled DGCs at 21 and 28 dpi. The amplitude of GABA events was 35.6 ± 1.5 pA, n = 10 at 21 dpi and 31.1 ± 2.3 pA, n = 5 at 28 dpi in *Fmr1* KO, and 37.3 ± 5.1 pA, n = 8 at 21 dpi and 33.2 ± 3.6 pA, n = 6 at 28 dpi in control ($p>0.05$ at 21 and 28 dpi). The frequency of GABA events was 0.43 ± 0.06 Hz, n = 10 at 21 dpi and 0.91 ± 0.08 Hz, n = 5 at 28 dpi in *Fmr1* KO, and 0.31 ± 0.07 Hz, n = 8 at 21 dpi and 1.2 ± 0.34 Hz, n = 6 at 28 dpi in control ($p>0.05$ at 21 and 28 dpi). These initial data suggest that the

development of GABAergic synapses is not altered in the first four weeks of maturation. In ongoing experiments we are recording from neurons at 28 and 42 dpi to determine if inhibitory and excitatory synaptic input to more mature DGCs is altered in *Fmr1* KO mice. Together these studies will be the first to systematically determine the effects of loss of FMRP on the maturation of newborn neurons in *Fmr1* KO mice.

Disclosures: C. Remmers: None. **A. Contractor:** None.

Poster

296. Molecular and Cellular Mechanisms in Fragile X Syndrome

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Topic: A.07. Developmental Disorders

Support: FRAXA Research Foundation

NIH NS064967

Title: Modulation of mitochondrial efficiency and its potential application in the treatment of fragile x syndrome.

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Abstract: Fragile X syndrome is caused by loss of function of the gene encoding Fragile X mental retardation protein (FMRP), an RNA-binding protein. That leads to abnormally elevated protein synthesis. It has been reported that loss of FMRP causes abnormally high survival rates in the brain leading to excessive numbers of neurons and insufficient synaptic pruning, associated with a significant elevation in levels of the anti-apoptotic mitochondrial protein Bcl-xL. Moreover, Bcl-xL and FMRP target to mitochondria and depletion of FMRP disrupts mitochondrial membrane potential. We find that the elevated levels of protein translation in FMRP KO mouse neurons can be reduced by treatment with modulators of the ATP synthase, which increases the efficiency of mitochondrial metabolism. Total protein translation can also be affected by manipulating the levels of Bcl-xL. The present study examines the mechanism of normalizing enhanced protein translation in FMRP KO by pharmacological or molecular enhancement of the efficiency of mitochondrial metabolism.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

Title: Disrupted synaptic transmission and protein homeostasis in an Angelman Syndrome (AS) mouse model

Authors: ***G. LI**[1,2], M. ANDERSON[2], M. PIECHOWICZ[2], L. ZHANG[2], X. MA[2], J. WU[3], S. QIU[2];
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Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder characterized by developmental delays, intellectual disabilities, impaired language and speech, and movement defects. Most AS cases are caused by dysfunction of a maternally-expressed E3 ubiquitin ligase (UBE3A, also known as E6 associated protein (E6-AP)) in neurons. Currently, the mechanism on how loss-of-function of the enzyme influences the nervous system development remains unknown. We hypothesize that impaired metabolism of proteins, most likely those related to E6-AP substrates, may alter the developmental trajectory of neuronal structures including dendrites, spines and synaptic proteins, which leads to disrupted activity/experience-dependent synaptic plasticity and maturation. To test this hypothesis, we conducted a detailed investigation on neuronal morphology and electrophysiological properties in prefrontal cortex layer V pyramidal neurons. We found smaller soma size with increased basal dendritic processes in the maternal *Ube3a* deficient mice (m^-/p^+; 'AS' mice) at postnatal 4 weeks. Surprisingly, both excitatory and inhibitory miniature postsynaptic currents (mEPSCs and mIPSCs) on these neurons are decreased. Western blot analysis revealed that autophagy pathway may be disrupted and the function of Golgi apparatus and apoptosis may be enhanced in AS. Currently, we are investigating whether abnormal protein homeostasis accounts for the impaired neuronal morphology and synaptic deficits observed in L5 neurons, and whether restoring normal protein homeostasis may correct the morphological and physiological phenotypes in AS mice.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

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BIH

DAAD

Title: Microcephaly, intellectual disability, mid-hindbrain malformation a novel phenotype

Authors: *E. RAVINDRAN[1], H. HU[2], N. KRAEMER[1], O. NINNEMANN[1], L. MUSANTE[3], E. BOLTSHAUSER[4], D. SCHINDLER[5], A. HÜBNER[6], H.-H. ROPERS[3], C. HUBNER[1], A. KAINDL[1];
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Abstract: During development, the localized expression of genes drives proper segmentation and development of various brain regions and several proteins are known to contribute to localized gene expression. Disturbances in the expression of these proteins are known to contribute to mid-hindbrain malformations. We will present a novel mid-hindbrain malformation phenotype in two affected children of a consanguineous family of Kurdish-Turkish descent and delineate the genetic cause. We identified the mutation in the causative gene through whole exome sequencing. The effect of mutation on mRNA and protein levels were checked through qPCR and Western blot, respectively. The rescue experiments were performed to serve as a proof of principle. With our study, we report for the first time, a novel mid-hindbrain malformation in humans.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: Angelman Syndrome Foundation Grant

Title: Anatomical underpinnings of decreased white matter volume in angelman syndrome model mice

Authors: *M. C. JUDSON, C. L. THAXTON, A. C. BURETTE, A. L. PRIBISKO, A. M. RUMPLE, B. PANIAGUA, R. J. WEINBERG, B. D. PHILPOT;
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Abstract: Angelman syndrome (AS) is a severe neurodevelopmental disorder characterized by profound deficits in cognition, motor function, and speech. Microcephaly is highly comorbid in AS – penetrant in 80-90% of cases – indicating problems with brain growth and development that belie grossly normal brain architecture. Accordingly, structural neuroimaging of children with AS has revealed deficits in white matter volume and integrity for which the underlying anatomy remains unclear. Here we utilize AS model mice to explore relationships between brain growth, white matter volume, and the ultrastructural integrity of white matter tracts. We find that AS model mice exhibit microcephaly with onset during the second postnatal week, mirroring the altered brain growth trajectory of people with AS. This trajectory culminates in reduced brain weight (8-10%) and generalized reductions in white matter volume (12-15%) in adult AS model mice. Electron microscopic analyses of the anterior corpus callosum and sciatic nerve reveal largely normal myelination and little or no evidence of axon degeneration; however, axon caliber is significantly reduced in these tracts in adult AS model mice (~20%), explaining deficits in white matter volume and, perhaps, abnormal nerve conduction. Our findings implicate deficits in axon growth and development in the pathogenesis of AS, and support the possibility that structural neuroimaging could serve as a noninvasive biomarker for gauging the efficacy of AS therapeutics.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

Title: Altered EEG spectral power in the NS-Pten knock-out model of cortical dysplasia with epilepsy

Authors: *S. AVILA[1], A. REGNIER-GOLANOV[3], Y. A. DABAGHIAN[2], L. NGUYEN[4], A. BREWSTER[4], N. SUNNEN[4], A. E. ANDERSON[5];
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Abstract: Cortical dysplasia is a devastating disease often associated with catastrophic epilepsy. NS-*Pten* knock-out (KO) mice model aspects of the human disorder, including abnormal electroencephalography (EEG) recordings with frequent epileptiform activity (spikes, polyspikes, and seizures) and disruption of normal background EEG activity. Previous studies reported suppression of epileptiform activity and subjectively identified improvements in the background EEG in KO mice following rapamycin treatment. Quantitative EEG analysis showed the frequency spectra were different between KO and WT. In this study, to characterize EEG differences between the KO and wild type (WT) mice, we used analysis of prevalent amplitudes and power spectra of the EEG at 4, 8 & 13 wks. Mice were video-EEG monitored for 2 to 4h at 4, 8, and 13 weeks of age (Nicolet v32 Amplifier). 500,000 data points of baseline interictal were sampled at 200Hz. To quantify the differences between the KO and WT animals, we studied changes in the Δ amplitudes (Hilbert transform) and in the frequency spectra (Fast Fourier transform) of the recorded EEG signal. The results were then averaged for KO and WT groups. NS-*Pten* KO have a normal EEG at 3-4 weeks of age and start to develop spikes and seizures along with higher background noise. At 4 weeks the profiles of the amplitude distribution were similar between WT and KO with Δ amplitude KO: 33μV vs WT: 42μV ($p>0.05$, n=4-5). At 8 and 13weeks of age, Δ amplitude shifted toward wider amplitude distribution (8 weeks, Δ amplitude KO: 63μV vs 36μV; 13weeks, Δ amplitude KO: 60μV vs WT 33μV, $p>0.05$, n=3-4). The frequency spectra did not differ by their profiles and their peaks at 4, 8 or 13 weeks between genotypes during baseline recordings. Power curves from KO and WT mice displayed peak power levels in the theta-alpha-beta range (6-18Hz, peak at 12Hz, n=11). At 13 weeks the power spectra were significantly higher in KO mice compared to WT ($p>0.05$). Our results demonstrated an increase in the overall power in all frequencies that could indicate insufficient synaptic pruning. Furthermore, the power spectrum and amplitude analysis provide tools to quantify the instability of the EEG signal typical for NS-*Pten* KO.

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Poster

297. Rare Genetic Developmental Disorders

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Title: Rare genetic variations in MEPE are associated with Otosclerosis and a Craniofacial bone disorder with facial paresis and mixed hearing loss

Authors: *I. SCHRAUWEN[1,2], L. TOMAS-ROCA[3], U. ALTUNOGLU[4], M. WESDORP[3], H. VALGAEREN[2], M. SOMMEN[2], M. RAHMOUNI[3], E. VAN BEUSEKOM[3], M. HUENTELMAN[1], E. OFFECIERS[5], I. DHOOGHE[6], R. ROBERT VINCENT[7], A. HUBER[8], C. GILISSEN[3], E. DE LEENHEER[3], C. CREMERS[3], B. VERBIST[3], A. DE BROUWER[3], G. PADBERG[3], R. PENNINGS[3], H. KAYSERILI[4], H. KREMER[3], G. VAN CAMP[2], H. VAN BOKHOVEN[3];
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Abstract: Hereditary Congenital Facial Palsy (HCFP) is an autosomal dominant disorder caused by uni- or bi-lateral maldevelopment of the VIIth cranial nerve (facial). Normal development of the middle ear bones is crucial for the correct development of the facial nerve canal. In this study, we identified a heterozygous frameshift variant c.1273del (p.Gln425Lysfs*38) in *MEPE* in a HCFP family with mixed hearing loss associated with diploeic thickening and sclerosis of the skull. *MEPE* encodes a matrix extracellular phosphoglycoprotein and plays an inhibitory role in bone mineralization. We next hypothesized that this gene might also be important in otosclerosis bone remodeling disorder and screened 91 individuals with familial otosclerosis. We identified two additional heterozygous frameshift variants in *MEPE*, c.209_212del (p.Lys70Ilefs*26) in two families with otosclerosis, and c.617del (p.Ser206Ilefs*3) in an isolated otosclerosis case. Furthermore, we screened 1398 unrelated cases with otosclerosis and 1447 random controls of white ethnicity. We observed the rare c.209_212del frameshift variant in eight affected individuals with otosclerosis only, but in none of the controls, and two other rare variants (c.184G>T; p.Glu62* and c.229G>A; p.Ala77Thr) were found in cases and not in the controls (p=0.0020). Our results pinpoint MEPE as a key player in temperal bone and ossicle mineralization, essential for facial nerve development and implicated in the pathogenesis of otosclerosis and other craniofacial bone disorders associated with mixed hearing loss.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH/NINDS R21 NS090040

R01 NS080598-S1

Title: A gain-of-function mutation in the human GRIK2 gene causes neurodevelopmental and intellectual deficits

Authors: *Y. F. GUZMAN[1], K. RAMSEY[2], J. R. STOLZ[1], V. NARAYANAN[2], G. T. SWANSON[1];
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Abstract: Kainate receptors are ionotropic glutamate-gated channels that exert a modulatory role on neuronal excitability and synaptic transmission. Loss-of-function mutations in genes coding for different kainate receptor subunits, the *GRIK* genes, have been associated with neurodevelopmental disease and intellectual disabilities in humans. The aim of this project is to study the effects of a mutation in the *GRIK2* gene of a nine-year old patient displaying Angelman-like symptomatology that includes ataxia, passive motor tone, a highly social demeanor, and learning disabilities. Genetic testing showed no alteration in the genes responsible for Angelman, Rett, Prader-Willi, and Fragile-X syndromes, or for spinal muscular atrophy and myotonic dystrophy. Whole-exome sequencing of the proband, however, revealed a heterozygous *de novo* single point mutation in *GRIK2,* the gene coding for the GluK2 subunit. The mutation is analogous to the Lurcher (Lc) mutation described for δ2 receptors and consists of a single nucleotide alteration that changes the encoded alanine to a threonine within a highly conserved motif in the third transmembrane domain of the GluK2 kainate receptor subunit.
As a first approach towards understanding the mechanistic basis for the human pathology, we characterized the biophysical properties of kainate receptors containing the GluK2-(A657T) mutation (referred to as GluK2^{Lc}). Electrophysiological recordings from HEK293T/17 cells expressing GluK2^{Lc} revealed a significant slowing in the rate of desensitization of homomeric glutamate-gated currents (average weighted tau = 55.8 ± 12.0 ms, n=4). Furthermore, heteromeric GluK2^{Lc}/GluK5 receptors exhibited a significant level of constitutive activation that became apparent upon desensitization with 10 mM glutamate application. In accordance with tonic activation of the mutant receptor, the open channel blocker, 1-Naphthylacetyl spermine (NASPM), significantly reduced the whole-cell holding current of cells expressing GluK2^{Lc}/GluK5 receptors. Kainate receptors are likely to be heteromeric *in vivo* and therefore we characterize the GluK2^{Lc} mutation as a gain-of-function alteration in channel properties. These results are the first time a gain-of-function mutation in a GRIK gene has been associated with developmental and learning disabilities. Future work will characterize the effects of this mutation on neuronal development and learning processes with the use of human iPSC-derived neurons and a GluK2^{Lc} mouse model.

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Poster

297. Rare Genetic Developmental Disorders

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Title: Interaction between Gabrb3 haploinsufficiency and prenatal LPS exposure exacerbates placental and fetal brain vulnerability in mice

Authors: *H. MOON[1], P. A. CARPENTIER[1], V. SARAVANAPANDIAN[1], U. HADITSCH[1], J. SU[1], M. L. CHIN[1], K. MUENCH[1], A. R. MOORE[1], A. BORMANN[2], N. NIMA[2], G. SUBRAMANYAM[3], M. RIVERA[1], T. D. PALMER[1];
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disability defined by social interaction deficits and stereotyped behaviors but the underlying pathophysiological mechanisms are poorly understood. ASD heterogeneity may result from dynamic interplay between genetic and environmental risk factors. Maternal immune activation (MIA) caused by prenatal infections, asthma, allergy, or other immunological mechanisms are known environmental risk factors that increase fetal vulnerability. Epidemiological studies also associate MIA with higher incidence of ASD and MIA-induced immune signaling is alone sufficient to alter fetal brain development in mouse models.
The present study highlights gene-environment interactions between ASD-related genetic susceptibility and MIA. The GABA type A receptor ($GABA_AR$) subunit beta 3 (*GABRB3*) in the human chromosomal loci 15q11-13 is implicated in Angelman Syndrome, Prader-Willi Syndrome and ASD. Peripheral $GABA_ARs$ are also involved in modulating immune signaling.

Here we show that *Gabrb3* haploinsufficiency combined with prenatal lipopolysaccharide (LPS)-mediated MIA accentuates placental vulnerability and exacerbates neurodevelopmental abnormalities in developing fetuses. *Gabrb3* heterozygous pregnant dams injected with LPS at embryonic day 12.5 showed elevated maternal cytokines/chemokines relative to wild-type controls. Increased placental necrosis and impaired proliferation of neocortical progenitor cells are found in LPS-treated *Gabrb3* heterozygous placentas and fetal brains, respectively. Alterations in neuronal subtype specification and abundance were also notable in the developing fetal cortex. Importantly, $GABA_AR$-selective agonists and antagonists were shown to modulate cytokine production in cultured macrophages. Gaboxadol, a $GABA_AR$-selective agonist, administered in combination with LPS significantly attenuated LPS-induced cytokine elevation, suggesting a possible convergence of immunomodulatory signaling pathways between $GABA_AR$/GABRB3 and LPS-induced MIA responses in the placenta and developing fetus. Together, our data provide evidence that *Gabrb3* haploinsufficiency and MIA may synergistically increase placental and fetal brain vulnerability by altering the immune microenvironment at the maternal-fetal interface. We anticipate that additional genetic risk factors may also elevate placental and/or fetal brain vulnerability to MIA and these gene-immune interactions may significantly contribute to the spectrum of phenotypes noted in ASD, schizophrenia, and other neurodevelopmental disorders.

Disclosures: **H. Moon:** None. **P.A. Carpentier:** None. **V. Saravanapandian:** None. **U. Haditsch:** None. **J. Su:** None. **M.L. Chin:** None. **K. Muench:** None. **A.R. Moore:** None. **A. Bormann:** None. **N. Nima:** None. **G. Subramanyam:** None. **M. Rivera:** None. **T.D. Palmer:** None.

Poster

297. Rare Genetic Developmental Disorders

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NHMRC Senior Research Fellowship GNT1032364

Title: Mutations in DCC cause agenesis of the corpus callosum and mirror movements in humans

Authors: *T. J. EDWARDS[1,2], A. P. L. MARSH[3,4], C. GALEA[6], K. POPE[3], A. PAOLINO[1], I. GOBIUS[1], J. BUNT[1], G. MCGILLIVRAY[7], R. J. LEVENTER[4], S. MANDELSTAM[8,4,5], E. H. SHERR[9], P. J. LOCKHART[3], L. J. RICHARDS[1];
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Abstract: The axon guidance receptor Deleted in colorectal cancer (DCC) and its ligand Netrin-1 (NTN1) are necessary for the formation of white matter tracts throughout the neuraxis in a number of model CNS systems (Lai Wing Sun et al., 2011). Mice homozygous for *Dcc* mutations display complete agenesis of the corpus callosum (ACC), as well as defects in the corticospinal tract (CST) and other cerebral commissures (Fazeli et al., 1997; Fothergill et al., 2014). In humans, DCC haploinsufficiency is associated with mirror movements (MM, OMIM#157600), but has not been associated with ACC (OMIM#217990, Srour et al., 2010). The corpus callosum is the largest forebrain commissure in humans, and facilitates the transfer of information between hemispheres. ACC is one of the most common brain malformations (incidence of 1/4,000 newborns); however, the genetics underlying cases of ACC are incompletely understood (Edwards et al., 2014). We utilized whole exome sequencing and a custom in-house gene panel to identify mutations in *DCC* in two families with varying degrees of MM and ACC. These mutations affect highly conserved residues located within the DCC/NTN1 binding interface, and both mutations were predicted to disrupt receptor/ligand interactions. Individuals from both families also displayed reduced crossing of the CST at the pyramidal decussation. We show for the first time in humans that *DCC* mutations result in ACC and MM associated with aberrant CST projections. These results demonstrate that DCC plays a highly conserved role throughout evolution in the formation of contralateral projections within the brain and CST.

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Poster

297. Rare Genetic Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NRF-2012R1A3A1050385

Title: Everolimus restores mTOR signaling disrupted by a novel TSC2 mutation and improves neuropsychiatric symptoms in a tuberous sclerosis patient

Authors: *J.-E. YANG[1], S.-K. HWANG[2], K. LEE[3], J.-H. LEE[4], J.-A. LEE[5], Y.-S. LEE[6], B.-K. KAANG[1], C.-S. LIM[1];
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Abstract: Tuberous sclerosis complex (TSC) is a rare genetic disease with multiple symptoms including neuropsychological deficits such as seizures, intellectual disability, and autism. TSC is caused by mutations in either the *TSC1* or *TSC2* genes, which regulate the mammalian target of rapamycin (mTOR) signaling pathway. Recent studies have suggested that mTOR inhibitors improve TSC-related deficits in rodent models of TSC. In addition, clinical trials are ongoing to test the efficacy of mTOR inhibitors against the psychiatric symptoms of TSC. In this study, we report a case study of a Korean TSC patient carrying novel *TSC2* mutation. We performed whole exome sequencing and identified a novel small deletion mutation in *TSC2.* The novel deletion mutant abnormally activated the mTOR signaling and everolimus, the mTOR inhibitor, rescued the mTOR signaling in HEK293T cells. Furthermore, everolimus treatment showed not only reduction in SEGA size, but also dramatically improvement of behavioral deficits including autism-related behaviors in the patient. In summary, we identified a novel small deletion mutation in *TSC2* associated with severe TSC in a Korean family that enhances the activation of mTOR signaling *in vitro*. Everolimus treatment not only restored mTOR signaling *in vitro*, but also improved behavioral deficits in the patient.

Disclosures: J. Yang: None. **S. Hwang:** None. **K. Lee:** None. **J. Lee:** None. **J. Lee:** None. **Y. Lee:** None. **B. Kaang:** None. **C. Lim:** None.

Poster

297. Rare Genetic Developmental Disorders

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Program#/Poster#: 297.10/D12

Topic: A.07. Developmental Disorders

Title: Neural correlates of impaired visuospatial cognition in children with Williams syndrome

Authors: T. NASH[1], J. CARRASCO[1], J. P. MIKHAIEL[1], O. RAVINDRANATH[1], L. YANKOWITZ[1], R. PRABAKARAN[1], M. SOTTILE[1], K. ROE[1], P. KOHN[1], J. S. KIPPENHAN[1], D. EISENBERG[1], M. D. GREGORY[1], C. B. MERVIS[2], *K. F. BERMAN[1]; [1]Section on Integrative Neuroimaging, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Dept. Psych and Brain Sci., Univ. of Louisville, Louisville, KY

Abstract: Williams syndrome (WS) is a rare neurodevelopmental disorder caused by microdeletion of ~25 genes on chromosome 7. Previous studies in adults with WS have identified alterations in frontolimbic circuitry and in the dorsal visual processing stream as neural correlates of the characteristic hypersociability and visuospatial construction problems. Here, in children with WS and normal-range IQs we tested white matter integrity with diffusion tensor imaging (DTI), neural function using an fMRI task that taps into dorsal and ventral processing streams (DS and VS), and the relationship of white matter integrity to visuospatial construction ability assessed by the Differential Ability Scales (DAS-II).

DTI data were acquired in 23 children with WS (mean age=11, range 6-18; 9 males) and 21 typically developing children (TDC; mean age=12, range 6-18; 9 males) and data were processed with TORTOISE. Each child's native space fractional anisotropy (FA) map was warped into study-specific template space and smoothed at 4 mm FWHM. Between-group analyses controlled for age and sex and were thresholded at $p<0.01$, FDR corrected. Average FA values for each child were extracted from DS regions that significantly differed between groups, and were examined in relation to the DAS-II Spatial cluster standard score (SCss).3T fMRI data were acquired in a subset of 14 children with WS (mean age=12, range 8-18; 4 males) and 17 TDC (mean age=14, range 8-18; 7 males). During fMRI, children judged either the location (DS) or the content (VS) of images on the screen. Both of these conditions were contrasted against a sensorimotor control condition. Analyses of each of these contrasts controlled for age and sex and were thresholded at $p<0.001$ uncorrected.

Children with WS had increased FA in the right orbitofrontal cortex but decreased FA in the corticospinal tract and in white matter adjacent to the intraparietal sulcus (IPS) bilaterally. Increased left IPS FA predicted higher SCss in TDC ($p < 0.05$). During the DS task, children with WS had significantly decreased activation in the IPS bilaterally as well as in the right middle temporal gyrus. During the VS task, children with WS had less deactivation of default mode network regions.

In children with WS, we found altered microstructural integrity in portions of white matter tracts that are related to the hypersocial personality and visuospatial problems in the syndrome. Structural integrity in white matter adjacent to the IPS predicted visuospatial abilities, indicating that the reduction in FA here in WS may play a role in the visuospatial cognition impairment. Future research will elucidate the developmental trajectory of these alterations.

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Poster

298. Limbic System Development

Location: Halls B-H

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Program#/Poster#: 298.01/D13

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Prenatal paracetamol exposure decreases anxiety-related behaviors and disrupts memory in mice

Authors: ***T. M. MILEWSKI**[1], R. A. ANTONAWICH[1], D. WOOD[1], P. T. ORR[1,2];
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Abstract: The over the counter drug paracetamol has long been in use, but little work has investigated the full range of cognitive and behavioral effects of this drug. In this study, mice were exposed to paracetamol prenatally, weaned and reared normally, and were tested as adults for anxiety-related behavior in an open field. These mice were also tested in an object recognition task. Mice were placed in an open field arena and allowed to explore freely for five minutes. Anxious behavior in the open field was assessed by quantifying both time spent in the center and time spent near the wall (thigmotaxis) of the arena. Mice exposed to paracetamol spent significantly less time near the wall ($t(49.692) = 2.297$, $p = .026$). There was a non-significant trend toward mice exposed to paracetamol spending more time in the center of the open field ($t(57) = 1.866$, $p = .067$). Overall, prenatal chronic exposure to paracetamol generated mice who were less anxious. This conclusion is supported by an analysis of grid crossings during the habituation phase of object recognition. Mice exposed to paracetamol made significantly more crossings into the inner grid squares ($t(56) = 2.812$, $p = .007$) and inner crossings were a greater proportion of total crossings for these mice ($t(56) = 2.767$, $p = .008$). During memory testing, neither group showed a significant side preference during training. During testing, control mice spent significantly more time with the novel object ($t(27) = 2.403$, $p = .023$),

indicating memory, whereas mice exposed to paracetamol did not show a preference for the novel object ($t(23) = .911$, $p = .372$), indicating that these mice did not remember the familiar object. Overall, prenatal paracetamol exposure results in adult mice which are less anxious and have disrupted memory.

Disclosures: T.M. Milewski: None. **R.A. Antonawich:** None. **D. Wood:** None. **P.T. Orr:** None.

Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: R01 MH091351

R01 HD060628

R01 MH105538

Title: Prospective associations between maternal interleukin-6 concentrations during pregnancy and newborn amygdala volume and connectivity.

Authors: *C. BUSS[1,2], A. M. GRAHAM[3], J. RASMUSSEN[2], M. D. RUDOLPH[3], C. H. HEIM[1,4], J. H. GILMORE[5], M. A. STYNER[5], S. ENTRINGER[1,2], P. D. WADHWA[2], D. A. FAIR[3];
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Abstract: Exposures to maternal infection and excess stress during pregnancy represent intrauterine conditions that have been associated with an increased susceptibility for schizophrenia and autism. While inflammatory cytokines are requisite for typical fetal brain development, animal models of maternal immune activation have demonstrated negative neurodevelopmental consequences of excess interleukin-6 (IL-6). Here, we test, in humans, the hypothesis that *in utero* exposure to elevated maternal IL-6 concentration predicts alterations in newborn limbic circuitry with a specific focus on amygdala anatomy and connectivity. Participants were recruited in early pregnancy and maternal blood samples were collected in each trimester. Newborns (N=85 underwent anatomical, diffusion tensor imaging (DTI) and functional connectivity (rs-fcMRI) magnetic resonance imaging (MRI) during natural sleep.

Newborn amygdala were segmented using a semiautomatic method to quantify amygdala volumes, and served as seed regions for rs-fcMRI analyses. Structural connectivity was characterized by fractional anisotropy (FA) along fronto-limbic white matter tracts (uncinate fasciculus and fornix). Prospective associations between average maternal IL-6 concentrations throughout pregnancy and newborn amygdala volume and functional connectivity, and fronto-limbic FA, were examined.
Higher maternal IL-6 concentrations were associated with larger right amygdala volume ($p=.002$), increased amygdala functional connectivity to right anterior insula and bilateral caudate (p-corrected<.05), and reduced FA in bilateral uncinate and left fornix (p-corrected<.05). These results provide converging evidence across multiple modalities for alterations in limbic-circuitry in the context of elevated *in utero* inflammation. The pattern of results suggests that inflammation may lead to acceleration in development of limbic circuitry involved in emotional reactivity and delays in development of circuitry involved in emotion regulation. The circuitry identified is highly relevant for psychopathology across the lifespan, and these results may therefore facilitate increased understanding of the connections between prenatal immune stress and offspring risk for mental health disorders.

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Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant MH083973-07

Title: Prevention of an infection-induced maternal adenosine surge during gestation prevents the development of schizophrenia symptoms in mice.

Authors: *D. M. OSBORNE[1], U. SANDAU[2], A. JONES[2], N. ETESAMI[2], M. YAHYA[2], D. BOISON[2];
[1]Legacy Hlth. Res., Portland, OR; [2]Legacy Res. Inst., Portland, OR

Abstract: Schizophrenia (SZ) is one of the most common psychiatric conditions affecting around 1% of the population, yet fully effective treatments are lacking, and pathogenic mechanisms are poorly characterized. There is now broad consensus that SZ is a

neurodevelopmental condition, with specific risk factors associated with specific time windows of gestation. In rodents the development of SZ can be studied by inducing a 'maternal infection' during gestation via the viral mimetic polyinosinic-polycytidylic acid (polyI:C). Because physiological stressors (such as infection) trigger a surge in adenosine, because adenosine is a regulator of immune and epigenetic functions, and because the fetus is protected through a placental adenosine barrier, we hypothesized that an infection-induced maternal adenosine surge initiates a cascade of events leading to the development of SZ symptoms in offspring upon reaching adulthood. If this hypothesis is true then prevention of the maternal adenosine surge should prevent the development of SZ in offspring. To test our hypothesis we administered polyI:C (2mg/kg) to pregnant mouse dams on gestational day 9 with or without co-administration of the adenosine degrading enzyme adenosine deaminase (ADA). Within an hour of polyI:C injection there was a decrease in placental adenosine kinase, while the developing offspring that did not receive ADA had a significant and persistent decrease in brain DNA methylation (5mc) consistent with adenosine's role as an inhibitor of DNA methylation. In adult male control mice from litters that received polyI:C treatment during gestation we found significant deficits in sensory motor gating, measured by prepulse inhibition (PPI). Importantly, we found that co-administration of ADA together with poly(I:C) reversed both the poly(I:C) induced epigenetic changes and the development of sensory motor gating deficits in adult offspring. Our data strongly support the interpretation that excessive adenosine exposure during gestation results in hippocampal-dependent SZ-like behaviors in adult offspring and suggest that adenosine-induced epigenetic changes play a major role in the pathogenesis of SZ.

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Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant MH105759

NARSAD

Title: Roles of thalamocortical interactions in mouse prefrontal cortex development

Authors: ***Y. NAKAGAWA**[1], A. PROUE, 55455[2], T. NICHOLS-MEADE[2], M. BENNYWORTH[1];

[1]Dept Neurosci, Univ. of Minnesota Dept. of Neurosci., Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The thalamus and the neocortex establish reciprocal connections during early development. Recent studies discovered that interactions between these two brain regions are crucial for the development of the sensory areas of the neocortex. However, it is unknown whether the thalamus plays a similar role in the development of other neocortical areas. The prefrontal cortex (PFC) is a center for higher cognitive functions and emotions, and defects of its development are implicated in many developmental brain disorders including schizophrenia and autism. In this study, we sought to determine if thalamocortical interactions are required for proper PFC development. In previous studies, we showed that the mice that lack the homeobox gene *Gbx2* in a thalamus-specific manner have defects in thalamocortical projections and that characteristic expression patterns of genes in primary sensory areas are altered at early postnatal stages so that these areas no longer exhibit clear boundaries with the surrounding higher-order sensory areas. Interestingly, however, expression of these molecular markers appeared unchanged in the PFC in thalamus-specific *Gbx2* mutant mice. This result prompted us to identify several genes that are preferentially expressed in the developing PFC. We found that one of these genes, *Cyp26b1*, which encodes an enzyme that degrades retinoic acid, was not properly induced in layer 6 of the medial and ventral PFC at early postnatal stages in *Gbx2* mutant mice. This result suggests that the thalamus may control retinoid signaling in the PFC by regulating the expression of *Cyp26b1*. In order to determine the roles of Cyp26b1 in PFC development and adult behavior, we further generated *Cyp26b1* knockout mice that are restricted to the frontal cortex including medial PFC. Preliminary analyses indicate that these mice show signs of impaired working memory, suggesting the role of the thalamus in regulating the sensitive developmental period of early postnatal PFC that is critical for normal behavior in the adult.

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Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Prenatal paracetamol exposure disrupts motor behavior in mice

Authors: *D. BIGLEY, JR[1], T. M. MILEWSKI[1], P. T. ORR[1,2];
[1]Neurosci., [2]Psychology, Univ. of Scranton, Scranton, PA

Abstract: Paracetamol is a common over-the-counter pain reliever, but the full extent of its effects on cognition and behavior is not known. Two previous correlational studies in humans suggest that paracetamol exposure can result in poorer gross motor development and increased risk of hyperkinetic disorders. Paracetamol is metabolized, through the enzyme FAAH, into AM404 which acts as a cannabinoid reuptake inhibitor, suggesting that motor effects of paracetamol may be mediated by cannabinoids. In this study, we experimentally manipulated prenatal paracetamol exposure and explored for an altered motor phenotype in the resultant pups. Paracetamol was added to the water of two sets of breeding trios, and two sets of breeding trios were kept as controls and given regular water. Regular drinking water was restored immediately after birth for all mice, and pups were weaned and raised to eight weeks in typical fashion. At eight weeks, the adult offspring were run on an accelerated and fixed-speed rotarod protocols and allowed to freely explore a large, empty arena for 5 minutes. Mice prenatally exposed to paracetamol spent significantly less time on the rod during the first ($t(58) = 2.007$, $p = .049$), ninth ($t(54.091) = 2.61$, $p = .012$) and tenth ($t(58) = 2.32$, $p = .024$) trials of the accelerated rotarod, and there was a non-significant trend toward these mice spending less time on the rod during the first fixed-speed trial at 6.5 rpm ($t(36.189) = 1.797$, $p = .081$). During free exploration, mice prenatally exposed to paracetamol made more grid crossings than control mice ($t(56) = 2.063$, $p = .044$), indicating hyperactivity. Overall, prenatal paracetamol exposure results in adult mice which are hyperactive and less coordinated than control mice.

Disclosures: **D. Bigley:** None. **T.M. Milewski:** None. **P.T. Orr:** None.

Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Conacyt CB-2010-01-0154645

Title: Ontogeny of Glutamatergic, GABAergic and Dopaminergic neurons of the embryonic mesencephalic nuclei A9 and A10

Authors: *D. A. RAMÍREZ DE LEÓN;
Lab. Biofísica Mol., Univ. Autónoma De San Luis Potosí, san luis potosi, Mexico

Abstract: The mesencephalic dopaminergic nuclei substantia nigra (A9) and Ventral Tegmental Area (A10) contain purely Glutamatergic neurons as well as Glutamatergic neurons that corelease Dopamine. The ability to release Glutamate by these neurons is given by the

expression of the vesicular Glutamate Transporter type 2 (VGluT2). The corelease of Glutamate by Dopaminergic neurons is implicated in the psychostimulant effects of cocaine and amphetamine, and plays an important pro-survival role during development. The earliest expression of VGluT2 in Dopaminergic neurons is reported until E14. However the ontogeny of purely Glutamatergic neurons is unknown. Considering that in the adulthood, the proportion of Glutamatergic neurons is about 14%, we searched for the moment in development in which the expression of VGluT2 appears in the mesencephalom of mouse embryos. Using single cell RT-PCR multiplex assays to detect the expression of tyrosine hydroxylase (TH), Dopamine Transporter (DAT), Glutamic Acid decarboxylase I (GAD), vesicular monoamine transporter type 2 (VMAT2), DOPA decarboxylase (DDC) and VGluT2, we found that 1) at E11.5, the embryonic stage in which Dopaminergic neurons appear in the mesencephalom, the expression of VGluT2 is already present; 2) at E11.0, Although TH was not yet expressed, the expression of VGluT2 was readily detected; 3) the proportion of purely Glutamatergic neurons (i.e. VGluT2 positive but negative to any marker for GABAergic or Dopaminergic neurons) decreased with the progression of embryonic development, whereas 4) the coexpression of VGluT2 with any marker for GABAergic or Dopaminergic neurons increased; 5) the Glutamatergic and GABAergic cophenotype appeared as early as E11.0; 6) Purely Glutamatergic neurons appeared at E11.0; 7) The Dopaminergic and GABAergic cophenotype appears as early as E11.5. Our results show a great heterogeny in the neuronal phenotypes expressed by embryonic mesencephalic neurons of the A9/A10 nuclei that appears as early as the first stages of midbrain development.

Disclosures: D.A. Ramírez De León: None.

Poster

298. Limbic System Development

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: R37-HD059288

R01-NS069629

Title: Organization of hippocampal mossy fiber pathway in the mouse

Authors: *G. XIONG[1,2], H. METHENY[2], K. FOLWEILER[2], A. S. COHEN[2,3];
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Philadelphia, PA; [3]Dept. of Anesthesiol. and Critical Care Med., Perelman Sch. of Medicine, Univ. of Pennslyvania, Philadelphia, PA

Abstract: Physiological recording along hippocampal mossy fiber pathway can be difficult. This may be partially due to the extremely complicated laminar organization of the microcircuitry. Here we injected biotinylated dextran amine into the dentate granule cell layer to initially anterograde label mossy fibers and their terminals; and subsequently infect the entorhinal cortex with GFP-tagged pseudorabies virus to transynaptically label area CA3 pyramidal neurons and their dendritic trees. Seven days after injection , we examined labeling in stratum lucidum of hippocampal slices cut at different slicing angles. We found that mossy fibers and their targeted pyramidal cell dendrites were well preserved in adjusted horizontal slices (Hippocampal-Entorhinal Cortex, HEC) but severely truncated in frontal (coronal) slices. In transverse hippocampal slices, the preservation of mossy fibers and pyramidal cell dendrites varied among cases, perhaps because of the slight difference in setting the hippocampi on stage for slicing. After immunofluorescent staining with an antibody against VGLUT1 in frontal slices, rosette arrangement of the mossy fibers could be observed encircling transversely truncated pyramidal cell dendrites. Confocal scanning laser microscopy demonstrated that each rosette consisted of 6 to 7 VGLUT1-positive puncta. Out of a mossy fiber rosette, one puncta might be labeled by biotinylated dextran. In HEC slices VGLUT1 positive puncta aggregated in patches along pyramidal cell dendrites at the proximal level, apposing thorny excrescences. The present study strongly suggests that HEC slices preserve that anatomic circuit and thus are the most suitable for physiological studies on focused on the hippocampal mossy fiber system.

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Poster

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Exosome-delivered miRNA-146a ameliorates peripheral neuropathy in diabetic mice

Authors: *B. FAN[1], X. LIU[1], M. CHOPP[1,2], A. SZALAD[1], L. WANG[1], W. PAN[1,3], Z. ZHANG[1];
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Abstract: Background: MicroRNA-146a (miR-146a) has been implicated in the pathogenesis of diabetic peripheral neuropathy (DPN). However, the therapeutic role of miR-146a in DPN has not been investigated. Exosomes are cell-derived vesicles and play an important role in mediating intercellular communication. The present study investigated whether tailored exosomes carrying elevated miR-146a improve neurological outcomes of DPN. Methods and Results: Tailored exosomes carrying elevated miR-146a were isolated from medium of cultured miR-146a knock-in mesenchymal stromal cells (MSCs) by ultracentrifugation. Diabetic db/db mice treated with naïve exosomes or db/db mice alone (n=8/group) were employed as controls. We administered exosomes (10^{10}) via a tail vein once per week for 4 weeks (n=8). Administration of tailored miR-146a-exosomes elevated levels of miR-146a in plasma (3.97±0.69 vs 0.97±0.11) and sciatic nerve (4.7±0.52 vs 1.01±0.08). Tailored miR-146a-exosome treatment significantly increased blood flow in the footpad (60.19±2.11 vs 51.45±4.16) and sciatic nerve (27.1±2.34 vs 18.29±1.43) compared to the db/db mice treated with naïve exosomes. Electrophysiological recordings demonstrated that tailored miR-146a-exosomes markedly increased motor nerve conduction velocity (MNCV) by 10%, and significantly decreased thermal stimuli threshold assayed by radial heat plate tests (12.2±1.9 vs 19.2±1.3sec, $p<0.05$). Moreover, tailored miR-146a-exosomes significantly ($p<0.05$) enhanced the number of intraepidermal nerve fibers (16.5±0.7 vs 12.9±0.8 fibers/mm) and g ratio (0.58±0.03 vs 0.61±0.03). Western blot analysis revealed that tailored miR-146a-exosomes substantially ($p<0.05$) suppressed proteins of diabetic proinflammatory target genes including IRAK1 (0.34±0.05 vs 1.01±0.19) and TRAF6 (0.33±0.03 vs 1.02±0.14) and also decreased serum cytokine levels of TNFα (1.7±0.5 vs 4.8 ± 0.9pg/ml) and IL-1β cytokines (59.5±11.3 vs 117.2±25.7pg/ml) measured by ELISA. Conclusions: Together, our data suggest that tailored miR-146a-exosomes have a potent therapeutic effect on DPN via suppressing diabetic-induced proinflammatory genes.

Disclosures: **B. Fan:** None. **X. Liu:** None. **M. Chopp:** None. **A. Szalad:** None. **L. Wang:** None. **W. Pan:** None. **Z. Zhang:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

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NSF HRD 0450339

Title: Myelination of prefrontal axons is accompanied by increased speed and integrity of cortical neurotransmission in rats

Authors: *A. SILVA-GOTAY[1], S. MCDOUGALL[2,5], W. M. VARGAS[3], G.-L. LI[4], H. N. RICHARDSON[2];
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Abstract: The anterior cingulate is a subregion of the prefrontal cortex involved in emotional and cognitive processing. Cells in the anterior cingulate project to—and receive fiber projections from—other cortical and subcortical structures via the anterior branches of the corpus callosum, or forceps minor. Enhancement of cognitive ability during adolescent development corresponds with increases in frontal white matter—the axonal fiber tracts comprised primarily of myelinated axons. Myelin is a lipid-rich coating wrapped around the axons of neurons and myelination of these axons during adolescence may serve to increase the speed of communication between cells. Alternatively, the developmental increases in white matter may correspond instead with other functions such as the integrity of firing, e.g., the probability that action potentials successfully propagate along the axon to the terminal. We tested whether a developmental increase in white matter corresponds with increased speed or with enhanced integrity of neural transmission. We examined the neurophysiological properties and microstructural changes in myelinated axons in the mPFC in developing rats. There was a significant increase in the number of myelinated axons in the mPFC from postnatal days (PD) 15-43. Between PD15-22, conduction velocity increased as well. The relationship between response latency and transmission distance depended on the level of stimulation used, suggesting that different levels of stimulation preferentially activate separate fiber populations. High stimulation intensity produced a positive relationship between latency and distance in all age groups. Conversely, stimulation at threshold intensity produced no correlation between latency and distance, but did produce a positive relationship between conduction *velocity* and transmission distance. This indicates that for this population of fibers, increased conduction velocity in longer fibers may serve as a mechanism to keep response latency constant over different distances so signals may be received at the same time. Disruption of myelination of these axons in developing animals could impair the speed, integrity, and synchronization of neural signals, which could have long-term effects on cognitive processing in adulthood.

Disclosures: A. Silva-Gotay: None. **S. McDougall:** None. **W.M. Vargas:** None. **G. Li:** None. **H.N. Richardson:** None.

Poster

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BBRF Young Investigator Award (A.M.)

Title: Longitudinal assessment of neuronal 3D genomes in mouse prefrontal cortex

Authors: *A. C. MITCHELL[1], B. JAVIDFAR[1], L. K. BICKS[1], R. NEVE[2], K. GARBETT, PhD[3], S. S. LANDER[4], K. MIRNICS[3], H. MORISHITA[1], M. WOOD[5], Y. JIANG[1], I. GAISLER-SOLOMON[4], S. AKBARIAN[1];
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Abstract: Neuronal epigenomes, including chromosomal loopings which bypass the linear genome to move distal cis-regulatory elements into spatial proximity of target genes, could serve as 'molecular bridges' linking present-day-behavior to distant exposures of the past. However, longitudinal modeling is challenging because conventional chromosome conformation capture assays essentially provide single snapshots, reflecting genome organization at the time of brain harvest and therefore are non-informative about the past. Here, we introduce 'NeuroDam' to assess past epigenome status in longitudinal context. Short-term expression of the bacterial DNA adenine methyltransferase Dam, tethered to the *Gad1* gene promoter in mouse prefrontal cortex neurons, resulted in long-term tagging of *Gad1*-bound chromosomal contacts bearing the artificial $G^{methyl}ATC$ mark. We show by NeuroDam that mice with persistent deficits in cognition and alterations in anxiety-related behaviors, 4 months after pharmacological blockade of NMDA receptor signaling, already were affected by disrupted chromosomal conformations emerging shortly after drug exposure. NeuroDam can be easily modified to retrospectively chart transcription factor occupancies and many other epigenomic determinants that until now remain unexplored in longitudinal context modeling normal and diseased human brain development and aging.

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Poster

299. Adolescent Development: Animal Models I

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Support: CIHR

STIHR

FRSQ

NSERC

Title: Maternal care modulates the febrile response to lipopolysaccharide through differences in glucocorticoid receptor sensitivity in the rat

Authors: *T. ZHANG[1,2], H.-B. NGUYEN[2], X. WEN[2], J. DIORIO[2], M. J. MEANEY[2,3], C. PARENT[2];
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Abstract: Early adversity is associated with an increased risk for infection in adulthood. The febrile response is a potent mechanism to combat infection. We found that variations in maternal care influence the febrile response to 50 μg/kg lipopolysaccharide (LPS) challenge in adult male rats while not affecting behavioural sickness responses. Offspring from low-licking/grooming (LG) mothers had an increased febrile response compared to offspring from high-LG mothers challenged with LPS. Low-LG offspring had reduced plasma IL-6 two hours post challenge compared to high-LG offspring. Binding of the transcription factor NFκB to the IL-6 promoter region in the anterior hypothalamus was greater in low-LG offspring treated with LPS than in high-LG offspring. IL-6 gene expression in the anterior hypothalamus was induced following LPS challenge in low-LG offspring but not in high-LG offspring at two hours post challenge. These findings suggest greater activation of thermoregulatory neurons in the anterior hypothalamus of low-LG compared to high-LG offspring following LPS challenge. No differences were found in plasma corticosterone levels following LPS challenge between high and low-LG offspring. Low-LG offspring had enhanced glucocorticoid receptors (GR) in the spleen compared to high-LG offspring which could indicate enhanced sensitivity to the effects of glucocorticoids. Challenge with RU-486 prior to LPS challenge eliminated differences in the febrile response between offspring of high and low-LG mothers. Individual differences in GR

sensitivity may modulate differences in the febrile response to LPS challenge, exerting a long-term influence on the capacity to recover from infection.

Disclosures: **T. Zhang:** None. **H. Nguyen:** None. **X. Wen:** None. **J. Diorio:** None. **M.J. Meaney:** None. **C. Parent:** None.

Poster

299. Adolescent Development: Animal Models I

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P01DA008227

Title: Adolescent social stress results in sex-specific transcriptional reprogramming throughout the reward circuitry in adult mice

Authors: ***D. M. WALKER**[1], I. PURUSHOTHAMAN[1], M. E. CAHILL[1], C. K. LARDNER[1], S. MACHLOVI[1], E. S. CALIPARI[1], H. M. CATES[1], R. C. BAGOT[1], C. J. PEÑA[1], G. E. HODES[1], M. A. DOYLE[1], E. RIBEIRO[1], S. J. RUSSO[1], P. J. KENNEDY[2], E. J. NESTLER[1]; [1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Psychology, The Univ. of California Los Angeles, Los Angeles, CA

Abstract: Adolescent social isolation (SI) alters neuronal morphology, physiology, and gene expression throughout the reward circuitry and increases preference for drugs of abuse in male rodents. However, few studies have investigated if there are sex differences in responses to adolescent SI as well as the molecular mechanisms underlying such long-term changes in reward-associated behaviors. Our preliminary data suggest that SI reverses or reduces sexually dimorphic reward- and anxiety-related behaviors. Therefore, we tested the hypothesis that SI results in persistent transcriptional changes that underlie such sex differences. Mice were socially isolated or group housed (GH) from postnatal day (P) 22 - P42, then GH until adulthood (~P90). Micropunches from 4 reward-associated brain regions (meAMY, VTA, NAc, and PFC) were collected and transcriptome-wide changes investigated by RNA-sequencing after acute or chronic cocaine or saline (7.5mg/kg) ($n = 5 – 8$/group). Those genes displaying a sex X stress interaction were significantly enriched in sexually dimorphic genes in all 4 brain regions ($p < 0.001$), an effect not observed in genes altered by SI alone, suggesting that sexually dimorphic

genes are preferentially altered by adolescent stress. Additionally, hierarchical clustering revealed that SI reversed baseline sex differences in gene expression in 3 of 4 brain regions, similar to the differences observed in behavior. Specifically, GH males cluster with SI females and vice versa for those genes displaying a sex X stress interaction in the meAMY (869 genes) and PFC (495 genes) only. In the VTA (276 genes), male SI clustered with female GH but male GH and female SI clustered separately suggesting that SI alters sex differences in the male but not female VTA. Interestingly, in the NAc (1444 genes) males and females clustered together, independent of adolescent stress. Finally, significant overlap of sexually dimorphic genes across all 4 brain regions was observed. To determine if sex differences in the response to cocaine were also reversed by SI, hierarchical clustering was used to analyze how those genes displaying a sex X stress interaction responded to the drug. Analysis revealed that sex differences in response to chronic but not acute cocaine are reversed by SI in the VTA. In the meAMY, SI females display a male-typical response to chronic but not acute cocaine. These effects were not observed in the PFC or NAc. These data suggest that adolescence is a sensitive period for development of sex differences in the reward circuitry and that adolescent stress causes sustained reversal of sexually dimorphic gene expression in key limbic brain regions.

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Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: NIDA Grant T.J.G., DA017949

Title: Storm, stress, and nicotine: Interaction of stress and nicotine during adolescence on adult learning and stress response systems

Authors: *E. HOLLIDAY[1], C. OLIVER[2], R. COLE[2], D. BANGASSER[2], T. GOULD[2]; [1]Univ. of Texas Med. Br., Galveston, TX; [2]Temple Univ., Philadelphia, PA

Abstract: Despite the known risks associated with tobacco use and decline of tobacco consumption in the last 50 years, nearly 18.1% of people in the United States are everyday smokers. One reason for persistent smoking rates may be due to adolescent onset of tobacco use.

In rodent models, nicotine treatment during adolescence leads to long-term deficits in hippocampus-dependent tasks later in life. Adults do not display long-term learning impairments. The results from this study suggest that these long-term impairments result from an interaction between acute stress and chronic nicotine during adolescence. This study examined the interaction of stress and nicotine in early adolescent (p23), late adolescent (p38) and adult (p54) C57BL/6 mice that were either shipped from an animal facility in Maine to our animal facility in Philadelphia (STRESS) or bred in our animal facility (NO STRESS). Follow up studies wanted to recreate the shipping stress in the laboratory using social and physical stressors and wanted to match CORT elevations to that seen in shipped mice. Following stress, all animals were implanted with osmotic mini-pumps to deliver 12.6mg/kg/day of nicotine or saline per day for a period of 12 days. Thirty days later animals were trained and tested in our contextual fear conditioning paradigm. Only early adolescent and late adolescent animals shipped in or subjected to our induced stress protocol and administered nicotine demonstrated persistent learning deficits. Additionally, blood was collected from animals not designated for behavioral tasks and analysis were run to determine the interactive effects of stress and nicotine on stress response systems. There were no interactions between stress and nicotine on baseline CORT levels at various time points but there were significant changes in GR and CRFR expression in subregions of the hippocampus. Finally, it was determined adolescent stress and adolescent nicotine attenuated stress response to an acute stressor applied in adulthood suggesting altered stress response signaling as a mechanism underlying the observed long-term learning deficits.

Disclosures: E. Holliday: None. **C. Oliver:** None. **R. Cole:** None. **D. Bangasser:** None. **T. Gould:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH Grant R15MH102717

NIH Grant R25NS080685

Title: MAGL inhibition decreases aggression after post-weaning social isolation in male and female rats

Authors: L. DAWUD[1], E. LOETZ[2], J. FONTENOT[2], D. TAUBER[1], I. BRALLIER[1], *S. T. BLAND[2];
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Abstract: Post-weaning social isolation (PSI), also known as isolation rearing, interferes with normal social development and produces a behavioral phenotype that includes increased aggression. PSI also alters the endocannabinoid system in brain regions including the medial prefrontal cortex. The MAGL inhibitor MJN110 increases levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the CNS, and we have previously shown that MJN110 impacts social behavior. Moreover, MJN110 differentially altered phosphorylation of mammalian target of rapamycin (p-mTOR) in neurons and astrocytes within the medial prefrontal cortex (mPFC). Here, we tested the hypothesis that MJN110 would decrease aggressive behavior after PSI. Male and female Sprague-Dawley rats were exposed to either 4 weeks of PSI or group housing starting on postnatal day (PND) 21, and on PND 49 underwent a 15 min trial of social interaction with a novel, same-sex juvenile rat. Prior to the social interaction trial, rats received either 0, 1, or 5 mg/kg of MJN110. The trials were recorded for later behavioral assessment, and brains were removed for p-mTOR immunohistochemistry. We observed that MJN110 dose-dependently decreased aggressive grooming while having no effects on overall social interaction or on play behaviors. Sex differences in the effects of MJN110 on p-mTOR were observed, with more robust alterations in p-mTOR in males than females. Increased 2-AG signaling may decrease aggression during adolescence, and this may involve the mPFC mTOR pathway.

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Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH Grant NS052819

NIH Grant T32GM007739

Title: The impact of BDNF Val66Met single nucleotide polymorphism on rodent social interaction across development

Authors: *A. LI[1], D. JING[2], R. YANG[2], F. LEE[1,2];
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Abstract: Brain-derived neurotrophic factor (BDNF) is crucial for neuronal differentiation, survival, and plasticity (Binder & Scharfman, 2004; Chao, 2003; Huang & Reichardt, 2001; McAllister, Katz, & Lo, 1999). A common human single nucleotide polymorphism (SNP) in the BDNF gene leads to a single amino-acid substitution (Val66Met) that reduces activity-dependent BDNF secretion and neuronal plasticity (Chen et al., 2004, 2005). Consequently, the BDNF Met allele has been associated with alterations in learning, planning, and memory (Dincheva, Glatt, & Lee, 2012), as well as susceptibility to many neuropsychiatric disorders (Hall, Dhilla, Charalambous, Gogos, & Karayiorgou, 2003; Neves-Pereira et al., 2002; Ribasés et al., 2003; Sen et al., 2003; Sklar et al., 2002; Ventriglia et al., 2002). However, the impact of BDNF Met allele on social behaviors has not been extensively studied. Using a knock-in mouse containing the BDNF Met polymorphism ($BDNF_{Met}$), we investigated social behaviors across development using both a traditional three-chamber paradigm and a novel free-interaction system. Results indicate a significant age-gene interaction ($F(2,47) = 3.260$, $p < 0.05$) with reduced social interaction in the $BDNF_{Met}$ mice at postnatal day (P)25 and P60, but not at P40. Similarly, our preliminary data show altered amygdala-hippocampus connectivity in the $BDNF_{Met}$ group at P25 and P60, but not at P45. Together these findings suggest that phenotypic expression of this BDNF polymorphism in social behaviors may vary depending on developmental expression of BDNF, as well as neural circuit maturation, and suggest that developmentally timed interventions may prevent the behavioral and neuroanatomical alterations associated with the BDNF Met allele.

Disclosures: **A. Li:** None. **D. Jing:** None. **R. Yang:** None. **F. Lee:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Title: Periodic enrichment affects the outcome of two social preference tasks in adolescent female rats

Authors: *H. JOHNSON, H. C. SKINNER, S. L. SANTIAGO, R. GUCWA, M. N. PAVELKA, K. L. PATTERSON, M. C. ZRULL;
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Abstract: Changes in behavior, often featuring increased interest in novelty and forms of risk taking, mark adolescence. For rats, changes in novelty preference include how adolescents interact with conspecifics as well as how they explore environments. Social preference (SP) can be influenced by environmental enrichment (EE), exposing an animal to a novel and enhanced environment on a regular basis, with EE apt to modulate SP by altering flexibility in novel situations. We investigated the impact of EE on SP in adolescent, female rats using two tasks. Long-Evans females (n=12) were exposed to EE for 1.5-h 18 times between postnatal days (pnd) 21 and 49. Age matched controls (n = 12) were held twice on 18 times. On pnd 49, rats performed one of two SP tasks: one involved placing a rat into a small cage with additional small cages that contained stimulus rats on either side of the experimental rat; and the other involved placing a rat in an open field with one (Trial 1) or two (Trial 2) stimulus rats. For each task, there was a sample trial, and, after a 30-min delay, Trial 2 occurred with one familiar and one novel stimulus rat. In both tasks, initiating contact and time spent with the novel stimulus rat on Trial 2 was of interest. Evoked activity in basolateral amygdala (BLA) was examined using c-fos immunohistochemistry. When separated from stimulus rats by a wire mesh wall, EE rats made equal portions of nose pokes at and spent equal time with familiar and novel stimulus rats (0.49, 0.52, SDs=0.10). In contrast, control rats made 22% more pokes at and spent 33% more time near the novel rat (p<.05). In the direct contact task, EE rats initiated contact with the novel stimulus rat 50% less often than control rats (p<.02), and while EE rats spent about an equal proportion of time interacting with novel (0.54, SD=0.13) and familiar (0.46) rats, control rats spent less time with the novel stimulus rat (p<.08). C-fos positive neuron densities indicated a history of EE suppressed evoked activity in BLA relative to control brains (-58%, p<.05). Behavioral data suggest that periodic EE during adolescence promotes adaptation to social novelty with female EE rats exhibiting balanced contact time with familiar and novel conspecifics relative to female rats without enrichment. EE rats initiate interaction less often than controls when direct contact with a novel rat is possible but spend more time interacting than controls. BLA data suggest social features of EE may reduce neural activation thought to contribute to emotional behavior in a novel social situation. If preference for contact with a new over a known conspecific is risky, then enrichment seems to suppress this type of risk taking behavior in our rat model.

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Poster

299. Adolescent Development: Animal Models I

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Program#/Poster#: 299.09/D29

Topic: A.09. Adolescent Development

Title: Animal models of severe traumatic brain injury and its clinical significanc

Authors: ***S. LU**[1], Q. YING[2], X. XU[3], Y. TANG[5], Y. JIAO[5], Q. WANG[5], X. WANG[4], X. ZHANG[4], N. LU[6];
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Abstract: To improve the treatment of S-TBI, establish replicable animal patterns of S-TBI in experimental rats, find out the optimum trauma-causing impact force through experiments in 80 rats, and observe the correlation between pathological sections of brain and clinical brain trauma at serial time points after trauma. An improved free fall apparatus was used to give impacts on the vault of skull of anesthetized rats with a 50-400g weight from a 20-50cm height, the rats were observed for 4hr to 12days after impact, and brain was collected after anesthesia to make 652 pathological sections. The optimum impact force was determined as 10000g/cm. There was a marked correlation between pathological changes and clinical manifestation, which had a reference value to the diagnosis and treatment of S-TBI. A comparison between simulated clinical closed decompression and open decompression indicated that the latter was more favorable for the rehabilitation of brain trauma. The optimum impact force was found in rats of severe traumatic brain injury through experiments; experimental animals were significantly correlative to clinical diagnosis and treatment; open decompression was more beneficial to brain rehabilitation.

Disclosures: **S. Lu:** None. **Q. Ying:** None. **X. Xu:** None. **Y. Tang:** None. **Y. Jiao:** None. **Q. Wang:** None. **X. Wang:** None. **X. Zhang:** None. **N. Lu:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Title: Social interaction during critical developmental periods affects development of the prefrontal cortex

Authors: *W. E. MEDENDORP[1], A. PAL[1], E. PETERSEN[1], U. HOCHGESCHWENDER[2], K. JENROW[3];
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Abstract: The prefrontal cortex has been shown to participate in several roles in the regulation of social behavior. Dysfunction in the prefrontal cortex can lead to aberrant social behaviors similar to those found, for example, in autism. Critical periods of heightened plasticity are essential for normal development of the prefrontal cortex, and dysregulation during these developmental stages can result in a range of symptoms that correspond to neurodevelopmental disorders.

Socially isolated mice have been established as a model for autistic-like behaviors such as aberrant social interactions. Social isolation can also contribute to other behavioral dysfunction, including anxiety- or depressive-like behaviors, and has been demonstrated to alter the underlying neurological function in the prefrontal cortex. This suggests that social interaction with other animals contributes to the development and emergence of normal social behaviors in mice. Since development of the prefrontal cortex continues well into adolescence, it is likely that the social enrichment during this critical development stage is necessary for normalized social behavior. Correspondingly, lack of social enrichment during this critical period in adolescence likely contributes to the underdevelopment of the prefrontal cortex, giving rise to social dysfunction and aberrant social behavior. We hypothesize that social isolation during adolescence effects aberrant behavior in adult animals through changes in prefrontal neuronal morphology and electrical activity.

Male and female mice of strain C57BL/6 were weaned at 3 weeks of age and segregated into single housed or group housed cages. A minimum of six weeks later several tests were performed to assess for changes in behavior. Specifically, mice were tested behaviorally using the three chamber test to assess for social dysfunction, open field to assess any evidence of anxiety behaviors, and the forced swim test to assess depressive behaviors. These mice were then surgically implanted with recording electrodes to perform *in vivo* electrophysiology. A separate group of mice is being used to assess morphology of the cortical neurons in the prefrontal cortex. These mice express green fluorescent protein (GFP) in neurons of the cortical layer, allowing anatomical tracing of arborization by confocal microscopy and assessment of general morphology and number of neurons.

Together our studies will reveal whether behavioral changes caused by early social isolation correspond to changes in the activity or morphology of the prefrontal cortex.

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Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Title: Enrichment affects exploration of a field containing familiarly and newly located objects by adolescent rats

Authors: *E. A. ARTZ, H. L. JOHNSON, H. C. SKINNER, S. J. SNOUSE, R. C. PIERCE-MESSICK, T. J. ARNOLD, D. E. COBB, M. C. ZRULL;
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Abstract: Enrichment experiences (EE) can advance informal learning about environments, objects, and conspecifics via opportunity for investigation and interaction. Adolescence is a time when exploratory and novelty seeking behaviors emerge, with EE likely to have an impact by affecting brain structures mediating these behaviors. We examined how EE affected aspects of exploration and novelty preference during an object in place (OiP) task in adolescent rats. Between postnatal days (pnd) 25 and 48, same-sex groups of Long-Evans rats (n=8 each) experienced 18, 1.5-h EE sessions in cages containing objects, ramps, and platforms. Age-matched controls (8 female, 8 male) were not enriched. At pnd 36 and 50, an OiP task was performed, allowing assessment of field and object exploration and preference for objects in constant or new locations. There were 3 trials, sample and 15- and 60-min delays, that took place in a gridded, 1-m square open field. Exploration and object contact times were recorded for each trial, with the locations of two objects switched before Trial 2 and of the other two objects switched before Trial 3. After testing, brain tissue was processed to view neural activity in lateral and basolateral amygdala (BLA) using c-fos as a marker. At pnd 36 across delays, EE rats spent more time with objects (+56%), more in center of the field not near objects (+67%), and less time in the field exterior (-19%) than non-EE controls (all p<.02). Controls spent more time (+23%) and less time (-32%) the switched objects at the 15 and 60 min delays (p<.05), and EE rats spent similar time with all objects across delay trials. At pnd 50, EE rats spent less time with objects (-21%, p<.01), more time in center of the field not near objects (+100%, p<.02), and similar time in the field exterior than non-EE controls. Both EE and control rats spent a similar proportion of object time with switched objects at 15 and 60 min delays. Microscopy revealed similar c-fos positive neuron densities in LA of EE and control brains but fewer task-activated neurons in BLA of EE brains (-39%, p<.05) compared to controls. For younger adolescent rats, EE promotes balanced initial investigation of regions in and features of a simple environment. For older adolescents, EE continues to promote balanced exploration of environment regions but reduces investigation of specific features within a space. Reduced BLA activation suggests EE may promote quick neural adaptation to novelty, which correlates with reduced attention to

objects in the OiP environment by older adolescent rats. Overall, EE appears to foster balanced investigation of an environment and gradually diminish preference for familiarly or newly located features.

Disclosures: **E.A. Artz:** None. **H.L. Johnson:** None. **H.C. Skinner:** None. **S.J. Snouse:** None. **R.C. Pierce-Messick:** None. **T.J. Arnold:** None. **D.E. Cobb:** None. **M.C. Zrull:** None.

Poster

299. Adolescent Development: Animal Models I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 299.12/D32

Topic: A.09. Adolescent Development

Support: the Marie Curie Reintegration-Grant (FP7-268247)

the Italian Ministry of Health Grants for Young Researchers (GR-2010- 2315883)

Title: Attentional control assessment in LgDel adolescent mice through a modified five choice serial reaction time task

Authors: *M. CIAMPOLI[1], M. MEREU[2], F. PAPALEO[1];
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Abstract: The 22q11.2 deletion syndrome (22q11.2DS) is the major known genetic-based vulnerability factor for developmental neuropsychiatric disorders such as schizophrenia and ADHD. These psychiatric disturbances are the most problematic, debilitating and enduring features in this syndrome. By late adolescence/early adulthood up to one-third of patients with 22q11.2DS develops schizophrenia. Adolescence is a critical period for the development and maturation of higher order cognitive functions, including the orienting of attention in the visual space. However, at the moment, no studies investigated the impact of 22q11.2 microdeletion on attentional control during this critical period in mice. This was mostly due to the lack of behavioral task able to test higher order cognitive functions in rodents during the short period of "adolescence". We set up and validated a new version of the 5-Choice Serial Reaction Time Task (5-CSRTT) in order to selectively measure in adolescent mice different cognitive functions such as impulsivity, processing speed, broad monitoring and distractibility. Results showed that 21-days old C57BL/6J mice were able to quickly and reliably acquire this task in less of twelve days. In particular, we found that our setting was able to catch selective alterations of impulsive control, reduced ability to maintain a broad focus of attention and vulnerability to distractors.

Next, we applied this test to the LgDel genetically modified mice carrying the same genetic microdeletion as in 22q11.2DS. LgDel mice were able to acquire and perform the basic part of our modified 5-CSRTT. Remarkably, as observed in 22q11.2DS, LgDel mice showed selective higher distractibility than wild-type littermates, suggesting a vulnerability of attentional control in relationship to distracting cues. Omega-3 polyunsaturated fatty acids (PUFAs) have recently been indicated as a possible cognitive enhancer. In order to set the ground for the development of new preventive strategies, we tested the effects of chronic omega-3 PUFAs administration in LgDel adolescent mice. Omega-3 PUFAs was able to selectively rescue LgDel mice deficits in distractibility. Overall, our data pave the way for an early detection and therefore early intervention in psychiatric-related symptomatology to prevent and cure its debilitating features.

Disclosures: **M. Ciampoli:** None. **M. Mereu:** None. **F. Papaleo:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH: R01NS054272-04

Title: Adolescent binge ethanol exposure alters cholinergic cell populations, but not functional acetylcholine release

Authors: *G. M. FERNANDEZ, J. E. SANDERS, L. M. SAVAGE;
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Abstract: Adolescence is a malleable time period during which maladaptive alcohol consumption patterns are often initiated. Previous research has found variable effects of adolescent binge- like ethanol (EtOH) exposure on spatial learning and memory, despite a 30% reduction in forebrain cholinergic neurons. Using an adolescent intermittent ethanol exposure model (20% EtOH v/v, 5 g/kg, intragastric gavage, 2 days on/2 days off), rats were exposed to binge-like levels of EtOH from P25-P55. Cholinergic functioning was assessed using a spontaneous alternation protocol during which acetylcholine release was measured 3 weeks following EtOH exposure (P80). Across treatment, animals developed metabolic EtOH sensitization, as demonstrated by significantly increasing blood EtOH concentrations (125.8 mg/dL to 146.4 mg/dL). Despite exposure to binge-like levels of ethanol during adolescence, EtOH-treated rats had similar rates of spontaneous alternation compared to control rats. Functional hippocampal and prefrontal acetylcholine release increased during maze testing, but

there were no differential release patterns as a function of previous EtOH exposure. Additionally, no group differences were found during novel object recognition testing. Immunohistological staining for choline acetyltransferease (ChAT) indicated a 20% reduction in ChAT positive cells within the medial septum/diagonal band of EtOH exposed rats. Our results indicate that binge EtOH exposure initiated during early adolescence does not impair cholinergic functioning, despite a reduction in cholinergic staining, when assessed during early adulthood.

Disclosures: G.M. Fernandez: None. **J.E. Sanders:** None. **L.M. Savage:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: UU Grant SM.YI.2013.11.GS

Title: Deprivation of social play behavior results in decreased inhibition in adult prefrontal cortex and associated behavioral changes in rats

Authors: A. OMRANI[1], M. SPOELDER[2], R. VAN DORLAND[3], C. CORNELIS[2], *L. J. VANDERSCHUREN[2], C. J. WIERENGA[3];
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Abstract: Sensory input during early life is critical for proper development of the sensory cortex. It has been proposed that experience-dependent development of neuronal connectivity also occurs in brain areas involved in complex cognitive functions, such as the prefrontal cortex (PFC). By analogy, we hypothesized that optimal PFC development requires complex input, such as social interaction. Therefore, we examined how early social experiences shape PFC circuitry and function. We focused on a particular form of social interaction that is highly abundant during post-weaning development, i.e. social play behavior. It is thought that social play behavior facilitates social and cognitive development, especially the ability to flexibly use cognitive capacities under changeable circumstances. Young rats were deprived from social interaction for 3 weeks during the period in life when the occurrence of social play behavior peaks (P21-42, ISO rats), followed by resocialization (housing in pairs) until adulthood. In a probabilistic reversal learning task, which depends on integrity of the PFC, ISO rats achieved more reversals than controls, as a result of enhanced sensitivity to recently rewarded actions. ISO

rats also showed enhanced intake of sucrose as well as an increased motivation for sucrose under a progressive ratio schedule of reinforcement. In parallel, we recorded synaptic currents in layer 5 cells in acute slices from medial PFC. While we observed no differences in excitatory synaptic currents between ISO and control rats, we found that the frequency of miniature inhibitory synaptic currents (mIPSCs) was reduced in adult ISO rats. This reduced inhibitory transmission was accompanied by an increase in mIPSC rise time. Intrinsic excitability of layer 5 cells was not different. Our data suggest that deprivation from social play behavior during post-weaning development induces a long-lasting change in the balance between excitation and inhibition in the PFC, by reducing inhibitory synaptic transmission onto layer 5 neurons. This reduction in PFC inhibition may contribute to altered behavioral flexibility and sensitivity to reward. Together, our results demonstrate that early social experiences have long-lasting consequences for brain and behavior.

Disclosures: A. Omrani: None. **M. Spoelder:** None. **R. Van Dorland:** None. **C. Cornelis:** None. **L.J. Vanderschuren:** None. **C.J. Wierenga:** None.

Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH Grant NS080889

Title: Neonatal vincristine administration evokes delayed mechanical pain hypersensitivity in the developing rat

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Abstract: Chemotherapeutic agents induce peripheral neuropathy and severe pain in children, often necessitating a dose reduction in the chosen cancer treatment, as widely reported to occur in adults. Recent preclinical evidence suggests that peripheral nerve or tissue damage during the neonatal period leads to long-term changes in nociceptive processing. However, the degree to which pediatric chemotherapeutic regimens influence pain sensitivity throughout development remains unknown, in part due to the lack of an established animal model of chemotherapy-induced neuropathy during early life. Therefore, the present study investigated the effects of early life exposure to vincristine (VNC), commonly used in the treatment of pediatric cancers, on mechanical and thermal pain sensitivity in the developing rat.

Male and female Sprague Dawley rats received daily i.p. injections of 15, 30, 45, or 60 µg/kg VNC, or equivalent volumes of saline, with a five-day on, two-day off schedule starting on postnatal day (P)10 for a total of 10 injections. Mechanical reflex withdrawal thresholds were measured prior to each injection and then at weekly intervals until P56. Withdrawal latencies in response to noxious heat and cold stimuli were evaluated weekly between P26 and P56. VNC at 15 and 30 µg/kg did not significantly alter mechanical or thermal withdrawal thresholds at any time point compared to saline-treated littermate controls. Since the daily administration of 45 and 60 µg/kg VNC resulted in death following the third dose, we modified our dosing protocol and administered 5 i.p. injections of 60 µg/kg vincristine (or saline) every other day starting at P10. Rats routinely survived this VNC dose, although both male and female VNC-treated rats displayed reduced weight gain compared to saline controls. More importantly, VNC rats demonstrated significantly lower mechanical withdrawal thresholds compared to control groups beginning at P26. Sex did not influence the effects of VNC exposure on pain behaviors, as the delayed mechanical hypersensitivity was observed in both male and female littermates after neonatal VNC. Meanwhile, the responses to noxious heat and cold, as well as gross motor function, were unaffected by VNC in both sexes. Overall, the present results demonstrate that high dose administration of VNC during the early postnatal period selectively evokes a mechanical hypersensitivity in rats that is slow to emerge during adolescence, providing further evidence that aberrant sensory input during early life can have lifelong consequences for pain processing.

Disclosures: **K.A. Schappacher:** None. **M.L. Baccei:** None.

Poster

299. Adolescent Development: Animal Models I

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OD P51OD011132

Title: Effects of estrogen on brain structural maturation during adolescence: prefrontal, amygdala and temporal cortex in female macaques

Authors: *F. LOMBARDI[1], J. R. GODFREY[1], B. R. HOWELL[1,2], M. STYNER[3], M. E. WILSON[1,2], M. M. SANCHEZ[1,2];
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Abstract: Adolescence is a developmental phase of considerable brain growth and remodeling, occurring in parallel with increases in emotional and stress reactivity. A likely factor regulating these changes in females is puberty-induced increases in gonadal hormones, particularly estradiol (E2). E2 organizational effects could contribute to brain structural remodeling, although it is difficult to disentangle these hormonal effects from effects of chronological age in human studies. Non-human primates constitute translational animal models due to their brain, neuroendocrine, developmental and complex social behavior similarities with humans. In this study we used socially-housed female rhesus monkeys (*M. mulatta*) to disentangle brain structural effects of E2 during adolescence from chronological age by investigating the effects of delayed puberty on brain development. Animals were socially housed, allowing us to assess the effects of rank, and associated differences in social stressor exposure, on these changes in middle ranking animals. We focused on prefrontal cortex (PFC), amygdala (AMYG), and temporal-parietal-occipital (TPO) region of the superior temporal sulcus (STS) due to their known roles in processing socioemotional stimuli.
Structural MRI scans were collected at pre- and peri-puberty in 21 juvenile female rhesus macaques. Thirteen of those animals received monthly injections of a gonadotropin releasing hormone agonist (GnRH, Lupron) to delay puberty. We examined total intracranial volume (ICV), and grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) volumes, as well as AMYG, PFC GM and WM, and TPO GM volumes. To assess functional correlates of brain structure changes, social and anxiety behaviors as well as measures of stress neuroendocrine function (basal and stress-induced cortisol levels, and glucocorticoid negative feedback sensitivity) were collected. Results showed that suppression of E2 by Lupron treatment resulted in smaller GM and WM volumes, both total and in PFC, as well as smaller ICV and AMYG volumes. Smaller AMYG volumes predicted decreased stress reactivity. E2 suppression also resulted in stronger glucocorticoid negative feedback. In addition, there was a significant relationship between social rank and PFC GM volume, with higher social rank predicting larger volumes, even within the narrow range of middle ranking animals. These results provide evidence that during adolescence the female primate brain undergoes significant structural changes and are linked to changes in stress neuroendocrine function that seem to be driven by developmental increases in E2 as well as exposure to social stressors.

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Poster

299. Adolescent Development: Animal Models I

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Support: NIH grant MH097236

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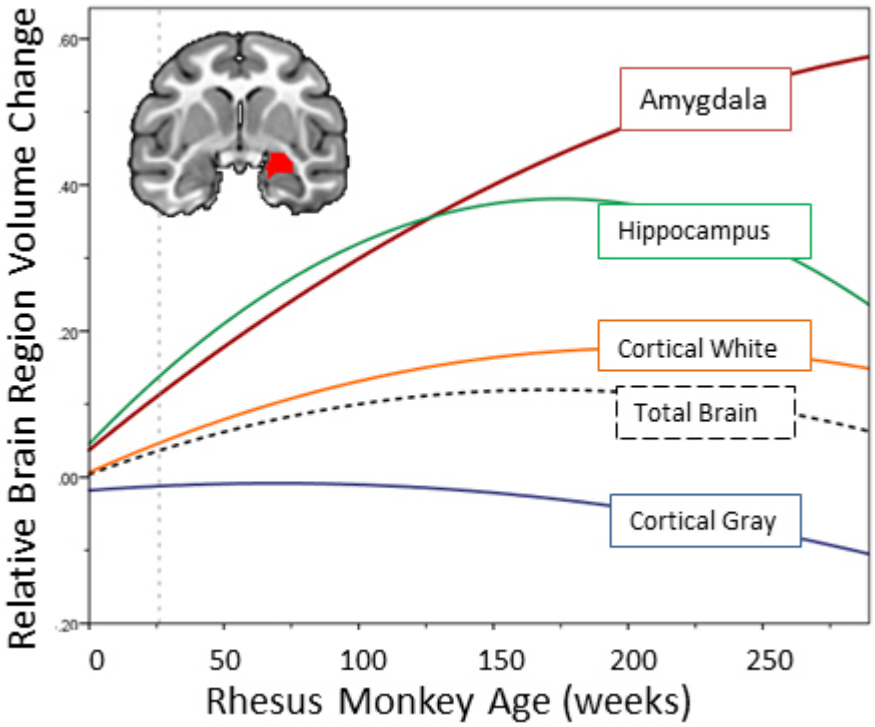
Title: Amygdala growth from youth to adulthood in the macaque monkey.

Authors: *C. M. SCHUMANN[1], J. A. SCOTT[2], A. LEE[3], E. FLETCHER[2], M. D. BAUMAN[1], D. G. AMARAL[1];
[1]Psychiatry and Behavioral Sci., UC Davis MIND Inst., Sacramento, CA; [2]Neurol., [3]UC Davis, Sacramento, CA

Abstract: BACKGROUND: The amygdala modulates the brain's socioemotional networks. Functionally, this system is among the latest to mature in primates. In human cross-sectional MRI studies, the amygdala appears to undergo a protracted growth trajectory by continuing to increase in size throughout adolescence, coinciding with acquisition of complex socioemotional learning. This study was carried out to determine if the primate amygdala does indeed undergo protracted development relative to other brain regions. Since this study would take over 20 years to do in humans, we utilized a nonhuman primate model, rhesus macaques, with MRI's collected longitudinally over 5 years. We compared amygdala growth relative to that of total brain, cortical gray matter, cortical white matter, and hippocampus. METHODS: Longitudinal T1-weighted MRI scans were collected on naturalistically-reared male and female rhesus macaques (n=24) at age 6 months, 9 months, 1 year, 3 years, and 5 years. Amygdala and hippocampal volumes were measured from manual delineations and cortical regions with semi-automated segmentation. RESULTS: Amygdala volume increases 49% (+/-12%) from age 6 months to 5 years, a relative gain greater than overall brain growth (8% +/- 6%). The amygdala best fit a linear trajectory model, in contrast to hippocampus, which plateaus near 3 years of age (relative volume gain 38%). Amygdala volume, but not hippocampus, is larger in males than females (8.14%, p=0.013). Cortical gray matter volume slightly declines (-7% +/-3%). White mater is similar to amygdala with positive age-related changes detectable through early adulthood (17% +/-3%). CONCLUSION: The protracted increase in amygdala volume in the macaque monkey mirrors that reported in humans. This is likely due to increasing connectivity with larger socioemotional networks and extended neuronal maturation that occurs beyond that of other brain regions.

Understanding this protracted growth trajectory is essential for developing targeted therapeutics for neurodevelopmental and psychiatric disorders in which the amygdala is implicated.



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Poster

299. Adolescent Development: Animal Models I

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Topic: A.09. Adolescent Development

Support: CNPq

CAPES

FAPESP

Hospital Israelita Albert Einstein

Title: Physical activity during adolescence and neural reserve: a study of the brain levels of brain derived neurotrophic factor (BDNF), adrenocorticotropic hormone (ACTH) and corticosterone.

Authors: *A. D. DOMINGUEZ CARVALHO[1,2], A. B. VICTORINO[2], A. A. DE ALMEIDA[2], J. S. HENRIQUE[2], F. R. CABRAL[3], L. B. TORRES[3], R. M. ARIDA[2], S. GOMES DA SILVA[3]; [1]Exercise Neurophysiolgy, [2]Neurology/Neurosciences, Federal Univ. of Sao Paulo, Sao Paulo, Brazil; [3]Hosp. Israelita Albert Einstein, Sao Paulo, Brazil

Abstract: Background: There are reports that life experiences at early ages such as physical activity in childhood and adolescence can reduce the future risk of brain disorders and enhance lifelong brain functions. However how early physical activity promotes such effects are not well understood. A possible explanation is that physical exercise can stimulate neuronal growth, resulting in a neural reserve which could be extracted throughout the life course. Purpose: The present study was designed to investigate the hypothesis of neural reserve induced by early physical activity. To do this, we evaluated the cortical and hippocampal levels of BDNF, ACTH and corticosterone during the aging course of rats submitted to physical exercise during adolescent period. Methods: Forty- eight male Wistar rats were distributed into exercise (EX, n=24) and control (CTL, n=24) groups. Rats from EX group were submitted to an aerobic exercise program during the 21st and 60th postnatal day (P21-P60). Afterward, cortical and hippocampal levels of BDNF, ACTH and corticosterone from the EX and CTL groups were investigated at different life stages: 0 (P60), 30 (P90), 60 (P120) and 90 (P150) days after last exercise session. The BDNF, ACTH and corticosterone levels were quantified by mean of Luminex xMAP system (MAGPIX technology). Results: After the last physical training session (at P60), a significant increase of hippocampal BDNF ($p = 0.002$) and a decrease of cortical ACTH ($p = 0.03$) were detected in EX group in relation to CTL group. At P90, a significant decrease in hippocampal BDNF level was observed in the Ex group when compared to the CTL group ($p = 0.016$). At P150, levels of hippocampal ACTH ($p = 0.003$) and cortical corticosterone ($p = 0.043$) were lower in Ex group than in CTL group. Conclusion: Our results indicate that changes in the cortical and hippocampal levels of proteins and hormones linked to cellular growth and stress may occur throughout of life of rats exercised in youth.

Disclosures: A.D. Dominguez Carvalho: None. **A.B. Victorino:** None. **A.A. de Almeida:** None. **J.S. Henrique:** None. **F.R. Cabral:** None. **L.B. Torres:** None. **R.M. Arida:** None. **S. Gomes da Silva:** None.

Poster

300. Postsynaptic Organization and Structure I

Location: Halls B-H

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Topic: B.07. Synaptic Transmission

Support: KAKENHI: 25290012

KAKENHI: 24120718

Title: GABAergic/non-GABAergic synaptic inputs to striatal medium spiny neurons

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Abstract: We analyzed GABAergic and non-GABAergic synaptic inputs along 3D-reconstructed dendritic segments of on a whole cell-recorded medium spiny (MS) cell of the rat striatum, using the serial electron microscopy with postembedding GABA immunohistochemistry. Among 108 spines in the 9 dendritic segments (total length: 113.1 μm, range: 2.8 - 30.8 μm), we found 71 spines receiving the non-GABAergic synaptic inputs, presumably excitatory glutamatergic terminals (non-GABAergic synapse density: 0.67 /μm). The two spines had two non-GABAergic synapse terminals simultaneously. Most of the non-GABAergic synapses were on the spine head (71 among 76 synapses). Ten GABAergic synapses were found on the dendritic shaft (n = 6), spine neck (n = 2), or spine head (n=2). Three spines with the GABAergic input also received the other non-GABAergic synaptic input. The GABAergic shaft synapse density was 0.05 /μm, and spine synapse density was 0.04 /μm. The inhibitory GABAergic synapses were 12 % (10 among 86 synapses) of all synaptic inputs to the MS cell.

To estimate total GABAergic and non-GABAergic synapse numbers, the total dendritic length was measured with the MS cell using Neurolucida reconstruction system. The total length of the dendrites was 2879 μm. The estimated non-GABAergic (presumably excitatory) synapse number was 1934 (1807 on spines and 127 on shafts). The estimated GABAergic inhibitory synapse number was 254 (101 on spines and 153 on shafts). The estimated number of spine innervated by both GABA-positive and -negative synapses was 76, and that by two GABA-negative synapses was 51.

Furthermore we found an FS cell showing a unique target preference by 3D reconstruction of its axon terminals from serial electron micrographs. All 16 axonal boutons exclusively innervated the spine head with no other additional synaptic inputs. The target spine head size was variable (volume: 0.08 ± 0.05 μm^3, 0.02 – 0.19 μm^3; surface area: 1.58 ± 0.83 μm^3, 0.49 – 3.26 μm^2; n = 16) . The synapse junction area (0.16 ± 0.09 μm^2, 0.03 – 0.36 μm^2; n=16) correlated with the spine volume or surface area. Most striatal FS cells make synapses on diverse surface domains (soma, dendritic shaft and spine). This finding suggests that striatal FS cells may be further diversified, based on the range of domain selectivity.

Disclosures: Y. Kubota: None. **Y. Kawaguchi:** None.

Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

Support: NIH R37NS036251

HHMI

P30DA018343

Title: Contacts between the ER and other membranes in neurons: an inter-organelle connectome

Authors: *Y. WU[1], S. XU[2], H. KENNETH[2], H. HESS[2], P. DE CAMILLI[1];
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Abstract: Close appositions between the membrane of the endoplasmic reticulum (ER) and other membranes (membrane contact sites, MCS) play important physiological functions. One such function, which applies to contacts between the ER and either the plasma membrane (PM) or mitochondria is the regulation of Ca^{2+} dynamics. Another function, thought to apply to appositions between the ER and all other membranous organelles including the PM, is the control of lipid homeostasis via the direct transfer of lipids between the two apposed bilayers, independent of membrane fusion and fission reactions. The abundance of such contacts in neurons has not been systematically analyzed. As a complement to ongoing studies in one of our labs of proteins that are localized at MCS and participate in lipid transfer (f.e. extended-synaptotagmins and ORP proteins), we have used 3 dimensional (3D) electron microscopy techniques [Focus Ion Beam-Scanning Electron Microscopy (FIB-SEM) and tilt imaging of serial sections] to reconstruct intracellular organelles and MCSs of portions of neuronal cell bodies, dendrites, and axons. Contacts (distance less than 30 nm) between the ER and other membranes, including the plasma membrane, were observed in all neuronal compartments. ER-plasma membrane contacts were particularly abundant in cell bodies, where they involved flat and large (width in the range of micrometers) cisternae often with a very narrow lumen (thin ER), corresponding to the previously described subsurface cisternae (Rosenbluth, JCB 13, 1962). However, smaller contacts were also numerous along dendrites and axons. Contacts between ER and mitochondria covered more than 4% of the mitochondrial surface and formed cages around mitochondria likely to have an impact on mitochondria motility. Collectively, our study reveals a striking degree of interconnections of membranous organelles and suggest that such connections may play more general role in neuronal physiology than appreciated so far.

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Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

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Title: Imaging of polarized light signal changes associated with neuronal activity in mouse hippocampus

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Abstract: We have expanded intrinsic optical signal imaging by incorporating the recording of polarization signals in addition to changes in overall transmissivity in acute mice hippocampal slices. Intrinsic optical imaging has been widely used to map patterns of brain activity *in vivo* in a non-invasive manner. Traditional intrinsic optical signals refer to changes in the transmissivity or reflectivity of brain tissue, which reflects volume changes in neurons and astrocyes in brain slices, and changes in blood flow and oxygenation of hemoglobin in the intact brain. Therefore, those signals report alternations in optical properties that include secondary results of neuronal activity in the tissue in addition to the changes that directly reflect neuronal activity. Polarization sensitive properties provide information about protein alignment and architecture of lipid membrane at the cellular level that are more directly affected by neuronal activity. Our polarization sensitive setup enables the evaluation of molecular alignments of protein assemblies/disassemblies and structures of membranous organelles. We detected reproducible spatial and temporal patterns of polarization change at stratum radiatum of area CA1 of hippocampus in response to stimulation of Schaffer collateral. The recovery time course of polarization signal change after nerve stimulation (40Hz, 50stim) was faster than that of transmittance light intensity change that was monitored through conventional intrinsic optical signal imaging. These polarization signals were partly blocked by the application of CNQX (10 microM) and D-APV (50 microM), and were completely abolished by TTX (1 microM). These signals are greatly enhanced by an application of the inhibitor of glutamate transporter, TBOA

(50 microM). Polarization signals are not affected by inhibitors of $GABA_A$ receptors (10 microM bicuculline) or group I metabotropic glutamate receptors (100 microM CPCCOEt). These results indicate that glutamatergic synaptic transmission is involved in the generation of polarization sensitive optical signals. Our results suggest that these polarization signals are generated via activity of postsynaptic glutamate receptors, following structural changes at the cellular and/or sub-cellular level, including transient changes in synaptic morphology, rearrangement of cytoskeletons, and remodeling of membranous structures. Our instrument development and applications expand intrinsic optical signal imaging to monitor synaptic activity in the brain in a non-invasive manner.

Disclosures: M. Koike-Tani: None. **S. Mehta:** None. **T. Tominaga:** None. **R. Oldenbourg:** None. **T. Tani:** None.

Poster

300. Postsynaptic Organization and Structure I

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Ministry for Health and Welfare Affairs Grant HI15C3026

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Title: Calsyntenin-3 mediates synapse development via neurexin/cbln complexes

Authors: *J. KO[1], H. KANG[2], J. KO[1], T. MORI[3], K. MATSUDA[4], S. JEON[1], M. YUZAKI[4], K. TABUCHI[3], J. UM[2];
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Abstract: Postsynaptic calsyntenin-3 (CST-3) is a synaptogenic adhesion molecule that induces presynaptic differentiation via presynaptic neurexins. Here, we show that expression of α- or β-neurexin splice variants with an insert at splice site #4 (SS#4) restores impaired CST-3 activity in neurexin-deficient neurons. In addition, CST-3 forms *in vivo* complexes with SS#4-positive neurexins, but quantitative cell-surface binding assays confirm no direct interaction of CST-3 with neurexins. Affinity chromatography analyses identified cerebellin-4 (Cbln4) as a candidate

CST-3 binding partner. Moreover, hexameric Cbln1 directly interacts with cadherin repeats of CST-3, and triple knockdown (KD) of Cblns reduced CST-3 activity in hippocampal neurons. Strikingly, endogenous CST-3 is mainly localized at inhibitory synapses, although CST-3 KD decreased both excitatory and inhibitory synaptic transmission *in vivo*. Furthermore, CST-3 protein is primarily monomeric. Collectively, our data suggest a revised model that monomeric CST-3 is linked to SS#4-positive neurexins/Cblns to organize inhibitory synapse development.

Disclosures: **J. Ko:** None. **H. Kang:** None. **J. Ko:** None. **T. Mori:** None. **K. Matsuda:** None. **S. Jeon:** None. **M. Yuzaki:** None. **K. Tabuchi:** None. **J. Um:** None.

Poster

300. Postsynaptic Organization and Structure I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 300.05/E9

Topic: B.07. Synaptic Transmission

Support: NIH grant MH097680

Simons Center for the Social Brain at MIT

Title: Shank modulates postsynaptic Wnt signaling to regulate synaptic development

Authors: *K. P. HARRIS, Y. AKBERGENOVA, R. W. CHO, M. S. BAAS-THOMAS, J. T. LITTLETON;
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Abstract: Prosap/Shank scaffolding proteins regulate the formation, organization, and plasticity of excitatory synapses. Mutations in *SHANK* family genes are implicated in autism spectrum disorder (ASD) and other neuropsychiatric conditions. However, the molecular mechanisms underlying Shank function are not fully understood, and no study to date has examined the consequences of complete loss of all Shank proteins *in vivo*. Here we characterize the single *Drosophila* Prosap/Shank family homolog. Shank is enriched at the postsynaptic membrane of glutamatergic neuromuscular junctions (NMJs) and controls multiple parameters of synapse biology in a dose-dependent manner. Both loss and overexpression of *Shank* result in a decrease in the total number of synaptic boutons at the NMJ and an increase in immature synaptic boutons (ghost boutons) that lack a postsynaptic scaffold. We find that Shank regulates synaptic maturity by modulating the internalization of the Wnt receptor Fz2 in the postsynaptic cell. Furthermore, a structure-function analysis of Shank reveals that conserved protein-protein interaction domains

have distinct roles in regulating synaptic bouton number. This study identifies Shank as a key component of synaptic Wnt signaling, thus defining a novel mechanism for how Shank contributes to synapse maturation during neuronal development.

Disclosures: K.P. Harris: None. **Y. Akbergenova:** None. **R.W. Cho:** None. **M.S. Baas-Thomas:** None. **J.T. Littleton:** None.

Poster

300. Postsynaptic Organization and Structure I

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Program#/Poster#: 300.06/E10

Topic: B.07. Synaptic Transmission

Support: Grant-in-Aid for Young Scientists Grant Number 16K18376

Title: Developmental regulation of NMDA receptor subunits expression by drebrin

Authors: *N. KOGANEZAWA, T. SHIRAO;
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Abstract: Drebrin has critical functions in synaptic plasticity; therefore it is thought to be in a responsible position of learning and memory. Drebrin plays an important role in formation of stable actin complex in dendritic spines via modulating the helix pitch of actin filaments. Drebrin has two isoforms, embryonic (E) and adult (A) isoforms, and we have recently shown that drebrin A is indispensable for normal synaptic plasticity and its roles cannot be complemented by drebrin E (Kojima and Yasuda et al., 2016). In addition, we have previously shown that NMDA receptor (NMDAR) activation induces a bidirectional shift in subcellular distribution of drebrin (Sekino et al., 2006), and have also reported that drebrin A is required for the homeostatic up-regulation of the NR2A subunits within spines (Aoki et al., 2009). Therefore it is of interest to further examine the relationship between drebrin and NMDAR, especially focusing on the difference of drebrin isoforms. We used primary cultured hippocampal neurons prepared from drebrin knockout (DXKO) mice. The both isoforms were genetically deleted in the DXKO mice. We first examined the accumulation of NMDAR subunits immunocytochemically. At 14 days in vitro (DIV), there were more labeled NR1 in DXKO neurons than in wild-type neurons whereas there was no difference in NR2B level. On the other hand, at 21 DIV, there was no difference in NR1 level whereas the labeled NR2A was slightly less in DXKO neurons. These data suggest that there are more NR1 containing receptors in DXKO neurons. NR1 subunit could form homomeric receptors, but highly active NMDARs are usually di-teteromeric or tri-heteromeric receptors which contain two or three distinct subunits. Therefore, it is suggested that

DXKO neurons have less functional NMDAR. Because drebrin has two isoforms, we further investigated which isoform induces this less functionality of NMDAR. For this experiment, we used drebrin A specific knockout (DAKO) mice. Our preliminary data showed that primary cultured hippocampal neurons prepared from DAKO mice have comparable level of NMDAR subunits to wild-type neurons. In DAKO mice, drebrin E expresses continuously even in adult brain instead of drebrin A. Taken together, drebrin E may play an important role in the developmental regulation of NMDAR function.

Disclosures: **N. Koganezawa:** None. **T. Shirao:** None.

Poster

300. Postsynaptic Organization and Structure I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 300.07/E11

Topic: B.07. Synaptic Transmission

Support: Seneca Postdoc Fellowship

Title: Quantitative analysis of synapse organization at nanoscale by cryo-electron tomography

Authors: **A. MARTINEZ-SANCHEZ**, Z. KOCHOVSKI, U. LAUGKS, W. BAUMEISTER, *V. LUCIC;
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Abstract: Cryo-Electron Tomography allows comprehensive imaging of cellular components within their natural environment at a single nanometer scale. We have developed a novel segmentation and analysis procedure that is particularly suited for dense filamentous networks, such as the postsynaptic density of excitatory central nervous system synapses. Results obtained by examination of neocortical rodent synaptosomes showed that protein distribution is highly heterogeneous, thus differing from both homogeneous and random distributions. In order to detect specific patterns, we analyzed separately layers comprising synaptic cleft and the regions of pre- and postsynaptic terminals close to the synaptic membranes. We found that layers have distinct organization and identified statistical properties that are preserved among different synapses, but also that the degree of clustering and the presence of regions with a low protein density show high variability. All together, our results directly show that synaptic complexes have distinct organization at nanometer scale and point to a dynamic nature of this organization.

Disclosures: **A. Martinez-Sanchez:** None. **Z. Kochovski:** None. **U. Laugks:** None. **W. Baumeister:** None. **V. Lucic:** None.

Poster

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Location: Halls B-H

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Program#/Poster#: 300.08/E12

Topic: B.07. Synaptic Transmission

Support: BBSRC

Title: The role of cortactin in AMPAR trafficking

Authors: *G. PARKINSON[1], S. E. L. CHAMBERLAIN[2], M. TURVEY[1], N. JAAFARI[1], J. G. HANLEY[1];
[1]Sch. of Biochem., [2]Sch. of Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking is a well-studied molecular mechanism that underlies the changes in synaptic strength associated with the alterations in neural circuits during learning events and pathologies. AMPAR associated proteins, including those that regulate the actin cytoskeleton, control AMPAR trafficking, and are therefore essential determinants of AMPAR synaptic expression and plasticity. Previous work in our laboratory has investigated the interplay between receptor trafficking and cytoskeletal rearrangement through the study of protein interacting with C kinase 1 (PICK1). We have demonstrated that PICK1-mediated inhibition of Arp2/3 mediated actin polymerisation is required for the internalisation of GluA2 containing AMPARs and hippocampal long term depression. Here, we investigate another regulator of actin dynamics and GluA2 binding partner, cortactin, and its role in AMPAR trafficking. In contrast to PICK1, cortactin promotes actin polymerisation via Arp2/3 complex activation. While cortactin has been proposed to function in various trafficking events, its role in AMPAR trafficking is unexplored. Therefore, it is of interest to determine how these actin interactors coordinate the cytoskeleton, receptor movement and each other to regulate synaptic plasticity. In this study, we utilise molecular, immunocytochemical and electrophysiological methods to provide evidence for a role of cortactin in maintaining basal AMPAR surface expression. Additionally, we employ biochemical techniques to further characterise the actin-cortactin-AMPAR interaction and elucidate how it is regulated during synaptic plasticity. Thus, we demonstrate that cortactin is a key regulator of basal AMPAR expression and is also implicated in activity-dependent AMPAR trafficking.

Disclosures: G. Parkinson: None. **S.E.L. Chamberlain:** None. **M. Turvey:** None. **N. Jaafari:** None. **J.G. Hanley:** None.

Poster

300. Postsynaptic Organization and Structure I

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Program#/Poster#: 300.09/E13

Topic: B.07. Synaptic Transmission

Support: Intramural Research Program NINDS, NIH

Title: Identification and characterization of proteins at the synaptic cleft

Authors: A. BURCH[1], J.-H. TAO-CHENG[2], *A. DOSEMECI[1];
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Abstract: Identification of synaptic cleft components has been hampered by the lack of a suitable preparation enriched in synaptic junctions devoid of adjoining peripheral membranes. Prior strategies for the isolation of synaptic junctions relying on detergents for the removal of peripheral membranes also resulted in substantial loss of membranes lining the cleft. Here we describe a novel detergent-free method for the preparation of a synaptic junction fraction that uses phospholipase A2. Limited digestion of synaptic plasma membrane (SPM) fraction with phospholipase A2 results in selective removal of peripheral membranes while junctional membranes remain relatively intact as observed by electron microscopy. Comparison of this 'synaptic junction' fraction to parent SPM and to total homogenate fractions by Western immunoblotting reveals significant enrichment of certain cell adhesion molecules including neurexins, neuroligins, N-cadherin, and SALM5 suggesting their preferential localization to the synaptic cleft. Other adhesion molecules, including SynCAM, NCAM, ephrin-B, EphA4, SALM2, and SALM4 do not show significant enrichment, indicating a more widespread distribution. Immuno-electron microscopy is applied to verify localization of selected putative cleft components. As expected, neurexins and neuroligins are found to be specifically localized to the synaptic junctions at pre-and postsynaptic sites, respectively. The synaptic junction preparation is further used to characterize post-translational modifications of cleft proteins with preliminary results indicating high levels of glycosylation. Ultimately, the preparation will be used for the estimation of the stoichiometry of cleft components.

Disclosures: **A. Burch:** None. **J. Tao-Cheng:** None. **A. Dosemeci:** None.

Poster

300. Postsynaptic Organization and Structure I

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Program#/Poster#: 300.10/E14

Topic: B.07. Synaptic Transmission

Title: Gephyrin splice variant dependent gamma aminobutyric type a receptor clustering

Authors: *Y. MERKLER[1,2], G. SCHWARZ[1,2,3];
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Abstract: At inhibitory synapses of the central nervous system, gephyrin is the main scaffolding protein to anchor and cluster gamma-aminobutyric type A receptors ($GABA_AR$) at postsynaptic densities, thereby ensuring fast and proper signal transmission. Gephyrin is a multi-domain protein with an N-terminal G-domain, a central C-domain and C-terminal E-domain. Despite its function in the CNS, gephyrin is ubiquitously expressed in all tissues due to its function in basic metabolism catalyzing the terminal steps in molybdenum cofactor biosynthesis. Tissue specificity of gephyrin is believed to rely on alternative splicing of the *GPHN* gene. A high diversity of gephyrin variants results from alternative splicing affecting the C-domain. Cassette C3 plays a role in molybdenum cofactor biosynthesis and is therefore highly expressed in liver and glia cells[1]. The C4 cluster harbors three alternatively spliced exons in rodents and an additional fourth one in humans, which are inserted at the same position between residues 288 and 289 (mouse numbering)[2]. Previous studies have reported the expression of cassettes C4a, C4c and C4d in the CNS[3]. In this study, we investigated the clustering of $GABA_AR$ by various gephyrin splice variants in cultured hippocampal neurons.
Following the depletion of endogenous gephyrin by shRNA-knock down in hippocampal neurons, we expressed different gephyrin slice variants and used confocal laser scanning microscopy to monitor the formation of gephyrin-positive clusters and determined their co-localization with either a presynaptic marker (vGAT) or α2 subunit-containing $GABA_AR$ clusters. On one hand, we found that gephyrin with either of the C4 splice cassettes is able to form postsynaptic clusters with similar properties as compared to gephyrin lacking a C4 cassette. However, presence of either of the C4 cassettes increased synaptic localization of gephyrin clusters and induced α2 subunit-containing $GABA_AR$ cluster formation. The opposite was found for gephyrin containing the C3 splice cassette, which increased size of gephyrin-positive clusters on the expense of the number of clusters localized at postsynaptic sites. Finally, using qRT-PCR we found a developmental expression of different gephyrin variants in hippocampal and cortical neurons.
[1] Smolinsky *et al.*, *J. Biol. Chem.* 2008, **283**:17370-17379

[2] Ramming *et al.*, *Proc Natl Acad Sci U S A.* 2000, **97(18)**:10266-71
[3] Paarmann *et al.*, *J. Biol. Chem.* 2006, **281**:34918-34925

Disclosures: **Y. Merkler:** None. **G. Schwarz:** None.

Poster

300. Postsynaptic Organization and Structure I

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: B.07. Synaptic Transmission

Support: EU grant No. 604102 (Human Brain Project)

Title: An automatic pipeline to optimize subcellular models of transynaptic signaling at inhibitory synapses

Authors: ***M. MIGLIORE**[1], C. A. LUPASCU[2], A. MORABITO[3], E. MERENDA[3], S. MARINELLI[3], C. MARCHETTI[3], R. MIGLIORE[1], E. CHERUBINI[3];
[2]Inst. of Biophysics, [1]Natl. Res. Council, Palermo, Italy; [3]European Brain Res. Inst., Rome, Italy

Abstract: Computational modeling of subcellular processes requires the tuning of many parameters that are difficult to determine from experimental data. We present an automatic approach to fit individual synaptic events recorded from voltage clamp experiments. Starting from any given kinetic model description (mod file) in the NEURON simulation environment, the procedure exploits user-defined constraints, dependencies, and rules for the parameters of the model to fit the time course of individual spontaneous synaptic events recorded experimentally. To test the efficiency of the pipeline, a custom Python code was implemented as a standalone task in the Collaboratory Portal of the Human Brain Project. The task is publicly available. Here we propose an use case scenario for testing the pipeline with a model implementing relatively simple gephyrin and gephyrin-dependent pathways, as a suitable example of a kinetic model of synaptic transmission. We used various sets of experimental data on GABAergic inhibitory synapses, recorded in hippocampal pyramidal CA1 neurons. The results suggest how and to what extent gephyrin-dependent subcellular pathways can shape synaptic events at the level of single cell, neuron, animal, or experiment. The correlation between the optimized model' parameters suggests how the transsynaptic signal can be strongly dependent not only on the postsynaptic gephyrin level, but also on the turnover rate of gephyrin-dependent presynaptic proteins, and on the extrasynaptic diffusion of neurotransmitter molecules. Small changes of these mechanisms can have large effects on the synaptic current, and these can be cell- or event-specific, with individual events that can be correlated with functional or pathological aspects at different levels.

Disclosures: **M. Migliore:** None. **C.A. Lupascu:** None. **A. Morabito:** None. **E. Merenda:** None. **S. Marinelli:** None. **C. Marchetti:** None. **R. Migliore:** None. **E. Cherubini:** None.

Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

Support: NIH Grant NS073700

IUPUI Biology Department

IUPUI UROP Program

Title: Consequences of mGluR5-dependent regulation of the spinophilin/sapap3 interaction.

Authors: ***C. W. MORRIS**[1], M. C. EDLER[2], A. J. BAUCUM, II[2,3];
[1]Chem. and Psychology, [2]Biol., Indiana Univ. Purdue Univ. at Indianapolis, Indianapolis, IN;
[3]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: Signaling perturbations in the basal ganglia result in a range of neuronal disorders. Specific molecular footprints that reside within the striatum—the major input nuclei of the basal ganglia—characterize many of these, however, understanding the molecular mechanisms regulating disease pathology remains of great interest. Moreover, indecorous inputs from the forebrain and midbrain result in altered synaptic protein phosphorylation and subsequent perturbations in synaptic protein complex formation and dendritic spine pathologies. Spinophilin, a protein phosphatase 1 targeting protein, functions to regulate the density of dendritic spines—which is attenuated in Parkinson's Disease (PD). Of these synaptic complexes, preliminary data demonstrates spinophilin interacts with SAP90/PSD95-associtated protein 3 (SAPAP3), and that this complex is significantly reduced in 6-hydroxy dopamine lesioned mice, a common model of PD. In itself, SAPAP3 has been associated with its ability to suppress obsessive-compulsive disorder (OCD)-like phenotypes in a metabotropic glutamate receptor 5 (mGluR5) dependent manner. Here, proteomic and pharmacological studies are performed to investigate the molecular events regulating the association of these proteins. Biochemically, we found that SAPAP3 associates with the coiled-coil region on spinophilin. Moreover, our preliminary data suggest that mGluR5 overexpression significantly increases the association of spinophilin and SAPAP3. Further studies will both delineate the mechanisms by which mGluR5 modulates this association as well as characterize the downstream functional and physiological consequences of modulating

this interaction. Data regarding this novel protein interaction may provide implications in underlying disease pathology, such as PD and OCD.

Disclosures: **C.W. Morris:** None. **M.C. Edler:** None. **A.J. Baucum:** None.

Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

Support: NIH Grant MH104736

NIH Grant NS039444

Title: Unraveling the inhibitory synapse proteome *In vivo*

Authors: ***A. UEZU**[1], D. J. KANAK[1], T. W. A. BRADSHAW[1], C. M. CATAVERO[1], A. C. BURETTE[2], R. J. WEINBERG[2], S. H. SODERLING[1];
[1]Duke Univ., Durham, NC; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: Inhibitory synaptic abnormalities result in multiple neurodevelopmental disorders that emerge in childhood, including autism spectrum disorders (ASD), epilepsy, neonatal hyperekplexia, and intellectual disability (ID). Like their excitatory counterparts, inhibitory synaptic properties are dynamic- their density, size, and receptor content is plastic during development and in response to activity. However compared to the excitatory synapse, the molecular components of the inhibitory synapse are not well defined, since this synaptic structure is not easily purified. This has impeded the systematic identification and analysis of the mechanisms underlying inhibitory synaptic function. Here we present a new *in vivo* chemico-genetic approach to isolate proteins of the inhibitory synapse. We find many proteins previously not known to target inhibitory synapses and we characterize these by biochemical, immunostaining, and electrophysiological approaches in neurons. One of the novel proteins, InSyn1, interacts with gephyrin and is localized to the inhibitory synapse in hippocampal neurons. Targeting of *InSyn1* in single neurons with the CRISPR/Cas9 system, results in a reduction in mIPSC frequency, suggesting InSyn1 is critical for functional inhibitory synapses. These data validate the feasibility of a novel proteomic approach to target synaptic structures and reveal new proteins important for the molecular machinery of the inhibitory synapse.

Disclosures: **A. Uezu:** None. **D.J. Kanak:** None. **T.W.A. Bradshaw:** None. **C.M. Catavero:** None. **A.C. Burette:** None. **R.J. Weinberg:** None. **S.H. Soderling:** None.

Poster

300. Postsynaptic Organization and Structure I

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 300.14/E18

Topic: B.07. Synaptic Transmission

Support: NSFC Grant

Title: Screening and functional analysis of neuroligin 1 interacting proteins

Authors: *R. DANG[1], A. LIU[1], W. XIE[1], Z. ZHOU[1], Z. JIA[2];
[1]Inst. of Life Sciences, Southeast Univ., Jiangsu, China; [2]Dept. of Physiology, Fac. of Medicine, Univ. of Toronto, Toronto, ON, Canada

Abstract: Neuroligin (NLG)1 is a postsynaptic cell adhesion protein that specifically localized on excitatory synapse. It can bind with both presynaptic Neurexins and postsynaptic PSD95, then functions as pre- and post-synaptic linkers and maintains the normal functions of the synapse. NLG1 is a type I membrane protein with a longer extracellular domain and a shorter intracellular domain, previous studies showed that the shorter intracellular domain can bind with various protein and has important functions. Conventional yeast two-hybrid system used C terminus of NLG1 as the bait. To screen for NLG1 binding proteins in its native condition, we performed DUALmembrane yeast two-hybrid system that can identify prey proteins using membrane-localized full-length NLG1. We found a number of novel NLG1 interacting proteins including Golgi apparatus membrane proteins, endoplasmic reticulum membrane proteins and transport vesicle proteins.

Disclosures: **R. Dang:** None. **A. Liu:** None. **W. Xie:** None. **Z. Zhou:** None. **Z. Jia:** None.

Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

Support: Project GENCODYS No. 241995

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Title: Ketamine-induced antidepressant effects and psychosis: A role for postsynaptic supercomplexes revealed using mouse genetic models and synaptome mapping.

Authors: *S. LEMPRIERE[1], J. NITHIANANTHARAJAH[2], F. ZHU[3], Z. QIU[1], N. H. KOMIYAMA[1], S. G. N. GRANT[1];
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Abstract: Ketamine is a widely-used anaesthetic and analgesic. At sub anaesthetic doses it causes a transient psychosis clinically indistinguishable from schizophrenia (Moore et al. 2011), this is followed by a long-lasting antidepressant response (Berman et al. 2000). Although it is known that ketamine is a non-competitive NMDA receptor antagonist (MacDonald et al. 1987), it is unclear how this receptor would mediate these different behavioural effects.
The NMDA receptor is assembled into supercomplexes with PSD95, Arc/Arg3.1 and other proteins via the critical role of the GluN2B cytoplasmic tail (Frank et al. 2016). Ketamine induces long-lasting (72hr) upregulation of PSD95 and Arc (Li et al. 2010), suggesting these molecules may be relevant to the dose- and time-dependent effects of ketamine.
To investigate the mechanisms used by the NMDA receptor to couple to PSD95 and Arc, we first examined ketamine-induced hyperlocomotion in mice carrying mutations in the cytoplasmic domains of GluN2A and GluN2B (Ryan et al. 2013) and knockouts of PSD95. Ketamine-induced hyperlocomotion was absent in mice lacking PSD95 and in mice with the cytoplasmic tail of the GluN2B NMDA receptor subtype replaced with that of the GluN2A receptor subtype.
Next we used a line of mice in which endogenous Arc was modified to express a fusion protein with Venus (Arc-Venus). These mice exhibit activity-dependent punctate postsynaptic labelling, which we quantify using high-throughput spinning-disk confocal microscopy in brain regions of adult mice. Since literature suggests that 10mg/kg ketamine induces a long-lasting (>24hr) antidepressant response (Ma et al. 2013), and 100mg/kg does not (Chatterjee et al. 2011), we treated Arc-Venus mice with these two doses. At 1hr the response of Arc to ketamine was the same for both doses: the density of Arc-Venus puncta was increased in dendritic sub-regions of hippocampus and in cortex. However, at 6hr post-ketamine the 100mg/kg dose produced a decrease in puncta number, while the earlier (1hr) increase was maintained in the 10mg/kg group.
These findings provide evidence for the involvement of the supercomplex formed by NMDA receptors, PSD95 and other proteins in the action of ketamine and the utility of genetically modified mice in dissecting signalling pathways in pharmacological studies.
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Ryan et al. (2013) Nat Neurosci, 16:25-32

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Poster

300. Postsynaptic Organization and Structure I

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Topic: B.07. Synaptic Transmission

Support: NIH Grant NS073700

IUPUI Biology Department

Title: Mechanisms underlying spinophilin-dependent regulation of the association of PP1 with the NMDA receptor

Authors: *A. BEIRAGHI SALEK[1], J. MCBRIDE[2], M. C. EDLER[1], A. J. BAUCUM, II[1,3]; [1]Biol., Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; [2]Northwestern Univ., Chicago, IL; [3]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: Normal brain function requires proper organization of downstream signaling pathways. This organization can be modulated by phosphorylation of synaptic proteins. Protein phosphorylation is a balance of phosphatases, such as protein phosphatase 1 (PP1), and kinases such as protein kinase A (PKA) and cyclin dependent kinase 5 (CDK5). This proper targeting of synaptic proteins is critical for their normal function and is perturbed in various disease states. Spinophilin is critical in targeting PP1 to various substrates, making it important in regulating the phosphorylation state and thus the function of synaptic proteins such as the NMDA-type glutamate receptors. NMDARs are abundant postsynaptic proteins that are critical for normal synaptic communication. It has been reported that NMDAR phosphorylation modulates channel function. Here we aim to understand the role that spinophilin plays in targeting PP1 to the NMDAR as well as mechanisms by which the spinophilin/NMDAR interaction are altered. We have found that the presence of spinophilin actually decreases the amount of PP1 bound to the GluN2B subunit of the NMDAR in a heterologous cell system. This effect required spinophilin binding to PP1 as it was not observed when a PP1 binding-deficient spinophilin mutant (F451A)

was expressed. Furthermore activation of endogenous PKA and/or overexpression of PKA catalytic subunit robustly increased the association between spinophilin and the GluN2B subunit of the NMDAR. Conversely, these associations were decreased when CDK5, along with its activator, p35, was overexpressed. Our future studies will evaluate the role of spinophilin in regulating the phosphorylation state of the NMDAR. Taken together, our data demonstrate that spinophilin can associate with the NMDAR in HEK293 cells and that protein kinases can biphasically modulate this association.

Disclosures: **A. Beiraghi Salek:** None. **J. McBride:** None. **M.C. Edler:** None. **A.J. Baucum:** None.

Poster

301. Modulation: Pharmacology

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Topic: B.07. Synaptic Transmission

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MOST 104-2321-B-010-021

MOST 104-2745-B-010-003

Title: Chemogenetic control of hilar mossy cell excitability regulates emotional behaviors

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Abstract: Hilar mossy cells (MCs), the glutamatergic neurons located in the hilus of the hippocampal dentate gyrus (DG), have been known to play an important role in network function and complex information processing such as pattern separation. However, their role in emotional behavior remains largely unexplored. To address this question, we bidirectionally manipulated the activity of MCs in the dorsal DG using a chemogenetic approach. Selective expression of designer receptors exclusively activated by designer drugs (DREADDs) was achieved by injecting a virus encoding Cre-dependent DREADDs into a mossy/CA3-Cre driver, a mouse line specifically expressing Cre recombinase in the hilar MCs and CA3 cells. By specifically expressing inhibitory DREADDs (i.e., hM4Di receptor) on the membrane of the MCs in the dorsal DG, we found that decreasing the activity of MCs increased the mouse anxiety level.

Conversely, elevating the activity of MCs by expressing excitatory DREADDs (i.e., hM3Dq receptor) decreased their anxiety level. In addition, we tested the effect of manipulating the MC activity during and after contextual fear conditioning. We found that decreasing the MC activity induced the fear-like behavior in the retrieval phase, although mice performed normally during learning phase. In contrast, elevating the MC activity reduced the freezing level in the retrieval phase. In summary, switching the MC activity of MCs by using chemogenetic manipulations, we demonstrated that MCs in dorsal DG would participate in
controlling the anxiety- and fear-like behaviors.

Disclosures: K. Wang: None. **C. Lien:** None.

Poster

301. Modulation: Pharmacology

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Program#/Poster#: 301.02/E22

Topic: B.07. Synaptic Transmission

Support: BMBF Grant 01EE1403C

National Science Council, Taiwan

Title: Acute and chronic noradrenergic effects on cortical excitability in healthy humans

Authors: *M.-F. KUO[1], H.-I. KUO[1,2], W. PAULUS[2], G. BATSIKADZE[2], A. JAMIR[2], M. A. NITSCHE[1,3];
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Abstract: Noradrenaline has been proposed as a critical neuromodulator in the central nervous system, which in turn is thought to influence functional recovery from neuropsychiatric diseases. Previous transcranial magnetic stimulation (TMS) studies have shown that acute selective noradrenaline reuptake inhibitor (NRI) application enhanced cortical excitability in humans. However, it usually takes prolonged treatment for selective NRI to get the therapeutic effects in clinical populations. The purpose of the study is to investigate the acute and chronic effects of selective NRI reboxetine on cortical excitability by applying different TMS protocols in healthy humans with a double-blinded, placebo-controlled, randomized crossover design. 16 subjects were assessed with different TMS measurements: motor thresholds (MTs), input-output curve (I-O curve), short-latency intracortical inhibition (SICI) and facilitation (ICF), I-wave facilitation,

and short-latency afferent inhibition (SAI) before and after placebo or reboxetine (8mg) administration. Afterwards, the same subjects took reboxetine (8mg/ day) consecutively for 21 days. During this period, TMS measurements were assessed before and after drug intake (placebo or reboxetine) on the day of experimental sessions. Both acute and chronic administration of reboxetine increased cortical excitability with regard to the enhancement of I-O curve, ICF, and I-wave facilitation, as well as decreased SICI and SAI compared to placebo condition. The results demonstrate that reboxetine enhances cortical excitability which might be a mechanism underlying the beneficial effect of reboxetine in neuropsychiatric diseases, as chronic reboxetine showed much more stable enhancement of ICF and I-wave facilitation. The finding might partially explain the prolonged treatment impact of selective NRI in clinical application.

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Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant MH 049469 to DHF

NIH Grant GM008541 to DHF

Title: Neurosteroid induction of NMDA and AMPA receptor trafficking

Authors: ***V. KUMARESAN**[1], K. SUGUNAN[1], R. M. BADOLATO[1], R. SINGH[1], J. LUEBKE[2], J. M. ADAMS[1], D. H. FARB[1];
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Abstract: Neurosteroids modulate both excitatory and inhibitory neurotransmission in the CNS. Recently, we reported that physiological concentrations of pregnenolone sulfate (PregS) (low picomolar) increase intracellular Ca^{2+} and CREB phosphorylation via a synaptic but not extrasynaptic NMDA receptor-dependent mechanism. We have previously reported that micromolar PregS stimulates the trafficking of GluN1 subunits to the cell surface of *Xenopus laevis* oocytes and cultured rat cortical neurons (7 DIV) as determined by electrophysiology and surface labeling respectively. Here we report that a 10^6 - fold lower concentration of PregS (50

pM for 10 minutes) increases surface GluN2A-containing NMDA receptors but surprisingly, with no change in surface GluN1 as determined by immunofluorescence. Increase in surface GluN2A-containing NMDA receptors is casein kinase 2 (CK2), L-Type voltage gated channel and GluN2B-containing NMDA receptor dependent mechanism. PregS does not alter the association of GluN2A with PSD 95, suggesting that the increase in GluN2A-containing NMDA receptors is initially extrasynaptic. PregS increases AMPA receptor mediated sEPSCs by ~2-fold without altering sEPSC amplitude or passive membrane properties. PregS also induces AMPA receptor trafficking, increasing surface and synaptic GluA1-containing AMPA receptors. Physiological concentrations of PregS therefore modulates critical aspects of synaptic plasticity by either increasing the frequency of neurotransmitter release or activating silent synapses. This research is a summary of the doctoral dissertation for Kavitha Sugunan Dissertation Research is presented

Disclosures: V. Kumaresan: None. **K. Sugunan:** None. **R.M. Badolato:** None. **R. Singh:** None. **J. Luebke:** None. **J.M. Adams:** None. **D.H. Farb:** None.

Poster

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Support: CONACYT-México, grant 219293

CONACYT-México CB-2011-01-166241

INFR-2012-01-187757

Title: Chronic toluene exposure alters medial prefrontal cortex synaptic transmission of adolescent rats

Authors: *M. I. TORRES-FLORES, S. L. CRUZ, E. J. GALVÁN;
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Abstract: Toluene is an organic solvent commonly misused by adolescents for its psychoactive effects. It has been reported that chronic toluene exposure produces learning and memory deficits, particularly in working memory. Toluene exerts its actions in the central nervous system by blocking NMDA receptors and increasing $GABA_A$-mediated responses. In addition, toluene alters the firing rate and the spike threshold of pyramidal neurons of medial prefrontal cortex (mPFC). Toluene also increases dopamine (DA) release, which modulates synaptic activity in the

mPFC. However, it is not known if toluene modifies synaptic transmission or the modulatory actions of DA in animals chronically exposed to this solvent. Male Wistar rats (22 days of age) were exposed to toluene (8000 ppm) 30 min, twice a day, for 10 days. Immediately after the last exposure, animals were euthanized to obtain coronal brain slices containing the mPFC. Extracellular field recordings were performed in mPFC layer V neurons. Our results show that chronic toluene exposure induces: a) increased synaptic responsiveness, evaluated by mean of input-output curves (I-O curves; n=9); b) increased excitatory postsynaptic potentials (fEPSP) in response to smaller presynaptic volleys (n=9), and c) decreased interneuron-mediated recurrent inhibition assessed by a paired-pulse protocol (n=9). In another group of experiments, stimulation with exogenous DA (100 µM, 10 min) induced a biphasic effect on the mPFC fEPSP; an early depression of synaptic responses followed by a long-lasting enhancement of the synaptic responses (n=9). In contrast, animals exposed to toluene exhibited a sustained fEPSP depression (n=9). In conclusion, our results show that chronic toluene inhalation induces hyperexcitability and reduces the GABAergic inhibition in the mPFC circuit. In addition, toluene alters mPFC dopaminergic modulation. These effects might underlie the cognitive dysfunctions observed in animals chronically treated with toluene.

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Poster

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Program#/Poster#: 301.05/E25

Topic: B.07. Synaptic Transmission

Title: Effect of Carbenoxolone on gap junction in the hippocampus of rats with epileptiform activity induced by 4-aminopyridine.

Authors: *C. VENTURA[1], R. BELTRÁN-RAMÍREZ[2], S. D. CONTRERAS-DELATORRE[3], G. ZARATE-RODRÍGUEZ[3], B. VILLANUEVA-AVALOS[3], N. S. MUÑOZ-FILIPPETTI[3]; [1]Ctr. De Enseñanza Técnica Industrial, Guadalajara, Jalisco, Mexico; [2]Sistemas de Información, Univ. de Guadalajara, Guadalajara, Mexico; [3]Ctr. de Enseñanza Técnica Industrial, Guadalajara, Mexico

Abstract: Epilepsy is a neuronal disorder that affects people worldwide which is characterized by the continued discharge and abnormally synchronic activity observed in a group of neurons. We have examined the influence of the gap junction and the gap junctional blocker, carbenoxolone (CBX), on epileptiform activity induced by 4-aminopyridine (4-AP) in the rat entorhinal cortex (EC) and the CA1 hippocampal region. A cannula and deep electrodes were

implanted into the brain to administer drugs and to monitor electrical activity. The deep electrodes will be implanted with a new automated system implantation of depth electrodes, to avoid manipulation of the people on the rat and avoid the generation of noise during the EEG record. This system implantation of depth electrodes will have a vertical movement to lead to different levels of depth this will give us the option to register several brain areas in the hippocampus. The injection of 4-AP (10 nmol) produced epileptiform discharge trains of high amplitude and frequency associated with seizure behavior rated between 0 and 5 in the Racine scale. On the other hand are evidence for the involvement of gap junctions (electrical synapses) in epilepsy, however, there are not studies related to the modulation on epileptiform activity by electrical synapses through an automated system implantation of depth electrodes. In this work we investigated the effect of carbenoxolone on epileptiform activity in the hippocampus of rats with seizures induced by 4-aminopyridine in order to determine the possible modulation of epileptiform activity by electrical synapses with an automated system implantation of depth electrodes. For these purpose intracranial EEG recordings from hippocampus were made in Wistar rats with seizures induced by EC 4-aminopyridine administration (10nmol), to determine the amplitude of the epileptiform activity, the duration of each seizure of the epileptiform activity, the frequency (Hz) of the seizure before and during carbenoxolone administration (dosage 50nmols) into the entorhinal cortex. During carbenoxolone injection a decrease in the amplitude of epileptiform activity in anterior hippocampus (80%) and posterior hippocampus (85%) was observed, as well in the duration for each seizure (85%, anterior hippocampus; 89%, posterior hippocampus), while the average of the seizure frequency decreased (89%, anterior hippocampus; 90%, posterior hippocampus) compared to data obtained before carbenoxolone administration. These findings suggest a possible modulation of epileptiform activity by electrical synapses, but are necessary to corroborate the present data with more additional studies.

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Poster

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Topic: B.07. Synaptic Transmission

Support: NIH Grant AA022937

Title: Restraint stress differentially alters Beta1- and Beta2-Adrenergic Receptor modulation of glutamatergic transmission in the Ventral Bed Nucleus of the Stria Terminalis

Authors: *Y. SILBERMAN;
Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA

Abstract: The bed nucleus of the stria terminalis (BNST) plays a critical role in physiologic responses to stress. Stress enhances norepinephrine signaling to the BNST, but the mechanism by which stress and norepinephrine modulate BNST glutamatergic neurotransmission is not fully understood. Previous work has established that stimulation of beta1-Adrenergic Receptors (β1-ARs) enhances glutamatergic transmission in the dorsal lateral subregion of the BNST (dBNST) via modulation of local corticotropin releasing factor (CRF) signaling. Few studies have, however, examined potential mechanisms by which β-AR activation may modulate glutamatergic transmission in the ventral lateral subregion of the BNST (vBNST). This study therefore examined mechanisms by which β1- and β2-ARs modulate spontaneous excitatory postsynaptic currents (sEPSCs) in the vBNST of adult male C57Bl/6J mice utilizing whole-cell patch clamp electrophysiology in acutely prepared brain slices. Mice were either stress naïve or exposed to a single 1 hour restraint stress followed by 30 min in their home cage prior to study. In stress-naïve mice, bath application of the β-AR agonist Isoproterenol (3μM, 10 min) significantly enhanced vBNST sEPSC frequency (88.0 ± 14.4% increase from baseline, $p<0.001$) with no significant changes to sEPSC amplitude (6.0 ± 7.0 % decrease from baseline, $p>0.05$). Similar to previous findings in the dBNST, Isoproterenol enhancement of vBNST sEPSC frequency could be blocked by pretreatment with the CRFR1 antagonist NBI27914 (7.0 ±13.1% increase from baseline, $p>0.05$). In contrast to previous work in the dBNST, where restraint stress functionally occludes further effects of Isoproterenol, restraint stress did not alter Isoproterenol enhancement of vBNST sEPSC frequency (59.6± 20.4% increase from baseline, $p<0.05$; Isoproterenol effects in stress vs. naïve mice, $p>0.05$). Bath application of the β2-AR agonist, Clenbuterol (10μM, 10 min), did not alter vBNST sEPSC frequency in naïve mice (3.9±8.3% increase from baseline, $p>0.05$) but significantly inhibited sEPSC amplitude (20.8 ±4.9% decrease from baseline, $p<0.01$). In stress-exposed mice, Clenbuterol surprisingly enhanced sEPSC frequency (42.4±17.4% increase from baseline, $p<0.05$) even though basal sEPSC frequency is significantly higher in stress mice compared to naïve mice (7.5±1.3 Hz vs. 3.6±0.6 Hz, $p<0.05$). These results suggest dissociable pre- and post-synaptic effects of β1- and β2-AR stimulation on vBNST excitatory synaptic transmission in naïve mice and novel emergence of β2-AR mediated enhancement of presynaptic glutamate release in the vBNST of stress-exposed mice.

Disclosures: Y. Silberman: None.

Poster

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Topic: B.07. Synaptic Transmission

Support: RFBR-2014 14-04-00391

Title: Peptidergic modulation of spontaneous and evoked synaptic activity in ca1 pyramidal cells of rat hippocampal slices.

Authors: *V. G. SKREBITSKY[1], R. KONDRATENKO[2], I. POVAROV[2], S. KOLBAEV[2]; [1]Res. Ctr. of Neurology., Moskva, Russian Federation; [2]Res. Ctr. of Neurol., Moskva, Russian Federation

Abstract: The aim of this study was to investigate whether novel prolin- containing dipeptide Noopept (NP) influences synaptic transmission in central neurons. NP was synthesized as peptide analog of piracetam which is known as one of the first generation nootropics racetam group (Giurgea, 1972; Winblad, 2005). NP is similar to piracetam in its chemical structure and memory- enhancing ability but displays the effect in much lower concentration. In addition to nootropic activity NP also displays an anxiolitic effect (Ostrovskaya et al, 2006). We examined the effect of NP on spontaneous and evoked IPSCs in CA1 pyramidal cells in rat hippocampal slices using patch-clamp technique in whole- cell configuration. It was found that NP(1μM) increased spike- dependant release of GABA from terminals of inhibitory interneurons on CA1 pyramidal cells. The effect manifested itself in the increase of amplitude and frequency of spontaneous TTX-sensitive sIPSCs whereas TTX- non sensitive mIPSCs remained unchanged. We also found that IPSCs evoked by Shaffer collaterals stimulation, either short -latency (feed-forward), or long- latency (feed-back) ones increased after NP application. We hypothesized that NP directly excited inhibitory interneurons which terminate on CA1 pyramidal cells. To check this hypothesis we performed current clamp egistration of several (n =5) interneurons resigning in stratum radiatum (SR). In all cases NP induced a 2-3 fold increase of spiking rate that was accompanied by depolarization of cell membrane to 3-5 mV. In the experiments with direct measurement of neuronal [Ca2+]i in hippocampal organotypic slices we revealed that NP selectively increased [Ca2+]i activity in SR interneurons without significantly changing it in stratum pyramidal neurons. Taken together these date clarify that the target of NP action is inhibitory interneurons located in SR of hippocampus.

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Poster

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Topic: B.07. Synaptic Transmission

Support: R21MH10069

R01MH096274

Title: L-proline, a metabolite linked to neuropsychiatric disorders and associated with the 22q11.2 deletion syndrome, specifically disrupts GABA-ergic transmission in the mPFC.

Authors: *G. W. CRABTREE[1], J. A. GOGOS[2];
[1]Physiol. and Cell. Biophysics, [2]Dept. of Neurosci., Columbia Univ. Med. Ctr., New York, NY

Abstract: Accumulation of L-proline within the CNS,has been repeatedly correlated with predisposition to psychotic disorders. Proline dehydrogenase (PRODH), which degrades L-proline, resides within the 22q11.2 deletion a strong genetic risk factor for schizophrenia further suggesting L-proline may be neuroactive and directly contribute significantly to neuropsychiatric symptomology. Despite the strength of these clinical data, targets affected by disease-relevant concentrations of L-proline have not been convincingly described. Using an mouse model with a severely hypomorphic *Prodh* allele and employing a range of biochemical and electrophysiological assays in acute brain slices, cultured neurons, and heterologous expression systems we assessed L-proline impact upon neuronal function. At layer II/III mPFC pyramidal neurons we found that both basic synaptic transmission and very short-term synaptic plasticity of both glutamatergic and GABA-ergic transmission were unaltered. Similarly, medium to high frequency evoked glutamatergic transmission was unaltered in *Prodh*-mutant mice. We found, however, that mice with elevated CNS L-proline showed specific disruptions of high-frequency GABA-ergic transmission, consistent with deficits in sustained GABA release. Here we show that L-proline is GABA-mimetic and can act at multiple GABA-ergic targets *in vitro*. L-proline can activate both GABA-A and GABA-B receptors although this is only observed at L-proline concentrations far above CNS disease-relevant levels. At disease-relevant concentrations, L-proline GABA-mimesis was limited to competitive blockade of glutamate decarboxylase (GAD) leading to reductions in GABA production. The disruptions of high-frequency GABA-ergic transmission we observed in *Prodh*-mutant mice could be mimicked in WT mice with acute pharmacological blockade of GAD. Further, sustained GABA release deficits observed in *Prodh*-mutant mice could be reversed by enhancing net GABA production through pharmacological blockade of GABA transaminase. Together these findings suggest a novel neural mechanism in neuropsychiatric disorders whereby disease-linked mutations lead to accumulation of neuroactive metabolites that disrupt precise molecular targets leading to circumscribed, well-

defined synaptic dysfunction. (This work was supported by NIH grants R21MH10069 and R01MH096274).

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Poster

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Topic: B.07. Synaptic Transmission

Support: sponsored by the Institut de Recherches Internationales Servier

Title: Electrophysiological signatures of modulation of GABA-A α5 receptor activity by S 44819 in human cortex

Authors: *G. DARMANI[1], C. ZIPSER[1], G. M. BÖHMER[2], F. MÜLLER-MAHLHAUS[1], P. BELARDINELLI[1], M. SCHWAB[3], K. DESCHET[4], U. ZIEMANN[1];
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Abstract: Combining transcranial magnetic stimulation (TMS) and electromyography (EMG) or electroencephalography (EEG) allows to record in real time changes of human cortex excitability. Active motor threshold (AMT) and stimulus intensity to evoke a motor evoked potential of 0.5mV (SI0.5mV) are two single-pulse TMS-EMG measures representing corticospinal excitability. Also, recent findings provided evidence that the amplitude of two negative EEG deflections elicited by a single TMS pulse over primary motor cortex (M1), namely N45 and N100, reflects GABA-A and GABA-B receptor activation, respectively (Premoli et al., 2014). These measures provide a novel way to characterize drug effects on the GABAergic systems in the human cortex non-invasively. *In vitro*, S 44819 is a potent and competitive selective antagonist of GABA-A receptors that interacts selectively at the GABA-binding site of α5 subunit. The GABA-A α5 receptor is located extrasynaptically and has been shown to be involved in tonic inhibition controlling pyramidal neuron excitability. Its overactivation contributes to increased tonic inhibition in the peri-infarct zone after ischemic stroke. Investigating to what extent S 44819 is able to decrease tonic inhibition *in vivo* in humans is of much interest, as this may lead to a clinical trial to test its efficacy to enhance functional recovery after stroke. Here, we investigated the pharmacological effects of S 44819 on cortical

and corticospinal excitability measured by TMS-EMG and TMS-EEG in 18 healthy male volunteers. A randomized, placebo-controlled, double-blind crossover phase I clinical study was conducted, testing a single oral dose of 50mg and 100mg of S 44819. At the dose of 100mg S 44819 significantly decreased AMT and SI0.5mV, i.e. increased corticospinal excitability in response to TMS. In addition, the 100mg dose of S 44819 induced a significant decrease in the N45 amplitude, an effect opposite to benzodiazepines, while the N100 amplitude remained unaffected. These findings suggest that the GABA-A α5 receptor plays a role in generation of the N45 potential. Results also suggest that S 44819 increases neuronal excitability by reducing GABA-A receptor mediated inhibition and thus might be a promising candidate drug for improving functional recovery in post-stroke patients.
Acknowledgements: *This study was sponsored by the Institut de Recherches Internationales Servier.*
Premoli I., Castellanos N., Rivolta D., Belardinelli P., Bajo R., Zipser C., Espenhahn S., Heidegger T., Müller-Dahlhaus F., Ziemann U. (2014). TMS-EEG signatures of GABAergic neurotransmission in the human cortex. J. Neurosci., 34(16):5603-5612.

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Poster

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Topic: B.07. Synaptic Transmission

Support: National Research Foundation of Korea(NRF) Grant No. 2015R1D1A3A01016360

Title: Effects of olanzapine and haloperidol on mtorc1 signaling, dendritic outgrowth, and synaptic proteins in rat primary hippocampal neuron under toxic conditions

Authors: M. SEO[1], H. CHO[1], C. LEE[1], Y. KIM[1,2,3], J. LEE[1,2,3,4], *S. PARK[1,3];
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Abstract: Purpose: The aim of the present study was to determine whether alterations in mTORC1 signaling are observable following treatment with olanzapine and haloperidol under toxic conditions. Additionally, we investigated whether these drugs affect dendritic outgrowth and synaptic proteins expression through mTORC1 signaling pathway.

Methods: Using Western blotting, we measured changes in the mTORC1-mediated proteins and synaptic proteins under toxic conditions induced by B27 deprivation, which causes hippocampal cell death. Dendritic outgrowth was determined by neurite assay.
Results: Olanzapine significantly increased phosphorylation levels of mTORC1, its downstream effectors (4E-BP-1, p70S6K, eIF4B, and S6), and upstream effectors (Akt and ERK), whereas haloperidol did not affect the levels of these proteins. Increased phosphor-mTOR induced by olanzapine was significantly blocked in the presence of specific PI3K, MEK or mTOR inhibitors. Olanzapine also increased dendritic outgrowth and synaptic proteins levels (BDNF, PSD-95, and synaptophysin); all of these effects were blocked by rapamycin. However, haloperidol had no effect in these regards.
Conclusions: In current study, we demonstrated that olanzapine activates the mTORC1 signaling pathway and increases dendritic outgrowth and synaptic proteins through mTORC1 signaling activation in rat primary hippocampal neurons.

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Poster

301. Modulation: Pharmacology

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Topic: B.07. Synaptic Transmission

Title: Electrophysiological characterization of S 47445, a novel positive allosteric modulator of AMPA type glutamate receptors

Authors: *L. DANOBER[1], T. SCHAER[2], K. KAMBARAT[2], F. MARGER[2], S. BRETIN[3], D. BERTRAND[2];
[1]Inst. De Recherches SERVIER, PIT-NPS, Croissy sur Seine, France; [2]HiQScreen Sarl, Geneva, Switzerland; [3]Neuropsychiatry Innovation Pole, Inst. de Recherches Internationales Servier, Suresnes Cedex, France

Abstract: Positive allosteric modulators (PAM-AMPAs) have been described and developed for the treatment of schizophrenia, depression or Alzheimer's disease (Ward et al., 2010; Pirotte et al., 2013). We previously report the in vitro characterization of the compound S 47445, a novel PAM-AMPA on different classes of ionotropic glutamate receptors (Danober et al., SFN 2014). Here, the modulatory function of S 47445 was conducted at recombinant human AMPA receptors using GluA1, GluA2 and GluA4 flip and flop variants expressed in *Xenopus* oocytes. GluA2 were expressed as heteromeric complexes with GluA1 and GluA4 subunits. Finally, the

identification of the allosteric binding site of the compound was assessed using an AMPA-kainate chimera approach encompassing segments from the AMPA GluA1flop subtype and the kainate receptors. S 47445 dose-dependently potentiated currents evoked by a saturated concentration of glutamate (100 μM) in oocytes expressing homomeric GluA1 and GluA4 flip and flop splice variants. EC_{50} values did not differ among the different GluA recombinants. However, some difference was observed for GluA4 subtypes as GluA4o presented a lower EC_{50} value compared to GluA4i subtypes ($p \leq 0.001$, unpaired T-test). Moreover, the amount of potentiation evoked by S 47445 (100 μM) was consistently much greater for flop variants (around 9 fold more) compared to flip variants at GluA1 AMPA receptors. Analysis of the glutamate evoked responses recorded in *Xenopus* oocytes or in HEK293 cells using patch clamp revealed that S 47445 reduced receptor desensitization. For the AMPA-kainate chimera approach, the selectivity of S 47445 towards AMPA receptors was first confirmed on human receptors, since S 47445 up to 100 μM did not modified glutamate-evoked current on human GluK2 kainate receptors. The potentiation of glutamate-evoked current also persisted after S 47445 application on AMPA/Kainate chimera GluA1(K2NTD), with exchange of amino-acid segment at the N-Terminal portion and presented an EC_{50} similar to those obtained on GluA1flop subtype (4.2 μM [1.3; 13.6], n=5). In contrast, the effect of S 47445 was lost in AMPA/Kainate chimerae GluA1(K2S1), with exchange of the amino-acid segment corresponding to the putative binding site of AMPA positive modulators. Altogether these data illustrate that S 47445 acts as a powerful allosteric modulator at all human AMPA subunits tested with greater amount of potentiation on flop variants. S 47445 was shown to slow AMPA receptor desensitization and bind to the same binding pocket located on the ligand-binding domain (LBD) near the first transmembrane domain as previously described for other PAM-AMPAs (Lynch, 2006).

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Poster

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Program#/Poster#: 301.12/E32

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS078184

Title: Effects of the antipsychotic drug loxapine on synaptic transmission in the superficial lamina of the dorsal horn

Authors: *K. EVELY[1,2], S. HAJ-DAHMANE[3], A. BHATTACHARJEE[4];
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Abstract: Loxapine is an antipsychotic drug that is clinically approved for the treatment of schizophrenia and is patented for its potential to treat pain. It is also a known opener of the sodium-activated potassium channel Slack (Kcnt1) and has been shown to ameliorate neuropathic pain behavior in Slack-dependent manner (Lu et al., 2015). Slack channels are expressed in the dorsal root ganglion and spinal cord dorsal horn that transmit pain information from the periphery to the brain. The underlying mechanism and level of the pain pathway responsible for the analgesic effects of loxapine remain unknown. Here, we investigate the effect of loxapine on glutamatergic synaptic transmission in the superficial, pain processing lamina of the dorsal horn. Transverse slices of the lumbar spinal cord were prepared and whole cell patch clamp recordings were performed. Intrinsic firing properties, as well as, spontaneous and evoked excitatory postsynaptic currents (EPSCs) were measured. Neurons were classified as inhibitory or excitatory based on previously described firing phenotypes (Punnakkal et al., 2014; Smith et al., 2015). Regardless of classification, bath application of loxapine (50μM) decreased the frequency but not the amplitude of spontaneous EPSCs, indicating a decrease in the network activity of lamina II. To investigate primary afferent synapses, we measured the amplitude EPSCs evoked by electrical stimulation of the dorsal root entry zone. We found that bath application of loxapine increased the amplitude of EPSCs in neurons classified as inhibitory but not excitatory. This suggests that at primary afferent synapses, loxapine acts to strengthen input to inhibitory interneurons within lamina I/II. Ongoing studies will investigate the underlying mechanism and incorporate Slack knockout mice to determine whether channel activation contributes to the effects of loxapine reported here.

Disclosures: K. Evely: None. **S. Haj-Dahmane:** None. **A. Bhattacharjee:** None.

Poster

301. Modulation: Pharmacology

Location: Halls B-H

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Program#/Poster#: 301.13/E33

Topic: B.07. Synaptic Transmission

Support: AFM - 17117

ANR-14-CE13-0037-03

Title: Dystrophins in the cerebellum: a first look at the role of Dp71 role in Purkinje neurons and Bergmann glia.

Authors: *R. HELLERINGER, O. JOLY, M. BELMAADI-CHERKAOUI, H. DANIEL, C. VAILLEND, M. GALANTE;
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Abstract: In Duchenne muscular dystrophy (DMD), the genetic loss of dystrophin leads to fatal muscle weakness. In addition to muscle, dystrophins are largely expressed in the nervous system (yielding full-length dystrophin and its truncated isoforms) and their loss may explain the cognitive disturbances reported in DMD patients consisting in low IQ mental retardation and verbal processing impairment.
Dp71, a short isoform of dystrophin, is the most prominent dystrophin in the adult brain. The brain mechanisms that involve Dp71, as well as the functional outcome of Dp71 deficiency, are still poorly understood and appear to be quite complex, as Dp71 likely endorses multiple functions in brain due to its expression in both neuronal and glial cells. In fact a fraction of Dp71 is expressed at glutamatergic synapses and normally interacts with adapter proteins associated with glutamate receptor complexes. Nonetheless, Dp71 is mainly located in glial cells where it is required for proper localization of Kir4.1 and AQP4 channels to plasma membrane suggesting that its loss may affect extracellular K^+ buffering and water balance.
In this work, we want to study the role of Dp71 in the cerebellum as clinical studies recently highlighted a role of cerebellar-dependent cognitive dysfunctions in DMD patients. We first focused on the glutamatergic transmission at parallel fiber/climbing fiber synapses onto Purkinje neurons, the only output element of the cerebellar cortex. Patch clamp experiments and pharmacology revealed that electrically evoked excitatory post-synaptic currents (EPSCs) are altered in Purkinje neurons from Dp71-null mice. Furthermore, our immunohistochemistry results had shown that in the cerebellar cortex, Dp71 seems to localize in Bergmann glia processes, in particular at perivascular end-feet and at the *glia limitans*. On the basis of the pivotal function of these radial glial cells in the control of extracellular ion homeostasis, we are investigating the ability of Bergmann cells to buffer extracellular ions in WT and Dp71-null mice combining patch clamp, optogenetics and ion-sensitive microelectrode recordings.
This work will allow a preliminary evaluation of the impact of Dp71 loss on cerebellar neuron and glial functions.

Disclosures: R. Helleringer: None. **O. Joly:** None. **M. Belmaadi-Cherkaoui:** None. **H. Daniel:** None. **C. Vaillend:** None. **M. Galante:** None.

Poster

301. Modulation: Pharmacology

Location: Halls B-H

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Program#/Poster#: 301.14/E34

Topic: B.07. Synaptic Transmission

Support: MDA Grant 295271

NIH grant NS090644

Title: Synergistic effects on calcium entry and transmitter release of a potassium channel blocker and a calcium channel gating modifier at the NMJ

Authors: R. LAGHAEI[1], M. WU[2], A. PUGLIONESI[2], T. TARR[2], M. DITTRICH[1], *S. D. MERINEY[3];
[1]Pittsburgh Supercomputing Ctr., Carnegie Mellon Univ., Pittsburgh, PA; [2]Neurosci., [3]Univ. Pittsburgh, Pittsburgh, PA

Abstract: We have previously reported the development of novel, first-in-class, Cav2 gating modifiers (GV-58) that prolong channel deactivation. These compounds effectively increase calcium flux during an action potential by stabilizing the open state of the channel. At synapses, this effect increases neurotransmitter release, and these compounds are being developed as potential treatments for diseases that cause neuromuscular weakness. Because these calcium channel gating modifiers require that the channel be in the open state before they can modify gating, and since action potentials are normally very brief (1-2 msec), only a fraction of channels are typically modified. This is relevant to potential treatment of neuromuscular diseases like Lambert-Eaton myasthenic syndrome (LEMS) since these patients are currently managed using a potassium channel blocker (DAP) that broadens the presynaptic action potential. DAP provides modest symptomatic relief for LEMS patients, and we hypothesize that a combination of DAP plus our calcium channel gating modifier would work synergistically to provide a stronger and more complete relief of neuromuscular weakness. Before moving into in vivo animal models, we have explored the dose-response relationship of this hypothesis in a previously validated MCell model of the presynaptic neuromuscular active zone. The advantage of this computer modeling approach is that we can explore various combinations of these two pharmacologic agents, and study the spatio-temporal dynamics of presynaptic calcium influx and the subsequent impact on transmitter release. Within MCell, we modeled DAP effects by increasing the amplitude (5-10%) and prolonging the decay time (5-15%) of the presynaptic action potential. To model the effects of GV-58, we edited our calcium channel gating scheme to include drug bound states with kinetic rates that resulted in modeled calcium current that matched our patch clamp recordings of calcium current modulation by GV-58. Then we used these two modifications in both control and LEMS MCell model active zone architectures to evaluate the effects on transmitter release.

In addition, we extracted from the model the spatio-temporal dynamics of buffered calcium diffusion within the nerve terminal, and also the stochastic dynamics of calcium ion binding to synaptotagmin-based calcium sensors on docked synaptic vesicles. We find that broadening the presynaptic action potential increases the effectiveness of our gating modifier, and we are currently studying the dose-response details of this synergistic effect using our MCell model before moving to in vivo testing in animals.

Disclosures: **R. Laghaei:** None. **M. Wu:** None. **A. Puglionesi:** None. **T. Tarr:** None. **M. Dittrich:** None. **S.D. Meriney:** None.

Poster

301. Modulation: Pharmacology

Location: Halls B-H

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Program#/Poster#: 301.15/E35

Topic: B.07. Synaptic Transmission

Support: IBS-R002-D1 to E.K.

Title: Trans-synaptic zinc mobilization improves social interaction in two mouse models of autism through NMDAR activation

Authors: ***H. LEE, V**[1], E.-J. LEE[2], T.-N. HUANG, 305-701[3], C. CHUNG[2], W. SHIN[2], K. KIM[2], J.-Y. KOH[4], Y.-P. HSUEH[3], E. KIM[2];
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Abstract: Genetic aspects of autism spectrum disorders (ASDs) have recently been extensively explored, but environmental influences that affect ASDs have received considerably less attention. Zinc (Zn) is a nutritional factor implicated in ASDs, but evidence for a strong association and linking mechanism is largely lacking. Here we report that trans-synaptic Zn mobilization rapidly rescues social interaction in two independent mouse models of ASD. In mice lacking Shank2, an excitatory postsynaptic scaffolding protein, postsynaptic Zn elevation induced by clioquinol (a Zn chelator and ionophore) improves social interaction. Postsynaptic Zn is mainly derived from presynaptic pools and activates NMDA receptors (NMDARs) through postsynaptic activation of the tyrosine kinase Src. Clioquinol also improves social interaction in mice haploinsufficient for the transcription factor Tbr1, which accompanies NMDAR activation in the amygdala. These results suggest that trans-synaptic Zn mobilization induced by clioquinol

rescues social deficits in mouse models of ASD through postsynaptic Src and NMDAR activation.

Disclosures: **H. Lee:** None. **E. Lee:** None. **T. Huang:** None. **C. Chung:** None. **W. Shin:** None. **K. Kim:** None. **J. Koh:** None. **Y. Hsueh:** None. **E. kim:** None.

Poster

301. Modulation: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 301.16/E36

Topic: B.07. Synaptic Transmission

Title: Functional endpoint assays to assess neurotoxicity with human iPSC-derived neurons

Authors: S. DELAURA[1], *E. M. JONES[2], K. KIM[1], C. KANNEMEIER[1], R. LEWIS[1], K. MANGAN[1], B. SWANSON[1], C. CARLSON[1];
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Abstract: Human cell types differentiated from induced pluripotent stem cells (iPSC) offer a unique source of cellular material for toxicity screening. Several examples have been presented recently on the use of iPSC- derived cardiomyocytes and hepatocytes, for example, in safety toxicology studies. Equally as important, however, is comparative neurotoxicity assessment in neuronal cell types for safety toxicology and uncovering molecular mechanisms underlying excitotoxic cell death pathways. Advances in iPSC technology provide access to previously unattainable cell types from the human brain opening new opportunities to address the shortcomings and limitations of rodent primary cells and immortalized cell lines Here we highlight examples using human iPSC- derived neurons in image- based screens, as well as on multi- electrode arrays (MEA) to assess the effects of both developmental and environmental neurotoxicants. In our recent studies, we demonstrated the neurotoxic effects of the excitatory neurotransmitter glutamate and related compounds across a panel of cell types, including iPSC-derived GABAergic and glutamatergic cortical neurons, as well as midbrain dopaminergic neurons. For comparison, the cytotoxicity of a broad spectrum kinase inhibitor, staurosporine (STS), was also evaluated. To achieve robust signals across these different iPSC- derived neuron types, we have optimized the cell culture protocols (i.e., media, time in culture, cell plating density, etc.). Under the various conditions tested, we confirmed differential responses for glutamatergic compounds (e.g. glutamate, NMDA, AMPA, and kainic acid) versus STS, suggesting the toxicity responses were due to excitotoxic effects of neuronal synaptic receptors and not other mechanisms. Importantly, toxicity induced by glutamate could be reversed with antagonists of the AMPA and NMDA receptors, DNQX and D- AP5, respectively. Additionally,

with an emphasis on analysis to decipher the complexity of neuronal activity in a dish, we were able to quantify the development of highly network- level bursting behaviors in the cultures. Overall, these iPSC- derived neurons exhibit functional glutamate pathways that respond appropriately to known agonists and antagonists, thus providing biologically relevant models for identifying emerging targets for excitotoxicity research. Together with the developmental and environmental toxicity studies, these data establish a clear utility for these each of these cell types in neurotoxicology studies.

Disclosures: S. DeLaura: None. **E.M. Jones:** None. **K. Kim:** None. **C. Kannemeier:** None. **R. Lewis:** None. **K. Mangan:** None. **B. Swanson:** None. **C. Carlson:** None.

Poster

301. Modulation: Pharmacology

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 301.17/E37

Topic: B.07. Synaptic Transmission

Support: NRF-2015M3C7A1028790

Title: pre- and postsynaptic dopamine receptors differentially modulate the subicular inputs to layer V pyramidal neurons in medial and lateral entorhinal cortex

Authors: *H. KIM, J. KWAG;
Korea Univ., Seoul, Korea, Republic of

Abstract: Medial entorhinal cortex (MEC) and lateral entorhinal cortex (LEC) are known to process spatial and non-spatial information, respectively. Interestingly, layer V pyramidal neurons in both MEC and LEC receive inputs from subiculum (Sb), which conveys information to the hippocampus. We previously demonstrated that DA-Rs modulate the properties of Sb-MEC synapse (Sb-MEC) and Sb-LEC synapse (Sb-LEC) but whether such dopaminergic modulations are due to DA-Rs in pre- or postsynaptic loci are yet unknown. To distinguish synaptic location of DA-Rs, we measured the paired-pulse ratio (PPR, 2 stimuli at 50 Hz) of excitatory postsynaptic currents (EPSCs) and synaptic integration (SI, 10 stimuli at 30 Hz) of excitatory postsynaptic potentials (EPSPs) through whole-cell voltage- and current-clamp recordings by stimulating distal Sb. SI was analyzed by calculating area of summated EPSPs (mV·s). To activate DA-R, 50 μM dopamine (DA) was used and 10 μM SCH 23390 (D1-R antagonist) and 100 nM Raclopride (D2-R antagonist) were applied in the presence of DA to study the roles of DA-R subtypes. Two-tailed paired Student's *t*-test was used for all significance tests.

To investigate whether there is a presynaptic DA-R modulation of synaptic transmission, PPR was analyzed. Sb-MEC showed paired-pulse facilitation (PPF), which was preserved after DA application (Control *vs* DA: 115.21±13.92% *vs* 105.22±25.04, n=5, p>0.05). In contrast, Sb-LEC showed paired-pulse depression (PPD) but DA application converted PPD to PPF (Control *vs* DA: 67.78±2.47% *vs* 118.0±7.14%, n=5, $p<0.01$), suggesting that Sb-LEC is modulated by presynaptic DA-R. When D1-R or D2-R antagonist were applied in the presence of DA, in our preliminary experiment, D2-R antagonist blocked the DA effect (DA *vs* D2: 118.0±7.14% *vs* 64.80±7.25%, n=5, $p<0.01$), while D1-R antagonist had no effect (DA *vs* D1: 152.06±27.43% *vs* 159.81±12.42%, n=3, $p>0.05$) at Sb-LEC.
To study postsynaptic DA-R modulation, of Sb-EC synapse, SI was analyzed by calculating summated EPSP area (mV·s). SI of Sb-MEC was reduced by DA (Control vs DA: 5.74±0.47 *vs* 4.30±0.50, n=11, $p<0.05$), but not in Sb-LEC (Control *vs* DA: 6.14±1.14 *vs* 4.93±1.0, n=12, $p>0.05$). DA effect of SI at Sb-MEC was blocked by D1 antagonist (Control *vs* D1: 5.20±0.80 *vs* 5.08±1.33, n=5, $p>0.05$), but not by D2-R antagonist (Control *vs* D2, 5.93±0.60 *vs* 3.70±0.60, n=5, $p<0.05$) in Sb-MEC, indicating that SI at Sb-MEC is modulated by postsynaptic D1-R. Overall, here we found that Sb-LEC was modulated by presynaptic D2-R while Sb-MEC was modulated by D1-R, which may have implications in understanding how DA influences spatial and learning information processing in the entorhino-hippocampal circuit.

Disclosures: H. Kim: None. **J. Kwag:** None.

Poster

301. Modulation: Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 301.18/E38

Topic: B.07. Synaptic Transmission

Support: NSERC 195814317

Title: Corticosterone and neuromodulators display similar rapid effects on inhibitory cortical networks

Authors: *C. A. WOTTON, E. F. QUON, L. K. BEKAR;
Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Stress is associated with activation of the hypothalamic-pituitary-adrenal axis that results in liberation of corticosterone into the blood to act on all tissues of the body including the brain. In addition, stress activates neuromodulatory networks throughout the brain to alter information flow and processing to optimize the fear-fight-flight response for survival. Given

that both neuromodulators and corticosterone are present in the brain during stress responses, we assessed the impact of corticosterone, norepinephrine and serotonin alone or in combination on frequency transmission in a simple cortical network in acutely isolated mouse brain slices. We used a paired-stimulation paradigm to enable evaluation of effects on excitatory as well as inhibitory activity, as paired-stimulation suppression in the cortex is known to be dependent on recruitment of inhibitory circuits. Application of norepinephrine to the slice perfusate produced a decrease in the amplitude of both pulse 1 and pulse 2. In contrast, application of either serotonin or corticosterone decreased the amplitude of pulse 1, while increasing pulse 2. All three lead to a significant increase in the paired-pulse ratio. The striking similarities in corticosterone and serotonin effects suggest a potential common mechanism. Indeed, application of corticosterone and serotonin in the presence of the $GABA_A$-receptor antagonist, bicuculline, affected the responses similarly, now showing a decrease in both pulse 1 and pulse 2 like that of norepinephrine. Additional experiments were performed with serotonin in the presence and absence of corticosterone to evaluate commonality between their mechanisms of action. Corticosterone alters the direction and amplitude of pulse 2 in the serotonin response, resulting in a response that resembles that of norepinephrine. Taken together, this suggests that both corticosterone and serotonin alter $GABA_A$ signaling pathways to exert their modulatory action. Given that chronic stress leads to depression and that altered serotonin signaling is central to the etiology of depression, the similarity in corticosterone and serotonin neuromodulatory actions highlights an interesting interaction that may lead to future treatment options for depression.

Disclosures: **C.A. Wotton:** None. **E.F. Quon:** None. **L.K. Bekar:** None.

Poster

301. Modulation: Pharmacology

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Program#/Poster#: 301.19/F1

Topic: B.07. Synaptic Transmission

Support: NIH Grant GM58055 (HCH)

Departments of Anesthesiology of Weill Cornell Medical College

Kurume University School of Medicine

Title: α_2-Adrenergic receptor and isoflurane modulation of presynaptic Ca^{2+} influx and exocytosis in hippocampal neurons

Authors: *Z.-Y. ZHOU[1], M. HARA[1,3], H. C. HEMMINGS, Jr.[1,2];
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Abstract: Evidence indicates that the anesthetic-sparing effects of α_2-adrenergic receptor (AR) agonists involve α_{2A}-AR heteroreceptors on non-adrenergic neurons. Since volatile anesthetics inhibit neurotransmitter release by reducing synaptic vesicle (SV) exocytosis, we hypothesized that α_2-AR agonists inhibit non-adrenergic SV exocytosis and thereby potentiate presynaptic inhibition of exocytosis by isoflurane. Quantitative imaging of fluorescent biosensors of action potential (AP) evoked SV exocytosis (synaptophysin-pHlourin) and Ca^{2+} influx (GCaMP6) were used to characterize presynaptic actions of the clinically used α_2-AR agonists dexmedetomidine (DEX) and clonidine (CLO), and their interaction with isoflurane, in cultured rat hippocampal neurons. DEX (0.1 μM, n = 10) or CLO (0.5 μM, n = 8) inhibited AP-evoked exocytosis (54 ± 5% and 59 ± 8% of control, respectively; $p < 0.001$). Effects on exocytosis were blocked by the subtype-nonselective α_2-AR antagonist atipamezole or the α_{2A}-AR selective antagonist BRL 44408, but not by the α_{2C}-AR selective antagonist JP 1302. DEX inhibited exocytosis and presynaptic Ca^{2+} influx without affecting Ca^{2+} coupling to exocytosis, consistent with an effect upstream of Ca^{2+}-exocytosis coupling. Exocytosis coupled to both N-type and P/Q-type Ca^{2+} channels was inhibited by DEX or CLO. DEX potentiated inhibition of exocytosis by 0.7 mM isoflurane (to 42 ± 5%, compared to 63 ± 8% for isoflurane alone; $p < 0.05$). Our data suggest that hippocampal SV exocytosis is inhibited by α_{2A}-AR activation in proportion to reduced Ca^{2+} entry. These effects are additive with those of isoflurane, consistent with a role for α_{2A}-AR presynaptic heteroreceptor inhibition of non-adrenergic synaptic transmission in the anesthetic-sparing effects of α_{2A}-AR agonists.

Disclosures: Z. Zhou: None. **M. Hara:** None. **H.C. Hemmings:** None.

Poster

302. Network Interactions and Signal Propagation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 302.01/F2

Topic: B.10. Network Interactions

Title: Modulation of entorhinal cortical input to hippocampal granule cells through activation of local inhibitory network in the dentate gyrus

Authors: *Y. MIRCHEVA, M. R. PERALTA, III, K. TOTH;
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Abstract: Entorhinal cortex (EC) projections to the dentate gyrus (DG) are the main excitatory entry in the hippocampus. Input integration is strongly influenced by local inhibitory networks of interneurons situated in the molecular layer of the DG. Direct excitatory signals in DG granule cells (GCs) interplay with slow and fast inhibition signals in order to achieve high computational power during information processing. In this study we investigated how activation of the local inhibitory circuits through perforant path (PP) stimulation, could impact EC input integration in GCs. Slow inhibition is of particular interest as it has the potential to reduce granule cell's excitability and might be involved in shaping their preferential "burst" firing pattern. Our results show that activation of EC inputs can trigger a prolonged inhibitory response that can abolish GC firing for duration of 1-2 seconds. Given the possible functional consequences that such a slow inhibitory signal could have on information processing and transfer, we further investigated the mechanism and the properties of PP-evoked long lasting hyperpolarization. Long lasting hyperpolarization (LLH) was first observed *in vitro* after direct activation of the perforant path terminals (5 pulses at 50 Hz). PP excitatory responses in GCs were followed by prolonged IPSP (16.14 ± 0.73 mV; 1946 ± 159.7 ms, n=6). Optogenetic isolation of EC input and light activation of PP, similarly induced long lasting hyperpolarization that could last up to 2s. This slow inhibition has the potential to alter GCs firing pattern by critically decreasing the probability of the cell to emit action potentials, suggesting that LLH is a mechanism for regulating dentate gyrus excitability. Using a pharmacological approach we investigated LLH properties in detail. It is mediated by the co-activation of postsynaptic $GABA_A$, $GABA_B$ and mGluR1 receptors. Moreover, LLH is activity-dependent, being most efficiently induced by high frequency PP stimulation. Interestingly, LLH develops in an age-dependent manner: LLH is most prominent in adult mice (2047 ±68 ms; 7.3 ±0.9 mV, n = 4) and absent in juvenile animals (393±67.9 ms; 1.9 ±0.2 mV). These results demonstrate the presence of an exceptionally slow hyperpolarization in GCs due to activation of postsynaptic GABA receptors and mGluR. We hypothesize that this slow inhibition is induced through PP activation of local interneurons of the feed-forward inhibition circuit. Further investigation of the mechanism and the cells at its origin as well as its physiological relevance, will improve our current understanding t of the complex interactions at the EC-DG gateway.

Disclosures: **Y. Mircheva:** None. **M.R. Peralta:** None. **K. Toth:** None.

Poster

302. Network Interactions and Signal Propagation

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Program#/Poster#: 302.02/F3

Topic: B.10. Network Interactions

Support: BBSRC BB/J0153691/1.

Title: Adenosine provides fine-tuned and highly localised negative-feedback control of spatiotemporal activity in the neocortex

Authors: *M. J. WALL[1], A. NEWTON[2], M. G. THOMAS[2], M. J. E. RICHARDSON[2];
[2]Ctr. of Systems Biol., [1]Univ. of Warwick, Coventry, United Kingdom

Abstract: Neocortical networks support a multitude of well-defined activity states. For many of these states, the mechanisms of how they are activated, sustained and terminated still remains unclear. Negative feedback via the endogenous neuromodulator adenosine is likely to have a significant influence on network activity as there is dense adenosine-receptor expression in the neocortex and gathering evidence for its role in controlling synaptic strength. In this study we have combined electrophysiology, microelectrode biosensors, fast-scan cyclic voltammetry together with mathematical and computational modelling to quantify the spatiotemporal properties of adenosine release, transport, action and removal during physiological and pathological network activity. We found that both low-rate and high-rate network activity can be modulated by adenosine receptor activation, illustrating a wide dynamic range for the adenosine signaling mechanism with little receptor saturation. During network activity adenosine was found to be released in a neocortical layer-dependent manner. Additionally, we found that the concentration of extracellular adenosine is a highly local variable determined by activity over distances less than 100 um. Modulating the removal of adenosine by either inhibiting adenosine deaminase or blocking uptake by equilibrative transporters significantly changes the time course of adenosine kinetics and can result in the accumulation of adenosine in the extracellular space. Mathematical modelling constrained by these experimental findings suggests that high receptor density coupled with efficient adenosine removal underlies a negative-feedback mechanism with a fine spatiotemporal control of local network activity.

Disclosures: **M.J. Wall:** None. **A. Newton:** None. **M.G. Thomas:** None. **M.J.E. Richardson:** None.

Poster

302. Network Interactions and Signal Propagation

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Program#/Poster#: 302.03/F4

Topic: B.10. Network Interactions

Support: T.0015.13 to VS

IAP P7/10 to VS

Title: Effect of electrode morphology on the frequency spectrum of local field potentials in the rat ventral tegmental area

Authors: C. DELAIRESSE[1], G. BECKER[1], A. PLENEVAUX[1], *V. M. SEUTIN[2], S. KOULCHITSKY[1];
[1]Univ. of Liege, Liege, Belgium; [2]Univ. Liege, Liege, Belgium

Abstract: Implantable microelectrode arrays for chronic neural recordings receive an attention by a number of research groups investigating ensemble spike activity and local field potentials. Due to that interest, various types of arrays have been developed. In the present study we compared the LFP signals recorded from the ventral tegmental area (VTA) of freely moving male Wistar rats using the two types of arrays: microelectrode microwire arrays, and silicon-based planar probes. The microwire electrodes have a three-dimensional recording surface around their tips. This presumably allows them to record the signal from all angles with similar accuracy. In contrast, the electrode contacts of the planar probe are patterned on one side of the silicon shaft (often named top-side, the other one being named back-side). This configuration probably allows to record the signal from the top-side of the shaft, while the signal from the back-side may get attenuated. Microwire arrays used in this study consisted of 8 sharpened platinum iridium wires, coated with parylene-C, except for the tip allowing the recording (Alpha Omega GmbH, Israel). Planar probes (ATLAS Neuroengineering, Belgium) had 16 iridium oxide electrode contacts implemented in the 4 silicon shafts, 4 electrode contacts per shaft. The recording was performed using a wireless system (W-Basic-System, Multi Channel Systems MCS GmbH, Germany). The probes were implanted in two orientations: top-side facing the midline, and top-side facing the lateral plane of the brain. For verification of the recording area the rats were anaesthetized and perfused with 4% paraformaldehyde containing 1 % gadolinium. The brains were removed from the skull and placed in a specific material for MRI scanning. Frequency spectra of LFPs recorded by the microwire arrays, and by the planar probes oriented to the lateral plane of the brain contained a prominent peak in the theta range (6-8 Hz). In contrast, the signals recorded using the planar probes oriented to the midline lacked such a peak and were more variable. We attribute the observed difference to the geometry of the recording arrays.

Disclosures: **C. Delairesse:** None. **G. Becker:** None. **A. Plenevaux:** None. **V.M. Seutin:** None. **S. Koulchitsky:** None.

Poster

302. Network Interactions and Signal Propagation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 302.04/F5

Topic: B.10. Network Interactions

Title: Probing the ventral fronto-temporal pathway in the language dominant hemisphere - an intraoperative cortico-cortical evoked potential study

Authors: *T. NAKAE[1], R. MATSUMOTO[2], T. KUNIEDA[4], Y. ARAKAWA[1], T. KOBAYASHI[1], T. INADA[1], Y. TAKAHASHI[1], S. NISHIDA[1], K. KOBAYASHI[3], A. SHIMOTAKE[3], M. MATSUHASHI[5], R. INANO[1], Y. YAMAO[6], T. KIKUCHI[1], K. YOSHIDA[1], A. IKEDA[2], S. MIYAMOTO[1];
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Abstract: Background: Recent intraoperative high frequency electrical stimulation of the white matter has indicated an active role of inferior fronto-occipital fascicle (IFOF) in semantic processing. Although IFOF is regarded as a major fiber in the ventral language pathway, by definition it has no terminations in the temporal lobe which is heavily involved in semantic aspects of language. Our objective is to delineate functional connectivity between the inferior frontal gyrus (IFG) and the three temporal gyri (STG, MTG, ITG) to clarify this puzzling situation by means of an electrical tract tracing method of cortico-cortical evoked potential (CCEP).
Methods: Subjects are 13 patients (11 tumor, 2 epilepsy) who underwent intraoperative monitoring for lesionectomy around the language cortices or fibers in the language dominant hemisphere (IRB C573). Single-pulse stimuli (0.3 ms, 15 mA, alternating polarity, 30 x 2 trials) were applied to the IFG through the two adjacent subdural electrodes (3-7 stimulus sites per patient). CCEP was recorded from subdural electrodes (16-24 electrodes per patient) placed on the lateral temporal area. Remote CCEP fields were identified by focusing on the early negative potential (N1: peak latency < 100 ms). Electrodes with > 80% of the maximum responses were included for analysis. The locations of stimulus and response sites were analyzed in terms of the anterior-posterior dimension.
Results: Stimulation of the posterior part of IFG (< 40 mm from the precentral sulcus (PreCS)) elicited CCEP from all the three gyri (STG, MTG, ITG). In contrast, the anterior part of IFG (> 40 mm from PreCS) elicited CCEPs only in MTG and ITG. Linear regression line analysis revealed significant positive correlation between the stimulus sites (the distance from PreCS: range 3-58 mm) and the response sites (that from the temporal pole: range 37-93 mm) in MTG

(R^2 = 0.36) and ITG (R^2 = 0.71).
Conclusion: IFG connection to STG was confined to the posterior part of IFG, and most likely represented the dorsal fiber pathway. Connections to MTG/ITG were observed from all parts of IFG. Different from the current dichotomy of UF vs. IFOF, the anterior-posterior gradient between IFG stimulus sites and MTG/ITG response sites implicates the continuum of the two fibers, namely, more "fanning" nature of the ventral fronto-
temporal pathway as originally Dejerine addressed (1895). Further investigation, such as comprehensive white matter electrical stimulation, is warranted to clarify the role of the ventral fronto-temporal fibers in language and semantic functions.

Disclosures: **T. Nakae:** None. **R. Matsumoto:** 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; KAKENHI 26282218, 15H01664, 15H05874. Other; Endowed department by UCB, GSK, NihonKoden, Otsuka. **T. Kunieda:** Other; Otsuka Pharmaceutical. **Y. Arakawa:** None. **T. Kobayashi:** None. **T. Inada:** None. **Y. Takahashi:** None. **S. Nishida:** None. **K. Kobayashi:** None. **A. Shimotake:** None. **M. Matsuhashi:** None. **R. Inano:** None. **Y. Yamao:** None. **T. Kikuchi:** None. **K. Yoshida:** None. **A. Ikeda:** 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; KAKENHI 26282218, 15H01664, 15H05874. Other; Endowed department by UCB, GSK, NihonKoden, Otsuka. **S. Miyamoto:** Other; Otsuka Pharmaceutical.

Poster

302. Network Interactions and Signal Propagation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 302.05/F6

Topic: B.10. Network Interactions

Title: The capacity of active memory

Authors: ***E. P. FRADY**[1], G. ISELY[1], F. T. SOMMER[1], P. KANERVA[2];
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Abstract: Although the mechanisms of working memory are unclear, many theories suggest that information about the past is maintained through recurrent neural activity. However, the information capacity that can be maintained in neural activity is unknown. We explore the

information capacity in two computational models of working memory, holographic reduced representations (Plate, 2003) and hyperdimensional computing (Kanerva, 2009). These models use key-binding schemes to encode working memory traces in high-dimensional vectors, where arbitrary memory items can be directly decoded. We illustrate how to superimpose memory items in a linear recurrent neural network based on these computational algebras. As more items are superimposed, cross-talk reduces the quality of decoding memory items which ultimately limits the memory capacity. We analytically derive the fundamental capacity limits of these systems and verify their applicability by simulation experiments. We found an approximately linear relationship between the number of elements stored and the number of neurons required to maintain the information at a given tolerance: roughly 10-15 neurons are needed for each bit of information stored in active memory. Further, we show that our results also apply to more sophisticated machine learning models by training recurrent neural networks and LSTMs to perform computational tasks which require large working memories. The proposed information capacity estimates how many neurons a network requires to solve a problem based on the problem's computational complexity (e.g. the amount of information needed maintained in working memory). Our results demonstrate that performance drops drastically as this condition is violated. All told, the presented results form a theoretical foundation for analyzing the computational properties of neural activity both in experiments and machine-learning, and might also be important to understand human performance in working-memory tasks.
Plate TA. Holographic Reduced Representation: Distributed representation of cognitive structure. Stanford: CSLI Publications, 2003.
Kanerva P. Hyperdimensional Computing: An introduction to computing in distributed representation with high-dimensional random vectors. Cognitive Computation 1(2):139-159, 2009.

Disclosures: **E.P. Frady:** None. **G. Isely:** None. **F.T. Sommer:** None. **P. Kanerva:** None.

Poster

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Topic: B.10. Network Interactions

Support: VA: 5I01BX001814-03

Title: Sleep in clock mutant mice, clock$^{\Delta 5\text{-}6}$, responds to light changes and sleep deprivation very differently

Authors: *B. QIN[1,2], A. AKLADIOUS[3], P. FENG[4,3];
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Abstract: *Clock* is one of the important genes that regulate circadian rhythms and sleep. Mouse with *Clock* mutation (*Clock*$^{\Delta 5\text{-}6}$) has been demonstrated to have normal activity rhythms with altered response to lighting (Debruyne et al., 2006). However, how *clock* deficiency impact sleep/wake regulation is not known. Therefore, we conducted a polysomnographic (PSG) study in wild mice (n=8) and mice with *Clock*$^{\Delta 5\text{-}6\ -/-}$ (n=10).
Electrodes for EEG and EMG recording were implanted under standard anesthesia. Two days of baseline PSG recording was conducted ten days after post-surgical recovery under normal light/dark cycle, i.e., LD12:12 (light was on at 9:00 am and off at 9:00 pm). Then, PSG recording was continued for 6 hours of total sleep deprivation by gentle handling started at light onset (Zeitgeber [ZT] 0) followed by 18 hours of recovery. To study the effect of clock mutation in response to long darkness, light was turned off continuously for 7 days, i.e., constant darkness (DD) from the fourth day of PSG recording. Then, we studied the light effect on both genotype animals by turning on lights constantly (LL) for 7 days. PSG data was analyzed as rapid eye movement (REM) sleep, non-REM (NREM) sleep and wake in 10-second epoch. Data scoring has been completed for one baseline day, sleep deprivation and recovery day, DD day 1 and 7 and LL day 1 and 7.
Baseline sleep/wake features of the *Clock*$^{\Delta 5\text{-}6\ -/-}$ mouse were the same as of wild mouse. However, the *clock* mutation mouse had significant less total sleep and NREM sleep in post-sleep deprivation recovery period than the wild mouse. Sleep states in wild mouse and *Clock*$^{\Delta 5\text{-}6\ -/-}$ mice were similar in the first DD day but significantly different in the 7th DD day. In DD7, total sleep ($p<0.001$) and NREM sleep ($p<0.001$) was significantly less in the *clock*$^{\Delta 5\text{-}6\ -/-}$ mouse in the period of ZT5-8. The mean of REM sleep was higher in the *clock* mutation mouse in ZT 13-16 but not significant. When turn to LL period from DD period, mice with *clock* mutation had significantly ($p=0.028$) less total sleep and NREM sleep ($p=0.021$). In the last LL day, clock mutation mouse had significantly ($p=0.024$) more total sleep and significantly more NREM sleep ($p=0.019$) in the ZT13-16 time period indicating that clock deficiency reduced activity stimulation induced wake response. Interestingly, differences of REM sleep between wild and mutation groups were significant only in LL7, ZT0-4 period. EEG power analysis and micro-features of sleep will be analyzed in the future.
In summary, although rhythmic sleep/wake state remains the same in both the wild and the *clock* mutation mice, the capability of the mutation mice response to the challenge of sleep deprivation and light change is altered.

Disclosures: B. Qin: None. **A. Akladious:** None. **P. Feng:** None.

Poster

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Topic: B.10. Network Interactions

Support: Chinese Academy od Sciences

National Natural Science Foundation of China

Title: Network's critical state and its relation to the inverted-U profile of dopamine related working memory performance

Authors: G. HU, X. HUANG, T. JIANG, *S. YU;
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Abstract: It has been known that the working memory (WM) performance is modulated by dopamine (DA), with a characteristic inverted-U profile. That is, too strong or too weak of DA's activation in the prefrontal cortex (PFC) would be detrimental for WM, while an intermediate level of DA activation is required to maintain an optimal WM performance. In addition, it has been reported that such an intermediate level of dopamine D1 activation in the PFC is associated with the network being organized close to a critical state, while too strong or too weak D1 activation pushes the system away from it. Given the advantages of the critical state in information processing, it has been then hypothesized that the role of dopamine in the PFC is to adjust the system's overall state, with the critical point as the desirable target. This may provide a deep insight into the mechanisms underlying DA's inverted-U profile in WM performance. In the current study, we aim to test this hypothesis in a network model. To this end, we adapted a neural network model that was proposed to perform the WM task though calcium-dependent short-term synaptic plasticity. According to experimental as well as modeling results, dopamine's modulation was simulated as inserting a nonlinear function on the efficacy of excitatory synapses, which are slightly different between the pyramidal-pyramidal synapses and pyramidal-interneuron synapses. By systematically changing DA's 'concentration', we observed that the system operated close to the critical state with an intermediate level of DA activation, while a too high or too low concentration of DA renders the system sub-critical. Importantly, this critical state demonstrated the maximized sensitivity in WM task. That is, under such a condition, the network was able to memorize the weakest external input. In-depth analyses further revealed the mechanisms, including the balance between the excitation and the inhibition, underlying such a phenomenon. Our results provide a mechanistic link between the observed inverted-U profile for DA-related WM performance and DA-dependent critical state in the PFC network, suggesting that the former can be satisfactorily explained by considering DA's role in modulating the overall state of the PFC network around the critical point.

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Poster

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Topic: B.10. Network Interactions

Support: The Swartz Foundation

ONR grant N00014-13-1-0297

Title: Enhanced signal propagation and nonnormality in a large scale circuit model of the primate cortex

Authors: ***M. JOGLEKAR**[1], J. MEJIAS[1], G. R. YANG[1], X.-J. WANG[1,2];
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Abstract: Stable transmission of signals in a multi-area feedforward network represents a long-standing challenge: as a signal propagates across areas, it may either die out or explode (Diesmann et al., Nature 1999, Moldakarimov et al., PNAS 2015). In previous studies on signal transmission, areas are identical and inter-areal connection strengths are uniform and not constrained by data. We re-examined the problem of signal propagation using a newly developed large-scale network model of the primate cortex (Chaudhuri et al., Neuron 2015). The model is based on the recently published dataset of directed and weighted connectivity of the macaque cortex from Henry Kennedy's group at INSERM, France (Markov et. al., Cereb. Cortex 2014). This network displays complex feedforward and feedback projections with a wide range of connection strengths (spanning five-orders of magnitude), posing new questions about signal propagation. We quantified effective signal transmission in terms of the amount of attenuation of the peak firing rate as the signal propagates to areas higher in the brain's hierarchy. We found two regimes of stable signal propagation. The underlying principle governing both the regimes is strong long-range excitation that boosts inter-areal signal transmission, followed by strong delayed inhibition that stabilizes the network dynamics. In this sense, this work represents a generalization of the concept of balanced amplification (Murphy et al., Neuron 2009) from local circuits to large-scale cortical systems. Consistent with Murphy et al. showing that this mechanism is characterized by the non-normality of the network connection matrix, in our large-scale model, an improvement in propagation is correlated with an increase in the non-normality measure (Henrici, Numer. Math. 1962) of the large-scale connectivity matrix. Our regimes

improve signal propagation by 100 fold, from an attenuation of 1/10,000 (Chaudhuri et al., Neuron 2015) to 1/100.

Disclosures: **M. Joglekar:** None. **J. Mejias:** None. **G.R. Yang:** None. **X. Wang:** None.

Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: NIH Grant MH108022

Title: Pacap modulates amygdalar-bnst interactions in control of anxiety

Authors: Y. LI, R. ANDERO, K. J. RESSLER, *V. Y. BOLSHAKOV;
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Abstract: Previous studies provide experimental evidence for the role of pituitary adenylate cyclase-activating polypeptide (PACAP)-mediated signaling in regulation of anxiety in both experimental animals and human subjects. It has been demonstrated that PACAP may regulate anxiety-related behavioral processes through its actions in two interacting brain regions, the amygdala and the bed nucleus of the stria terminalis (BNST). However, synaptic and network mechanisms of PACAP-mediated effects in the brain are poorly understood. In this study, we addressed specific questions about the nature of PACAP-induced synaptic and network-level modifications in BLA-BNST circuits, contributing to control of anxiety states. We expressed channelrhodopsin-2 (ChR2) under control of the neuron-specific promoter CaMKIIα in BLA neurons and photostimulated the corresponding fibers synapsing on neurons in two BNST subdivisions, ovBNST and adBNST, known to regulate anxiety in opposite directions—activation of ovBNST was shown to induce anxiety, whereas activation of adBNST is anxiolytic. Consistent with our finding that PACAP-containing fibers were observed in ovBNST only, we found that PACAP potentiated excitatory synaptic responses at inputs to ovBNST but not at inputs to adBNST, selectively increasing the synaptically-driven spike output of ovBNST neurons in response to activation of projections from the BLA. The enhanced firing of ovBNST neurons resulted in inhibition of adBNST, since ovBNST neurons, projecting to adBNST, are GABAergic. By using a combination of retrograde tracing, immunohistochemistry and in vivo optogenetics, we demonstrated that neurons in the parabrachial nucleus (PBn) in the brainstem are the source of PACAPergic innervations of the ovBNST. Thus, we showed that neuropeptide PACAP contributes to regulation of anxiety states by differentially affecting synaptic efficacy at

BLA projections to different BNST subdivisions, and, therefore, modifying the signal flow in BLA-ovBNST-adBNST circuits in such a way that adBNST is inhibited. This would explain the ability of PACAP in BNST to trigger anxiety, as direct optogenetic inhibition of adBNST was shown to be anxiogenic.

Disclosures: **Y. Li:** None. **R. Andero:** None. **K.J. Ressler:** None. **V.Y. Bolshakov:** None.

Poster

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Topic: B.10. Network Interactions

Support: NYU Start Up Package

Title: Hippocampal modulation of sensory integration in entorhinal cortex

Authors: ***M. ELMALEH**[1], R. ZEMLA[2], M. DUFOUR[2], A. HAIRSTON[2], S. SUNDAR[2], J. BASU[2];
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Abstract: As we navigate the world our perception of sensory stimuli is not only defined by our ongoing experiences, but also by the summation of our previous experiences, preserved as memories. The hippocampus, a deep brain structure long considered the seat of learning and memory, receives input crucial to this function from an adjacent cortical area, the entorhinal cortex. The entorhinal cortex acts as a hub, processing sensory and associational input from many brain regions, and projecting to the hippocampus to enable memory formation. While we know a great deal about this forward circuit, we know very little about the reciprocal input from the hippocampus to the entorhinal cortex. We hypothesize that the hippocampus modulates the integration of sensory information occurring in the entorhinal cortex, providing a mechanism by which previous memories within the hippocampus shape the future processing of new experiences. In this study we examine the functional connectivity of the hippocampal back-projections and the role of this pathway in modulating activity within the entorhinal cortical microcircuit, using whole-cell electrophysiology, optogenetics and viral tracing approaches *in vitro* in the adult mouse. Furthermore, we are exploring the importance of these backprojections in hippocampal-dependent behavioral paradigms including contextual fear conditioning and novel object recognition using loss of function approaches and two-photon imaging.

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Poster

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Topic: B.10. Network Interactions

Support: The Hartwell Foundation

Title: Using multielectrode arrays to investigate the spontaneous firing patterns and functional connectivity in large neural assemblies

Authors: ***T. FENG**, N. X. KODAMA, R. FERNANDEZ GALAN;
Case Western Reserve Univ., Cleveland, OH

Abstract: The brain is active even in the absence of stimulation. Its spontaneous activity is not random but highly structured and can be used to investigate properties of neuronal circuits, such as their functional connectivity and the presence of endogenous pacemakers, which are crucial to understand how neuronal circuits process information. To address this questions it is necessary to record from large populations of neurons simultaneously, and overcome the traditional tradeoffs between spatial and temporal resolutions of standard electrophysiological and imaging methods. We attempt to solve this problem using high-density, two-dimensional, multielectrode arrays (MEA) to investigate spontaneous activity from the mouse neocortex *in vitro*. The arrays consist of 120 electrodes evenly spaced with 100 um pitch over an octagonal area spanning over 1.2 x 1.2 mm^2. This technology enables the study of activity patterns with unprecedented temporal and spatial resolutions over a few cortical columns. Using spike-sorting algorithms, action potentials from multiple cells can be resolved from each electrode in the MEA, so that we can potentially identify on the order of hundreds of neurons in a single experiment. In preliminary studies, the neurons displayed different types of spontaneous firing patterns at different frequencies, typically below 10 Hz. Some neurons fired tonically, some transiently, and some in regular bursts. From the firing patterns we were able to investigate functional connections between neurons at different locations in the neocortex, and identify neurons with important roles in the network. In particular, the relative lags between action potentials from certain neuron pairs were well preserved over time, indicating direct connections between those neurons. Some other neurons behaved as potential pacemakers in the cortex. These results open up a new avenue to investigate the activity and functional connectivity of neural circuits.

Disclosures: **T. Feng:** None. **N.X. Kodama:** None. **R. Fernandez Galan:** None.

Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: The Hartwell Foundation

Title: Spherical harmonics reveal standing EEG waves and long-range neural synchronization in the sleeping brain

Authors: ***S. S. SIVAKUMAR**, A. G. NAMATH, R. FERNANDEZ GALAN;
Case Western Reserve Univ., Cleveland, OH

Abstract: Previous work from our lab has demonstrated how the connectivity of brain circuits constrains the repertoire of activity patterns that those circuits can display. Specifically, we have shown that the principal components of spontaneous neural activity are uniquely determined by the underlying circuit connections, and that although the principal components do not uniquely resolve the circuit structure, they do reveal important features about it. Expanding upon this framework on a larger scale of neural dynamics, we have analyzed electroencephalogram (EEG) data recorded with the standard 10-20 electrode system from 41 neurologically normal children and adolescents during stage 2, non-rapid eye movement (non-REM) sleep. We show that the principal components of EEG spindles, or sigma waves (10-16 Hz), reveal non-propagating, standing waves in the form of spherical harmonics. We mathematically demonstrate that standing EEG waves exist when the spatial covariance and the Laplacian operator on the head's surface commute. This in turn implies that the covariance between two EEG channels decreases with the inverse of their relative distance, a relationship that we corroborate with empirical data. Using volume conduction theory, we then demonstrate that superficial current sources are more synchronized at larger distances, and determine the characteristic length of large-scale neural synchronization as 1.31 times the head radius, on average. Moreover, consistent with the hypothesis that EEG spindles are driven by thalamo-cortical rather than cortico-cortical loops, we also show that eight additional patients with hypoplasia or complete agenesis of the corpus callosum, i.e., with deficient or no connectivity between cortical hemispheres, similarly exhibit standing EEG waves in the form of spherical harmonics. We conclude that spherical harmonics are a hallmark of spontaneous, large-scale synchronization of neural activity in the brain, which are associated with unconscious, light sleep. The analogy with spherical harmonics in quantum mechanics suggests that the variances (eigenvalues) of the principal components follow a

Boltzmann distribution, or equivalently, that standing waves are in a sort of "thermodynamic" equilibrium during non-REM sleep. By extension, we speculate that consciousness emerges as the brain dynamics deviate from such equilibrium.

Disclosures: S.S. Sivakumar: None. **A.G. Namath:** None. **R. Fernandez Galan:** None.

Poster

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Title: Propagation of spike timing and firing rate across multiple layers in networks of cultured neurons

Authors: *J. BARRAL, A. REYES, X.-J. WANG;
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Abstract: The sequence of action potentials produced by neurons defines a coding strategy to transmit information in neuronal networks. Information may either be represented as the average number of spikes per unit time (rate coding) or by their precise timing (temporal coding). The nervous system utilizes both strategies and there appears to be a continuum between these two extremes. A rudimentary feature of most neural networks consists of several neurons' pools arranged in a feedforward manner, like the multiple cortical areas. The feedforward network model provides a minimal framework for studying information processing in the brain and has been investigated by computer simulations. They revealed that synchronous spikes propagate faithfully only when each layer contains a sufficient number of neurons. In networks of lower density, spikes volley disperses and eventually dies out. However, experimental support is lacking to test this hypothesis.
Here, we studied experimentally how spikes propagated in modular networks. We reconstructed *in vitro* networks by culturing cortical neurons in microfabricated chambers with sequentially connected compartments and controlled the density of neurons in each layer. Excitatory neurons of the first layer were activated optogenetically which allowed precise control of the timing and magnitude of the input (packet) as well as the spatial spread and number of stimulated neurons. Spikes and membrane potential of neurons in subsequent layers were recorded using patch-

clamp. In sparse networks where synaptic connections are strong, spikes propagated through the network and became progressively more synchronous when the input packet delivered to the first layer was sufficiently narrow and large, in agreement with previous theoretical results on exclusively feedforward networks. However, in dense networks where connections are weak, spikes propagated reliably but were temporally more dispersed and delayed, in contradiction with previous results. In this case, the input/output firing rate relation was linear for small range suggesting that these networks could transmit firing rate information. To mimic the increasing number of inputs in progressively higher order brain regions, we varied the number of neurons systematically along the network. We found that propagation was more efficient in cultures where the number of neurons increased progressively with layer, suggesting that propagation could be amplified by networks of larger density. These results indicate that spike propagation depends quantitatively and qualitatively (timing vs rate) not only on the input pulse packet but also on network architecture and density.

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Poster

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KAKENHI/15K00413 toT.T.

Title: Network plasticity facilitating the neural excitation propagation between the perirhinal and entorhinal cortices as revealed by voltage-sensitive dye imaging

Authors: *R. KAJIWARA[1], Y. WAKAYAMA[1], Y. TOMINAGA[2], T. TOMINAGA[2];
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Abstract: The rhinal cortices, such as perirhinal (PC) and entorhinal (EC) cortices, are located on the bi-directional pathway between the neocortex (NC) and the hippocampus (HP). The synchronous neural activation via the rhinal cortices between the NC and HP is essential for the formation and recall of context-dependent memories. However, previous electrophysiological experiments in the guinea pig isolated brains indicated that propagation of neural activity between PC and EC occurs with an extremely low probability. However, we found that the area

35 of PC started to allow the neuronal signal propagation from the NC to HP under certain specific physiological conditions with the voltage-sensitive dye imaging. That is, the area 35 of PC might act as a gate. Here, we examined the neuronal mechanism of the gate by visualizing the pharmacological effect on neural propagation patterns in mouse brains and analyzed the network plasticity having a strong effect on the gate. To assess the physiological property of the neural transmission throughout the rhinal cortices, horizontal slices of mouse brains that contained the PC, EC, and HP regions were used for the VSD imaging. Depolarized response evoked by electrical stimulation at 40Hz delivered to the superficial layers in area 36 of the PC spread across a wide area of the PC, under partial suppression of the $GABA_A$ system by 1 μM gabazine. But this experimental procedure did not cause the spread of neural activities across the PC/EC gate. We examined the effect of slowly inactivating potassium conductance (ID) of late-spiking neurons predominating in area 35 that can inhibit the generation of action potentials necessary for the entorhinal activation by perfusing a low concentration (40 μM) of 4-aminopyridine (4AP). 4-AP enhanced the neural activity in PC and eventually the area 35 started to show prolonged and repetitive depolarization. Once area 35 showed the long and repetitive depolarization, that induced synchronized oscillatory activity in the EC neuronal circuit. In other words, the gate opened. Interestingly, washing out the 4AP cannot inhibit the entorhinal activation anymore, i.e., the gate stayed open for more than one hour. When once the PC/EC gate was open, even weak stimulation to the PC initiated the spread of the neural activity throughout the rhinal cortices. Thus, the enhancement of network activity across the PC/EC gate observed in the study can be said as a new class of the long-term plasticity of the network activity. Such plasticity might play a crucial role in the hippocampal memory processing and pathological phenomena something like the epilepsy.

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Poster

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Title: Propagation of neural activity induced by single-pulse electrical stimulation during various sleep stages

Authors: *K. USAMI[1], A. KORZENIEWSKA[1], R. MATSUMOTO[2], T. KUNIEDA[3], N. MIKUNI[5], K. KOBAYASHI[4], T. KIKUCHI[3], K. YOSHIDA[3], S. MIYAMOTO[3], R. TAKAHASHI[4], A. IKEDA[2], N. E. CRONE[1];
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Abstract: Introduction: Single-pulse electrical stimulation (SPES) to human cortex evokes phase-locked potentials called cortico-cortical evoked potentials (CCEP) or non-phase-locked high gamma responses (HGR: > 80 Hz). The latter index changes in neuronal firing and/or synchrony. These responses may be mediated through direct or indirect anatomical connections with the stimulation site, but it is unknown whether propagation occurs beyond these 1st order connections. Previous reports have indicated that effective connectivity breaks down during non-rapid eye movement (NREM) sleep compared to awake and rapid eye movement (REM) sleep. We investigated whether these different brain states affect the propagation of high gamma activity evoked by SPES.
Methods: Four patients underwent invasive presurgical evaluation for intractable partial epilepsy using subdural electrodes (IRB No.443 in Kyoto University). Repetitive single-pulse electrical stimulation (SPES) at alternating polarity was delivered to the cortical surface during wakefulness and different sleep stages, and electrophysiological responses, including CCEPs, were recorded from all electrodes except for the stimulation sites. We applied event-related causality (ERC) analysis based on short-term direct directed transfer function (SdDTF) to estimate the dynamics, directionality, and magnitude of propagation of neural activity among electrodes with HGR (80-150 Hz) at least 100 ms after stimulation. Finally, we compared the propagations we observed during wakefulness and different sleep stages.
Results: We observed different patterns of neural activity propagation during wakefulness vs. different sleep stages. In all patients, we observed more propagation in the awake stage. The electrodes that showed significant CCEPs appeared to be sources of neural propagation to electrodes with and without CCEPs. During wakefulness, magnitudes of propagation were higher than during NREM and REM sleep stages.
Conclusion: Event-related causality (ERC) analyses revealed different patterns of neural activity propagation during wakefulness and different sleep stages. These observations suggest that sites with significant CCEPs can serve as sources of neural propagation especially during

wakefulness. This secondary propagation may be suppressed during sleep, possibly reflecting a state with lower effective connectivity across large-scale cortical networks.

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Poster

302. Network Interactions and Signal Propagation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 302.16/F17

Topic: B.10. Network Interactions

Title: Hippocampal-Perirhinal oscillatory coupling "switched on and off" by light

Authors: *J. DINE, A. GENEWSKY, F. HLADKY, C. T. WOTJAK, J. M. DEUSSING, W. ZIEGLGÄNSBERGER, A. CHEN, M. EDER;
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Abstract: The neurophysiological processes that can cause theta-to-gamma frequency range (4-80 Hz) network oscillations in the rhinal cortical-hippocampal system and the potential connectivity-based interactions of such forebrain rhythms are a topic of intensive investigation. Here, using selective Channelrhodopsin-2 (ChR2) expression in mouse forebrain glutamatergic cells, we were able to locally, temporally precisely, and reliably induce fast (20-40 Hz) field potential oscillations in hippocampal area CA1 *in vitro* and *in vivo* (i.e., slightly anesthetized NEX-Cre-ChR2 mice). As revealed by pharmacological analyses and patch-clamp recordings from pyramidal cells and GABAergic interneurons *in vitro*, these light-triggered oscillations can exclusively arise from sustained suprathreshold depolarization (~200 ms or longer) and feedback inhibition of CA1 pyramidal neurons, as being mandatory for prototypic pyramidal-interneuron network (P-I) oscillations. Consistently, the oscillations comprised rhythmically occurring population spikes (generated by pyramidal cells) and their frequency increased with increasing spectral power. We further demonstrate that the optogenetically driven CA1 oscillations, which remain stable over repeated evocations, are impaired by the stress hormone corticotropin-releasing factor (CRF) *in vitro* and, even more remarkably, found that they are accompanied by concurrent states of enforced theta activity in the memory-associated perirhinal cortex (PrC) *in vivo*. The latter phenomenon most likely derives from neurotransmission via a known, but poorly studied excitatory CA1→PrC pathway. Collectively, our data provide evidence for the existence of a prototypic (CRF-sensitive) P-I gamma rhythm generator in area CA1 and suggest that CA1

P-I oscillations can rapidly up-regulate theta activity strength in hippocampus-innervated rhinal networks, at least in the PrC.

Disclosures: J. Dine: None. **A. Genewsky:** None. **F. Hladky:** None. **C.T. Wotjak:** None. **J.M. Deussing:** None. **W. Zieglgänsberger:** None. **A. Chen:** None. **M. Eder:** None.

Poster

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Topic: B.10. Network Interactions

Support: DOD Grant SCI-30225

Title: Patch clamp recordings of cellular and synaptic properties in adult mouse thoracic paravertebral ganglia

Authors: *M. L. MCKINNON, S. HOCHMAN;
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Abstract: Thoracic intraspinal preganglionic neurons project to sympathetic postganglionic neurons (SPNs) within paravertebral chain ganglia. While little is known of thoracic SPN functional properties, a major function is to control vasomotor tone. Paravertebral ganglia were traditionally thought to faithfully relay information from the spinal cord to the periphery, but this is an overly simplified viewpoint. To address this, we developed an *in vitro* approach for whole-cell recordings in intact thoracic ganglia (T3-T12) to characterize cellular and synaptic properties. Mean resting membrane potential was -57 mV. Average cell input resistance was 928 MΩ, and membrane time constant was 82 ms (n=8). These values are much higher than seen with sharp electrodes. A longer membrane time constant supports a greater temporal range for synaptic integration. In response to depolarizing current steps from rest, mean rheobase was 20 pA (n=3), and all exhibited a post-spike after-hyperpolarization. Some cells displayed post-inhibitory rebound spiking. While previous studies with sharp electrodes report phasic firing, we always observed sustained tonic firing and firing rate increased with increased current magnitude with a quasi-linear *f*-I relation. Sustained firing rates peaked at 17 Hz. Spontaneous EPSPs occupied a continuous range of amplitudes with a skewed distribution (mean=3.1mV, median=2.8mV). Several instances of temporal summation of EPSPs leading to spike recruitment were observed. We used ChAT::ChR2 mice to optogenetically recruit cholinergic axons to characterize synaptic responses. Blue light fiber-optic illumination typically elicited suprathreshold EPSPs and evoked responses fatigued dramatically with repeated stimulation.

The rate of recovery after fatigue was characterized using a paired pulse protocol. Synaptic fatigue lasted several seconds before synapses fully recovered (n=5). We also characterized multisegmental convergence with optogenetic activation of preganglionic axons in ventral roots. Thoracic postganglionic neurons received presynaptic innervation from several contiguous spinal segments, and synaptic response amplitude varied with more proximal segments having a greater amplitude. In summary, thoracic SPNs have greater capacity for synaptic integration and spiking than previously considered, largely due to a reduced membrane leak, but recruitment may be restricted by severe synaptic fatigue observed at preganglionic cholinergic synapses. These data highlight the role of thoracic ganglia as active participants in vasomotor signal conditioning rather than passive relays of information.

Disclosures: M.L. McKinnon: None. **S. Hochman:** None.

Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: Department of Defense SCI-30225

Title: Anatomy of mouse thoracic sympathetic chain ganglia and electrophysiological assessment of their multisegmental preganglionic input

Authors: *M. HALDER[1], M. CHOI[2], C. MACDOWELL[2], M. MCKINNON[2], M. SAWCHUK[2], S. HOCHMAN[2];
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Abstract: Paravertebral thoracic sympathetic postganglionic neurons (tSPNs) predominantly control vascular function in the upper/middle extremities yet their chain ganglia are practically inaccessible for physiological study. We developed an ex vivo adult mouse model that retains these chain ganglia in situ with intact ventral root (VR) connections to study pre- to post-ganglionic interconnections and undertook parallel anatomical studies to assess tSPN ganglia composition.We used thoracic VR stimulation to recruit segmental preganglionics to examine tSPN intersegmental population responses recorded with suction electrodes attached to individual ganglia. We observed multisegmental convergence of preganglionics onto individual thoracic ganglia. When recording from the T12 ganglia, we observed orthodromic responses after stimulation of T7-T12 VR. Overall, VR axons from the same segment usually evoked the largest responses in individual ganglia. Widespread divergence was also seen, particularly for

mid-thoracic segments. For example, T6 VR stimulation evoked responses in all recorded ganglia (T2-T12). We examined the sensitivity of evoked synaptic responses to stimulus frequency and nicotinic acetylcholine receptor (nAChR) antagonists. Evoked responses were reproducible at 0.1 Hz but progressively depressed at 1 and 10 Hz. The common nAChR ganglionic blockers hexamethonium and mecamylamine depressed evoked responses consistent with actions on α3β4-containing nAChRs. Tubocurarine also depressed responses but DHβE did not. We used immunohistochemistry to determine the numbers and soma diameters of choline acetyltransferase$^+$ (ChAT$^+$) cholinergic and tyrosine hydroxylase$^+$ (TH$^+$) noradrenergic neurons in thoracic ganglia T1-T13 (n=2). Neuron numbers peaked at T7 and T8. TH$^+$ neurons comprised 97% of tSPNs which had slightly larger diameters than ChAT$^+$ neurons (17.5 vs. 16.7μm, respectively). There were more ChAT$^+$ tSPNs in segments rostral to T6. When counts of select ganglia from other mice were included, a remarkable inter-animal variability in ganglion neuron numbers was seen. We conclude that; (i) preganglionic convergence and divergence patterns onto tSPNs are abundant and multisegmental, (ii) evoked synaptic responses have nAChR pharmacology consistent with actions on α3β4-containing nAChRs, and (iii) tSPN ganglia have highly variable neuron numbers but are composed almost entirely of TH$^+$ adrenergic neurons.

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Poster

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Topic: B.10. Network Interactions

Support: NRF- 2013R1A2A2A01067990

NRF 2015R1C1A2A01053318

NRF 2014K1A3A1A21001372

Title: Suppressed GABAergic signaling in the zona incerta causes neuropathic pain in a thoracic hemisection spinal cord injury rat model

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Abstract: Neuropathic pain can deteriorate quality of life in patients with a spinal cord injury(SCI), but the mechanism by which SCI causes dysfunctional pain signaling remains unclear. A reduction in gamma-aminobutyric acid(GABA)ergic signaling in the zona incerta(ZI) of a thoracic hemisection-SCI rat model has been suggested, but not clearly demonstrated. To test whether these GABAergic signals influence SCI-induced neuropathic pain, we recorded and compared *in vivo* single-unit, neuronal activity between hemisection-SCI and sham-treated rat models. Furthermore, we analyzed neuronal activity in these groups following treatment with a $GABA_A$ receptor agonist (muscimol) or antagonist (bicuculline). The hemisection-SCI rats show reduced hindpaw withdrawal thresholds, latencies, and decreased ZI neuronal activity, compared with those of the sham-treated controls. Importantly, muscimol treatment increased, whereas bicuculline decreased, the firing rates of the ZI neurons. The muscimol treated, hemisection-SCI rats also exhibited increased hindpaw withdrawal thresholds and latencies. Together, these data provide evidence that neuropathic pain after SCI is caused by decreased GABAergic signaling in the ZI. Furthermore, our data demonstrate that GABAergic drug infusion into the ZI could restore its inhibitory action and improve neuropathic pain behaviors.

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Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Title: Exact analysis of spike-timing and higher-order interactions of neurons at the threshold regime suggests network architecture underlying sparse population activity

Authors: *S. RASHID SHOMALI[1], M. NILI AHMADABADI[2], S. RASULI[3], H. SHIMAZAKI[4];
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Abstract: The accurate prediction of how spiking of a presynaptic neuron affects spike timing of a postsynaptic neuron in vivo has significant importance in a variety of questions in

Neuroscience. An exact solution for this problem under conditions resembling in vivo, however, is lacking due to the nonlinearity of the neuron's spike generation mechanism. Neural activity in vivo exhibits significant variability. It is suggested that this variability reflects neuronal activity near the threshold regime, where small fluctuations of presynaptic neurons can significantly affect postsynaptic spike-timing. Here, we analytically investigate impact of a signaling input on a leaky integrate-and-fire neuron that receives background noise at the threshold regime. The signaling input models a synaptic or an assembly of nearly synchronous synaptic activity that conveys information while the background noise represents ongoing activity of many weak synapses. We demonstrate that, at the threshold regime, it is possible to obtain an exact solution that explains how the signaling input changes the spike-timing distribution. We then use the result to predict higher-order interactions of population activity caused by shared signaling inputs under different network architectures. This prediction allows us to uncover network architecture behind the population activity. In particular, we suggest architecture behind sparse population activity observed in monkey V1 or rat hippocampal neurons, which involves higher-order interactions. Contrary to common intuition, we find it unlikely that common inhibition causes the sparse activity; instead, we quantitatively show that the observed activity can result from local excitatory common inputs by comparing the theoretical prediction with empirical results obtained from the monkey V1 neurons.

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Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: NIMH (5R37MH071739)

NINDS (5R01NS074785)

Title: 5ht3a+ interneurons inhibit pv+ interneurons to enhance signal fidelity in hippocampal area ca1

Authors: *B. SUUTARI, S. M. COHEN, A. SALAH, R. W. TSIEN;
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Abstract: Inhibitory interneurons control gating of information transmitted from CA3 to CA1 of the hippocampus. Fast-spiking, parvalbumin+ interneurons (PV+ INs) are critical for feedforward inhibition to counterbalance excitatory synaptic inputs and provide fast, reliable control of pyramidal cell excitability and spike timing. However, PV+ INs are but one example of the much larger and more diverse population of inhibitory interneurons in the hippocampus. Non-PV+ INs containing the ionotropic 5HT3a receptor (5HT3aR) also participate in direct inhibition of CA1 pyramidal cells. In addition, these interneurons may mediate pyramidal cell disinhibition by inhibiting other interneuron types, including PV+ interneurons. The role 5HT3AR+ INs play in regulating pyramidal cell excitability in the CA1 microcircuit has not been fully explored.
We investigated the effects of 5HT3a+ interneurons' activity on the CA1 microcircuit, focusing on their specific control of pyramidal cell excitability and spike timing. We find that upon application of a 5HT3a receptor specific agonist (mCPBG), pyramidal cells spike more reliably, more precisely and with shorter latencies in response to Schaffer collateral stimulation. Furthermore, ChR2 stimulation of 5HT3a+ INs reduces the feed forward disynaptic inhibitory current, which is responsible for controlling pyramidal cell spike probability and timing. Given the critical role of PV+ interneurons in generating feed forward inhibition, we directly examined their excitability and spike timing while stimulating 5HT3a+ interneurons with mCPBG. PV+ interneurons responded to 5HT3a+ interneuron stimulation with lower spike probabilities, longer latencies, and increased inhibitory drive. These effects were the inverse of those seen in pyramidal cells, consistent with a disinhibitory role for 5HT3a+ interneurons. To understand how 5HT3a+ INs contribute to pyramidal cell inhibition vs. PV+ IN inhibition, we crossed 5HT3a-Cre mice with Lhx6-GFP mice. In future studies, these mice will allow us to optogenetically excite 5HT3a INs and record direct inhibitory responses in MGE-derived interneurons (both SST+ INs and PV+ INs). To explore potential network properties of the PYR-PV+-5HT3a+ IN circuit, we have developed a computational model to explore the impact of varying 5HT3a+ IN intrinsic biophysical and synaptic parameters with Monte Carlo simulations.

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Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: NIH Grant F30 MH106253-02

Title: Propagation patterns in spontaneous activity are state and frequency-dependent

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Abstract: Spontaneous neural activity is widely known to exhibit correlation patterns described as resting state functional connectivity networks (Fox et al. 2005; White et al. 2011). The spatial topographies of these networks are stable over a wide range of frequencies (0.001-4 Hz) (Chan et al. 2015) and across different physiological states (e.g., wake vs. anesthesia) (Silasi et al. 2016). More recent work has demonstrated the existence of stereo-typed propagation patterns in spontaneous activity (Mitra et al. 2015; Mohajerani et al. 2010); however, it is not known how cortical propagation patterns compare across frequencies and states. Here, we apply wide-field optical imaging of a neuronally-expressed fluorescent calcium indicator in a *Thy1*/GCAMP6 transgenic mouse model to explore propagation patterns across a wide range of frequencies, 0.001-4 Hz, in both awake and ketamine/xylazine-anesthetized conditions. We find two distinct propagation regimes: one in the infra-slow frequencies (0.01-0.1 Hz) and one in delta frequencies (1-4 Hz). Infra-slow and delta activity generally propagate in opposite directions, although the propagation speeds between the two frequencies are different by an order of magnitude. Moreover, the directions of both infra-slow and delta propagation reverse across wake and anesthesia. Therefore, neural communication, as measured through propagation of activity, is radically re-organized across frequency and state.

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Disclosures: **A. Mitra:** None. **P.W. Wright:** None. **A.Z. Snyder:** None. **G.A. Baxter:** None. **A.Q. Bauer:** None. **J.P. Culver:** None. **M.E. Raichle:** None.

Poster

302. Network Interactions and Signal Propagation

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: B.10. Network Interactions

Support: Welcome Trust

Title: Closed-loop modulation of hippocampal gamma reveals a role of recurrent excitation in setting the oscillatory frequency

Authors: *E. NICHOLSON[1], D. KUZMIN[2], M. LEITE[2], T. AKAM[3], D. M. KULLMANN[2]; [1]Dept. of Clin. and Exptl. Epilepsy, UCL, London, United Kingdom; [2]Dept. of Clin. and Exptl. Epilepsy, Inst. of Neuology, UCL, London, United Kingdom; [3]Champalimaud Ctr. for the Unknown, Champalimaud Neurosci. Programme, Lisbon, Portugal

Abstract: Gamma oscillations in anatomically coupled brain areas can entrain one another they also show differences in their characteristic frequencies. Hippocampal CA3 oscillates at approximately 30 Hz, whereas CA1 can either be entrained to CA3, or oscillate at 50 Hz or higher frequencies, either from an intrinsic generator or by entrainment with entorhinal cortex. IPSC amplitudes and decay kinetics play a role in setting the frequency. However, a brief optogenetic excitatory pulse targeted to pyramidal neurons can advance or delay subsequent cycles of gamma depending on the phase at which it is delivered (Akam et al., 2012). This implies that phasic excitatory inputs to pyramidal neurons may also have an important role in setting the frequency of hippocampal gamma. We therefore asked whether continuous closed-loop optogenetic modulation of pyramidal neuron excitability could modulate the kinetic properties of gamma. The optogenetic actuator CIVI was expressed either in CA1 or in CA3 pyramidal cells by injecting an AAV with a Camk2a promoter. Hippocampal slices were perfused in a submerged recording chamber mounted on an upright microscope. Upon exposure to an increasing ramp of light delivered through the objective, the local field potential (LFP) recorded in the pyramidal cell layer exhibited a gamma oscillation. We used the instantaneous LFP, or its time derivative, to modulate the light intensity. In a subset of experiments we simultaneously recorded action potentials from individual pyramidal cells, or else recorded membrane currents while voltage-clamping neurons at the ionotropic GABAergic or glutamatergic reversal potential. Consistent with previous studies the frequency of gamma during unmodulated ramps was higher in CA1 than in CA3. Cycle-averaging whole-cell voltage clamp recordings revealed phasic glutamatergic and GABAergic currents in pyramidal neurons that reflect recurrent excitation and inhibition. Closed-loop modulation designed to change the phase and/or amplitude of phasic excitatory currents in pyramidal neurons led to characteristic changes in oscillatory frequency and amplitude. Advancing or delaying the phase of the excitatory drive

relative to inhibitory currents increased and decreased the gamma frequency, respectively. In addition, attenuating the amplitude of the phasic excitatory current increased the gamma frequency while reducing its amplitude. Conversely, amplifying the excitatory current reduced the gamma frequency while increasing its amplitude. We speculate that recurrent excitation among pyramidal neurons in CA3 contributes to the lower characteristic frequency of gamma compared with CA1.

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Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Support: Sloan Research Fellowship

Title: Gain control across cortical layers can be mediated by balanced oscillatory coupling.

Authors: *E. PETERSON, B. VOYTEK;
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Abstract: The balance of “bottom-up” information from the senses with “top-down” goals, attention, and memories is one the most fundamental and heavily studied examples of information conflict in the human brain. Despite substantial work establishing the reality of top-down/bottom-up exchange in sensory cortices, the mechanism underlying this interchange is unknown. Neural oscillations, especially kinds of phase-amplitude coupling (PAC), are thought to play a key role. We've previously described a new mechanism that can explain biophysically how PAC can control information flow. When external oscillations interact with an asynchronous neural population via balanced excitatory-inhibitory input this enhances information flow, by improving the ability of the downstream population to separate signal from noise. In contrast, when oscillatory coupling is unbalanced, driven either by excitation or inhibition, information flow is obstructed. We now put this general mechanism to work in a extremely detailed neural mass model, derived directly from the Blue Brain neocortical microcircuit. This model demonstrates how oscillations and PAC can selectively route top-down versus bottom-up information within the cortical circuit, modeled as tradeoff between thalamic or cortico-cortico connectivity.

Disclosures: **E. Peterson:** None. **B. Voytek:** None.

Poster

302. Network Interactions and Signal Propagation

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Topic: B.10. Network Interactions

Title: Directional propagation of ketamine-induced high-frequency oscillations between the striatum, hippocampus, and motor cortex

Authors: ***M. B. SCHMIT**[1,2], T. YE[1,3], M. J. BARTLETT[1,4,5], T. FALK[1,5,4], S. L. COWEN[1,3,6]; [2]Neurosci., [3]Psychology, [4]Neurol., [5]Pharmacol., [6]Evelyn F. McKnight Brain Inst., [1]Univ. of Arizona, Tucson, AZ

Abstract: High-frequency oscillations (HFOs, 110-160 Hz) are relatively understudied patterns of activity found in the hippocampus, cortex, and basal ganglia (Tort et al., 2013). Although HFOs couple strongly to theta oscillations (Tort et al., 2008) they appear to be distinct from gamma oscillations in their mechanism (Jackson et al., 2011). They are also distinguished from gamma oscillations in their high coherence across different brain regions and between hemispheres (Haufler and Pare, 2014). Other studies have shown HFO coherence between regions, but the directionality of this coherence has not been studied. We hypothesize that HFOs follow a circuit, spreading sequentially through brain structures. We investigated this hypothesis by implanting electrode arrays in the nucleus accumbens (NAc), striatum, M1, and hippocampus of healthy rats. Rats received 5 injections of ketamine (20mg/kg, *i.p*) every 2 hours over an 11-hour period. Local-field and single-unit activity was continuously recorded from awake and freely-behaving animals. We found that repeated exposure to ketamine induced strong, highly coherent HFOs across multiple brain regions. To analyze the directionality of HFO propagation between regions, we used Granger prediction applied to local-field signals restricted to the HFO band. We found that, following ketamine injection, Granger prediction was directional from the NAc to DLS ($p < 0.05$, t-test) and NAc to CA3 ($p < 0.0001$, t-test). No significant difference in prediction direction was observed between M1 and DLS ($p = 0.74$, t-test). This suggests that the NAc is the first step in the circuit, with high-frequency activity propagating from this region to the cortex, striatum, and hippocampus. To control for issues related to volume conduction, electrodes were re-referenced relative to electrodes ~700 μM from the target electrode. Results from this analysis suggest that HFOs in NAc and M1 precede HFOs in the DLS (NAc to DLS $p < 0.0001$, M1 to DLS $p < 0.0001$). This also supports the idea that HFOs are locally generated in the NAc and M1, and that these generators influence the power of HFO activity in other regions.

This is consistent with findings by Hunt et al. (2013) that inactivation of the NAc with tetrodotoxin eliminates HFOs induced by NMDA antagonists. Our data suggests further that M1 contributes to the coordination of HFOs in connected structures. Given these results, it is conceivable that ketamine's therapeutic effects for the treatment of depression (P.R. Diamond, et al. 2014) and levodopa-induced dyskinesia (Bartlett et al., 2016) result from altered network-level communication in the HFO band.

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Poster

303. Oscillations and Synchrony: Unit Studies

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Topic: B.10. Network Interactions

Support: NIH Grant R01MH085074

Title: Intracellular dynamics during self-generated theta oscillations in the whole hippocampal preparation

Authors: ***F. FURMANOV**[1,2], S. WILLIAMS[3], J. A. WHITE[1];
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Abstract: In rodents, theta (4-12 Hz) oscillations are generated during movement related behavior and REM sleep, and are specifically associated with the encoding of spatial information during place cell activity. However, the mechanisms underlying network theta oscillations remain unclear. Intrinsic cellular properties such as membrane resonance and post-inhibitory rebound spiking have been proposed to have an important role in theta generation. To determine if such properties are critical, we took advantage of a whole hippocampal preparation that self-generates stable theta oscillations for over an hour. Using two-photon microscopy to visualize and target specific neuron subtypes expressing genetically encoded fluorophores, we carried out intracellular whole-cell recordings of inhibitory and excitatory cells in subiculum during ongoing network theta generation. Intracellular measures in current- and voltage-clamp indicate that CaMKII+ pyramidal cells exhibit a near-simultaneous increase in excitatory and inhibitory conductance during the rising phase of the LFP oscillation, resulting in spikes that occur near the peak of the oscillation. Further analyses of intracellular data indicate that spike timing is driven

by intense synaptic activity and not intrinsic mechanisms involving resonance or rebound spiking. In GAD2+ inhibitory interneurons, we observed two types of responses during theta oscillations. The first response was associated with lower impedance cells and characterized by spike discharge near the peak of theta, resulting from large depolarizations generated by an increase in excitatory conductance. Identical responses were observed in recordings targeting PV+ interneurons. A second, higher impedance GAD2+ population generated more variable responses and received a mixture of phase-locked excitation and inhibition, resulting in spike generation either at the trough or peak of theta. Overall, our data indicates that spike timing in pyramidal cells during theta oscillations is set by synaptic input consisting of recurrent excitation from other pyramidal cells and inhibition from two distinct interneuron populations.

Disclosures: **F. Furmanov:** None. **S. Williams:** None. **J.A. White:** None.

Poster

303. Oscillations and Synchrony: Unit Studies

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Program#/Poster#: 303.02/F28

Topic: B.10. Network Interactions

Support: Medical Research Council (MC_UU_12024/4)

Title: Specialized contributions of individual GABAergic medial septal neurons to hippocampal network activity

Authors: ***G. UNAL**[1], M. G. CRUMP[1], T. J. VINEY[1], T. ELTES[2], T. KLAUSBERGER[1], P. SOMOGYI[1];
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Abstract: GABAergic neurons of the medial septum innervate the hippocampus and/or related extra-hippocampal cortical areas, and contribute to the coordination of network activity, such as theta oscillations and sharp wave-associated ripples (SWR), via a preferential innervation of GABAergic interneurons (Unal et al., 2015). Individual septal neurons show a wide range of activity patterns, which may be related to their different hippocampal/cortical target areas and/or different types of interneuron they innervate. We tested this hypothesis by extracellular single cell recordings and juxtacellular labeling of individual GABAergic medial septal neurons in vivo in urethane anaesthetized rats. We revealed that individual septal neurons display diverse hippocampal theta phase selectivity in their firing, and terminate in restricted parts of a single or limited number of hippocampal areas and layers. One group of neurons were silent during

SWRs, fired phase-coupled to the descending phase of the CA1 theta oscillatory cycle, and maintained their firing rate between theta and non-theta epochs. In contrast, a second group of neurons were active during SWRs, preferentially fired at the ascending phase of the CA1 theta cycle, and significantly increased their firing during theta oscillations compared to non-theta epochs. Individual medial septal GABAergic axons targeted only a limited range of interneurons in the hippocampus, as established on the basis of expression of molecular markers which are restricted to subsets of interneuron types. The results demonstrate great diversity in septal GABAergic neurons and suggest that they coordinate hippocampal and cortical activity between functionally related areas through highly specialized, target area and cell type-selective projections.
Unal G, Joshi A, Viney TJ, Kis V, Somogyi P (2015) Synaptic targets of medial septal projections in the hippocampus and extrahippocampal cortices of the mouse. J Neurosci 35:15812–15826.

Disclosures: **G. Unal:** None. **M.G. Crump:** None. **T.J. Viney:** None. **T. Eltes:** None. **T. Klausberger:** None. **P. Somogyi:** None.

Poster

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Topic: B.10. Network Interactions

Support: HHMI

Title: Cell-type specific participation in sharp-wave dynamics in the CA3 region of the hippocampus

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Abstract: Sharp-waves are network oscillations that occur during stationary wakefulness, slow-wave sleep, and under light anesthetics. These events emerge from recurrent network dynamics, reflecting the synchronous population activity of CA3 pyramids. Several reports have demonstrated that the CA3a/b sub-region is the preferential locus of initiation for sharp-waves, but little is known about the cellular and circuit mechanisms that give rise to these population events. To gain insight into the functional roles of CA3 cell-types during sharp-waves, we recorded juxtacellularlly, acquiring single units, multiunit activity (MUA), and the local field potential (LFP) from the CA3a/b sub-regions during the spontaneous occurrence of sharp-waves

in-vivo. We previously showed that there are at least two types of pyramidal neurons in CA3 (SfN abstract 576.09/B111), so juxtacellular units were loaded with biocytin for histological validation of cell-type based on the presence or absence of thorny excrescences and the fraction of spikes that were part of a burst (burst index). We analyzed juxtacellular activity relative to locally generated sharp-wave events detected in the LFP, which coincided with a transient increase in MUA measured at that location. We find that high burst-index cells, which lack thorny excrescences, emit a complex spike approximately 200-100ms prior to the peak of the LFP. This initial burst event lead to a slow accumulation of MUA up to a threshold point, at which an additional complex spike initiated a rapid, supralinear increase in MUA. This nonlinear increase in MUA consisted primarily of low burst index cells synchronously firing a single action potential, generating the LFP. These data suggest a revision to the classical picture of the CA3 recurrent network to include a functional segregation into thorny cells (with low burst index) that contribute single spikes to population activity, and athorny cells (with high burst index) that drive the accumulation of network activity to a threshold where a complex spike initiates a nonlinear increase in MUA.

Disclosures: N.P. Spruston: None. **D.L. Hunt:** None.

Poster

303. Oscillations and Synchrony: Unit Studies

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Topic: B.10. Network Interactions

Support: Sloan Fellowship Program

NSERC

UCSD Katzin Prize

Title: Spiking correlates and temporal variability of oscillatory frequency modulation

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Abstract: Neural oscillations are extensively studied due to the putative role of dynamic changes in oscillatory power in neural communication, cognition, and disease. Canonically, oscillations are binned into discrete and functional bands such as theta (4-8Hz) or alpha (8-12Hz). Coherence

in these frequency bands has been used to identify long-range cortical networks in MEG (magnetoencephalography) and ECoG (electrocorticography) recordings. Recently, evidence from both empirical and computational studies has suggested that oscillatory frequency within a single band can itself shift dynamically, impacting behavioral outcomes. For example, in anticipation of an upcoming visual stimulus, alpha frequency can speed up or slow down, within the 8-12Hz bandwidth, to optimize perceptual outcomes.
Here, we analyze the moment-to-moment variation of band-limited oscillatory center frequency, bandwidth, and power in relation to single- and multi-unit activity, across several datasets from three species (human, rat, and macaque). Center frequency and bandwidth are defined as the median and interquartile range of instantaneous frequency, respectively, within a sliding window. Corroborating recent findings, oscillatory frequency within a single band is non-stationary and varies rapidly, suggesting a reevaluation of the stationarity assumption of Fourier analysis on neural electrophysiological data. We report consistently negative correlations between center frequency and bandwidth, but variable relationship between center frequency and oscillatory power (and firing rate) across different brain regions. Furthermore, local networks can be identified from clusters of recording electrodes with shared temporal variation of oscillatory frequency. Finally, we investigate possible mechanisms of frequency dynamics via computational modeling, exploring how frequency shifting in a single network and coherence between networks can impact observed neural oscillations.

Disclosures: **R. Gao:** None. **E.J. Peterson:** None. **B. Voytek:** None.

Poster

303. Oscillations and Synchrony: Unit Studies

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Program#/Poster#: 303.05/F31

Topic: B.10. Network Interactions

Title: Bdnf val66met polymorphism impairs network oscillations in the hippocampus

Authors: *Y. HUANG;
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Abstract: Brain-derived neurotrophic factor (BDNF) is a key regulator of neuronal plasticity and cognitive functions. BDNF val66met polymorphism, a human single-nucleotide polymorphism in the pro-domain of BDNF gene, is common in human populations with an allele frequency of 20 to 30% in Caucasian populations, which is associated with deficits in hippocampus-dependent memory. As revealed in our results, reduction of total number of cells in hippocampal CA1 region of met/met mice was found. There was a trend of increase in the percentage of

parvalbumin (PV)-positive interneuron, yet the change of the number of PV-positive interneurons was not found. Hippocampal gamma and theta oscillations in CA1 region were impaired in met/met mice, whereas theta burst induced long-term potentiation (LTP) was not altered. In addition, the mRNA expression of GAD65 in the hippocampus was elevated in met/met mice compared with that in WT mice, but the expression level of GAD67 was not affected by BDNF val66met polymorphism. Moreover, hippocampal expressions of β2 and β3 subunit of GABA receptor were up-regulated in met/met mice compared with WT mice. Our results suggested that BDNF val66met polymorphism changes activity of subtypes of interneuron in the hippocampus, which may underlie neural network dysfunction and cognition impairment.

Disclosures: Y. Huang: None.

Poster

303. Oscillations and Synchrony: Unit Studies

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Title: Volitional control of beta-band oscillations by brain machine interface affects sensorimotor behavior of primates

Authors: *O. PELES[1,2], U. WERNER-REISS[1,2], H. BERGMAN[1,2], Z. ISRAEL[3], E. VAADIA[1,2];
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Abstract: It is widely accepted that beta-band oscillatory activity plays an important role in the facilitation of neural synchrony essential for complex tasks such as planning and execution of

sensorimotor behavior. Previous studies suggested that beta-band oscillations in the motor cortex increase during hold periods, attenuate during movement initiation and re-emerge thereafter. However, the exact function of these oscillations is not yet understood.
We investigated the effect of beta-band oscillations on behavior by training two macaque monkeys, implanted with arrays of 96 electrodes in the motor cortex, to volitionally enhance beta-band (20-30Hz) oscillatory activity at local selected sites by neural operant conditioning, using a real time brain machine interface (BMI) platform. The monkeys were engaged in a behavioral task involving color discrimination. In addition, we tested the effect of electrical intracortical micro-stimulation (ICMS) on these spatiotemporal patterns.
We demonstrate that beta-band cortical oscillations can be robustly increased and that many neurons were phase locked to these oscillations. Furthermore, we found that the LFP beta-power before the execution of the motor action was positively correlated with the reaction times, and negatively correlated with behavioral performance (success rate).
Our results suggest that during high beta epochs new motor actions take longer to initiate and the perception of sensory inputs is impaired. These findings may support the widely accepted hypothesis that beta oscillations are tied to preservation of current motor state and help in unraveling the functional role of cortical oscillations and their effect on neural synchrony.

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Poster

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Title: Extracting neural networks formed by consistent between-cell spike timing from unit recordings

Authors: *R. VAN DER MEIJ[1], E. MARIS[2], B. VOYTEK[1];
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Abstract: It is widely assumed that distributed and overlapping neuronal networks are fundamental to brain functioning. These networks are thought to form in an ad hoc manner and encode information by the temporal activity patterns of the neurons that form them. How this occurs however, is not well understood. A hypothesis that has received recent empirical support is that information can be represented by the temporally precise spiking pattern among neurons forming a spike-timing network (also denoted as a cell assembly). Finding networks formed by consistent between-cell spike timing is challenging however, because the amount of possible spiking patterns grows exponentially with the amount of cells being recorded. At the same time, recent evidence suggests that such networks, if present, are small, making detection additionally problematic. Here, we present a technique that is capable of extracting neural networks formed by consistent between-cell spike timing from large-scale recordings of spike trains. This technique, denoted as SPACE, makes use of the spatial and temporal structure of such networks to find and separate them. Networks are described by (1) a weighting per neuron, describing how strongly they are part of the network-specific spiking pattern: (2) a set of between-neuron time delays describing the spiking pattern, and; (3) a weighting per epoch (e.g., a trial) indicating how strongly this network was present on any given trial. The latter can be used to quantify network presence between experimental conditions, and its relation to e.g., stimulus parameters or behavioral performance. Using simulations, we show that the technique is capable of finding spike-timing networks in a noisy environment, is robust to spike jitter, and can separate networks involving the same neurons. As such, the technique could be a valuable tool for investigating the prevalence and function of patterns in between-cell spike timing and uncovering functional neuronal networks in an unbiased manner.

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Poster

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Title: Fast-spiking neurons organize large-scale coherent oscillations and are the main generators of local field potentials in human and monkey cortex

Authors: *A. DESTEXHE[1], M. LE VAN QUYEN[2], N. DEHGHANI[3], E. HALGREN[4], S. CASH[5], N. HATSOPOULOS[6], B. TELENCZUK[1];
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Abstract: We analyzed recordings from Utah-type multi-electrode arrays, in human temporal cortex and monkey motor cortex, during wake and sleep states. To investigate the respective role of different cell types, we separated cells between fast-spiking (FS) and regular-spiking (RS) neurons based on spike shape, some of which were confirmed by direct pairwise interactions between cells from neighboring electrodes (Peyrache et al., PNAS 2012; Dehghani et al., Sci. Rep. 2016). FS and RS cells had different firing rates and autocorrelation attributes. Additionally, we found marked differences in their participation to LFP oscillations in the beta and gamma frequency range. Almost half of the FS cells participated actively to the oscillations, while only a smaller subset (<10%) of RS cells did participate. Furthermore, FS cells displayed long-range correlations while RS cells tended to be correlated only locally. Interestingly, during slow-wave sleep, LFP oscillations were maximally coherent, while pairwise interactions between FS cells were also the highest. Finally, we also determined the spike-triggered average LFP (stLFP), after removing correlations by whitening. The whitened stLFP propagated at the axonal speed across the array, suggesting that it represents a unitary field. The stLFP was in general broader for RS cells, while for FS cells it was sharper, of larger amplitude, and peaked earlier than for RS cells. This suggests that, not only FS cells have more impact on the LFP, but the RS cells contribution to LFP could be di-synaptic through FS cells, in which case the LFP would be generated almost exclusively by FS cells. We discuss possible biophysical mechanisms for how the LFP, although principally generated by pyramidal cells dipoles, could be generated almost exclusively by inhibitory conductances. Together, these results show that in human temporal and monkey motor cortex, FS cells have a determinant role, both at the level of the LFP signal itself, but also in the organisation of oscillations, and their large-scale coherence.

Disclosures: **A. Destexhe:** None. **M. Le Van Quyen:** None. **N. Dehghani:** None. **E. Halgren:** None. **S. Cash:** None. **N. Hatsopoulos:** None. **B. Telenczuk:** None.

Poster

303. Oscillations and Synchrony: Unit Studies

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Topic: B.10. Network Interactions

Support: This research project was supported by Consejo Nacional de Ciencia y Tecnologia (CONACYT).

Title: Fast Oscillatory activity in the hippocampus can back-propagate electrotonically to the dentate gyrus

Authors: *F. ORTIZ[1,2], R. GUTIÉRREZ[1];
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Abstract: Fast ripples (FRs), which are intermittent high frequency oscillations (>250 Hz) are the hallmark of epileptic foci in the limbic system. While electrotonic coupling is a mechanism by which high frequency network activity (150-200 Hz) becomes synchronized, FRs depend mainly on chemical synaptic transmission. Although a synaptic back-projection from CA3 to the DG has been described, the mechanisms by which FRs emerge in the DG remain elusive. One possibility would be that electrical coupling between pyramidal and granule cells may be responsible for back-propagating signals. To address this, we used a traumatic brain injury model where the entorhinal cortex is disrupted *in vivo*, and that produces FRs in the hippocampus *in vitro*. We recorded extracellular field activity with a multielectrode array of 4096 microelectrodes from combined entorhinal cortex-hippocampal slices from young adult Wistar rats. We used the Granger causality test to determine the directionality of FRs propagation. Recordings were conducted under normal neurotransmission conditions (normal ACSF), after preventing chemical transmission (in low $[Ca^{++}]_o$; 0.2 mM) and, finally, after blockage of electrical transmission with mefluoquine (50 μm). Under these conditions, we assessed functional connections between neurons of CA3 and DG.
For a given experiment, FRs initiated in a restricted area of CA3b that was followed by activation of different areas of the DG before invasion of the adjacent CA3a and c regions. FRs propagation did not spread homogeneously from one site of origin, rather, activity appeared simultaneously in discontinuous sites and with different oscillatory frequencies. These results confirm the presence of a CA3-to-DG back propagation. Importantly, using the Granger causality test we confirmed that FRs in the hilus and DG were causally related to FRs originated in strata lucidum and radiatum of CA3, rather than in the pyramidal cell layer.
Perfusion of low $[Ca^{++}]_o$ ACSF suppressed FRs. Under these conditions, we used appropriate filters to record unitary activity characterized by firing of CA3 pyramidal cells and granule cells of the DG in an apparent random manner. Interestingly, functional bidirectional connections

from the DG to CA3 and from CA3 to DG could be verified by a robust correlation analysis. The perfusion of the connexin36-specific gap junction blocker mefluoquine suppressed about 50 % of functional connections. We conclude that FRs emerge in CA3 after which FRs appear in the DG and CA1 albeit with different frequencies and a measurable phase shift. Back propagation from CA3 to the DG via the mossy fibers may play a significant role in DG activation by CA3 activity.

Disclosures: F. Ortiz: None. **R. Gutiérrez:** None.

Poster

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MTA-SZTE Agykérgi Neuronhálózatok Kutatócsoport

Nemzeti Agykutatási Program

Title: Identified cellular correlates of neocortical ripple and high gamma oscillations during spindles of natural sleep

Authors: *G. TAMAS, V. SZEMENYEI, S. BORDÉ, R. G. AVERKIN;
Univ. of Szeged, Szeged, Hungary

Abstract: Ultra-high frequency network events in the hippocampus are instrumental in a dialogue with the neocortex during memory formation, but the existence of transient ~200 Hz network events in the neocortex is not clear. Our recordings from neocortical layers layer II/III of freely behaving rats revealed locally generated field potential events at ripple and high gamma frequencies repeatedly occurring at troughs of spindle oscillations during sleep with no correlation to ongoing hippocampal ripples. Juxtacellular recordings identified subpopulations of fast spiking, parvalbumin containing basket cells with epochs of firing at ripple (~200 Hz) and high gamma (~120 Hz) frequencies detected during spindles and centered with millisecond precision at the trough of spindle waves in phase with field potential events but antiphase to pyramidal cell firing. The results suggest that basket cell subpopulation timed, spindle nested, high frequency network events might provide repeatedly occurring neocortical temporal reference states potentially involved in mnemonic processes.

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Poster

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Topic: B.10. Network Interactions

Support: NINDS NS35915

Title: Intrinsic and extrinsic sources of GABAergic inhibition in the dentate gyrus

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Abstract: The hippocampus plays a crucial role in high order brain functions such as learning, memory and spatial navigation. The input structure to the hippocampus, the dentate gyrus (DG), appears to have a sparse coding scheme: the principal granule cells are rarely active during behavior. It is generally agreed that GABA receptor-mediated inhibition is necessary for maintaining this sparse activity. However, the exact sources of this GABAergic inhibition and their temporal and spatial organization are largely unknown. To address this question, we performed juxtacellular recordings in awake mice from rigorously identified hippocampal GABAergic cells that innervate the DG. The local interneurons we recorded belonged to four neurochemically defined categories of somatostatin (SOM), parvalbumin (PV), nitrogen-monoxide synthase (NOS) and cannabinoid type I receptor (CB1)-expressing cells. Strikingly, we also identified GABAergic neurons from each neurochemical group in the CA3 and CA1 regions that have axonal projections to the DG (DG_{GABA}EXT cells). Both local DG interneurons and DG_{GABA}EXT cells were strongly modulated by running-associated theta rhythm and gamma oscillation. We also found that all the local interneurons recorded in the hilus or in the granule cell layer increased their spiking frequency during dentate spikes, a synchronous population event thought to be triggered by population bursts in the entorhinal cortex. In addition, sharp-wave-ripples (SWRs), which are synchronous population events generated in the CA3/CA1 network, modulated the firing frequency not only of DG_{GABA}EXT cells but the spiking activity of local DG interneurons as well. The modulation of GABAergic cells during SWRs appeared to be bidirectional: most neurons increased their firing rates, while others robustly reduced their firing rate during SWRs. This reduction of spiking during SWRs is most likely due to perisomatic inhibition resulting from inhibitory-inhibitory connections. Our results show that the dentate granule cells are under the influence of both intrinsic and extrinsic sources of GABAergic

inhibition that are temporally organized by various oscillations and synchronous population patterns with local and extra-dentate origin. These findings suggest that the sparse coding in the dentate gyrus is maintained by a complex GABAergic control including contributions from hippocampal regions external to the dentate gyrus. Funded by NINDS NS35915.

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Poster

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Topic: B.10. Network Interactions

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Title: Changes in network dynamics and prefrontal spiking activity in a mouse genetic model of schizophrenia: implications for connectivity

Authors: *J. L. ZICK[1,2], K. SCHULTZ[4], T. I. NETOFF[1,3], M. V. CHAFEE[4];
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Abstract: Disordered connectivity appears to be a key pathophysiological feature of schizophrenia. Measures of temporal correlation between regions of the brain are consistently reduced in schizophrenic patients upon functional imaging; however, evidence for functional disconnection at the cellular level is limited. Here we describe a study in which we assessed functional connectivity between spiking neurons in a mouse genetic model of schizophrenia. Neural data were obtained from multielectrode recording arrays inserted into the infralimbic and prelimbic areas of awake Dgcr8$^{+/-}$ mice and their littermate controls. Heterozygous deletion of the Dgcr8 gene is used to model the 30-fold increase in risk for schizophrenia which is seen in human patients with DiGeorge syndrome. In order to investigate hypothesized alterations in spike timing in the disease state, we identified the distribution of interspike intervals between putative pre- and postsynaptic neurons. In our analysis we found a prominent “zero-lag” peak representing a large number of coincident (within 1 millisecond) action potentials between coupled cells in the control mouse data, but not in the Dgcr8$^{+/-}$ mice. This suggests a change in the timing of action potentials which was apparent in the absence of a change in firing rates,

precluding overall decreased activity as an explanation for reduced synchrony. Additionally, we found evidence of bursting activity consistent with hippocampal sharp wave modulation of prefrontal activity in the awake resting state. These events are significantly more frequent in Dgcr8$^{+/-}$ mice, suggesting that dysregulation of prefrontal-hippocampal communication may be a key feature of this model and of schizophrenia. In summary, these results suggest disordered functional connectivity within the prefrontal cortex and between prefrontal cortex and hippocampus which may be related to a Hebbian reduction in synaptic strength resulting from desynchronization of spiking activity.

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Poster

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Title: Association of ketamine, dopamine D4 receptor activation, and locomotion with gamma range activity in the prefrontal cortex and mediodorsal thalamus

Authors: *K. E. FURTH[1,2,3], A. J. MCCOY[1], J. R. WALTERS[1], A. BUONANNO[3], C. DELAVILLE[1];
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Abstract: An acute, subanesthetic dose of the NMDA antagonist ketamine is used to model early stage schizophrenia, and increases cortical gamma oscillation power (40-70Hz) in healthy humans and rats. As reduction of this ketamine-induced cortical gamma power has been proposed to predict antipsychotic drug efficacy, we tested the ability of a dopamine D4 receptor (D4R) agonist to decrease ketamine-induced gamma power. We also compared gamma oscillations induced by ketamine to those induced by walking in the medial prefrontal cortex (mPFC) and its major thalamic source of innervation, the mediodorsal thalamus (MD), at the

LFP and single neuron levels.
Following 10 mg/kg ketamine, recordings from chronically implanted electrodes in male Long Evans rats walking on a treadmill showed a 254% increase in gamma LFP power centered at 55Hz in the mPFC, associated with increases in pyramidal neuron firing rates (33%) and pyramidal spike-gamma LFP correlations (107%). Treadmill walking also increased gamma power (centered at ~51Hz), firing rates, and spike-gamma LFP correlations in the mPFC. Notably, these walking-induced increases were less pronounced than those induced by ketamine. By contrast, in the MD, ketamine decreased firing rates (-46%) and the correlation between spikes and gamma oscillations (-34%) and increased gamma power by 31%, whereas walking increased gamma power, firing rates and correlation between spikes and gamma oscillations. In addition, ketamine did not change the gamma range coherence between the mPFC and MD, suggesting that synchronized inputs from neither area is driving gamma activity in the other. D4R agonists and antagonists can modulate gamma power and cognitive performance in animals, making D4Rs pro-cognitive targets in schizophrenia. The D4R agonist A-412,997 increased gamma power in the mPFC and MD, and the D4R antagonist L-745,870 reversed this increase. When injected before ketamine, neither the D4R agonist nor antagonist altered ketamine-induced changes in gamma power or firing rates in the mPFC. However, the D4R agonist increased ketamine-induced gamma power in the MD and reversed ketamine's inhibitory effect on firing rates.
Results show that gamma oscillations induced by walking and ketamine are similar in the mPFC, hinting at a common circuit underlying both oscillations. However, the differences in neuronal correlates of walking- and ketamine-induced gamma oscillations in the MD suggest that these oscillations arise from different mechanisms. Thus, walking-induced gamma oscillations are more similar between the mPFC and MD thalamus, while ketamine-induced gamma oscillations show region-specific differences.

Disclosures: K.E. Furth: None. **A.J. McCoy:** None. **J.R. Walters:** None. **A. Buonanno:** None. **C. Delaville:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.01/F39

Topic: B.14. Neuro-Oncology

Support: AIRC IG 2015 Id.16713

Title: Mutual influence of ROS, pH_i and chloride current in cell cycle progression of glioblastoma cancer stem cells

Authors: I. VERDUCI[1], M. PERETTI[1], F. M. RACITI[1], R. WURTH[3], F. BARBIERI[3], T. FLORIO[3], *M. MAZZANTI[2];
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Abstract: Glioblastoma (GBM) is the most aggressive and lethal among the brain tumors and finding a possible therapy still represents a big challenge. Cancer stem cells (CSCs), a small fraction of self-renewing cells, are responsible for GBM origin, progression and recurrence, also originating non-CSC population composing the bulk of the tumor. The broad aim of our investigation is to find specific pharmacological targets to eradicate CSCs.
We have previously shown a pivotal role of the Chloride intracellular channel 1 (CLIC1) protein in the tumorigenic potential of CSCs isolated from grade IV human GBM and that CLIC1 is essential for proliferation and self-renewal of GBM CSCs. In physiological conditions this protein is mostly cytoplasmic but, under stress conditions, it translocates to the membrane, where it acts as an ion channel. CLIC1 is poorly expressed in the membranes of human differentiated GBM cells and in umbilical cord mesenchymal stem cells, underlining its CSCs specificity. CLIC1 role in CSC activity occurs during cell cycle progression. CLIC1 channel inhibition leads to a partial but significant arrest of GBM CSCs in G1 phase. Previous reports showed both pH_i and reactive oxygen species (ROS) fluctuation during cell cycle. In recent investigation, the influence of pH_i changes and cytoplasmic oxidation on CLIC1 membrane translocation was demonstrated. We now report the relationships that links these three elements during G1/S phase transition. Cytoplasmic alkalization promoted by the Na^+/H^+ (NHE1) exchanger is able to enhance both CLIC1 membrane localization and chloride current. During the transition from G1 to S phase, pH_i levels increase simultaneously with CLIC1 membrane localization and the increase of chloride current. Furthermore, impairment of NHE1 function along G1 phase progression does also alter CLIC1-mediated current.
Since ROS production by NADPH oxidase is also involved in CLIC1 activity and G1/S phase progression, our current hypothesis is that CSCs undergo a tight regulation in which ROS, pH and chloride currents create a virtuous (vicious?) loop which enhances CSCs viability and is responsible for GBM aggressiveness. Giving its specificity on CSC membranes, CLIC1 represent, among the three regulatory proteins, the most suitable novel pharmacological target.

Disclosures: I. Verduci: None. **M. Peretti:** None. **F.M. Raciti:** None. **R. Wurth:** None. **F. Barbieri:** None. **T. Florio:** None. **M. Mazzanti:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.02/F40

Topic: B.14. Neuro-Oncology

Support: MOST 104-2314-B-038-031

Title: Inhibition of glioma progression by a novel compound of anthraquinone

Authors: *K.-Y. CHEN[1], S.-J. KANG[2], Y.-H. CHIANG[3], J.-C. WU[3], H.-S. HUANG[4]; [2]Dept. of Med. Lab. Sci. and Biotech., [3]Dept. of Surgery, Col. of Med., [4]The Ph.D. Program of Cancer Biol. and Drug Discovery, [1]Taipei Med. Univ., Taipei, Taiwan

Abstract: Aim:
High grade gliomas are the most common type of malignant brain tumor, and cause significant morbidity and mortality. Anthraquinone drug displayed antiproliferative activity and used for carcinoma treatment. However, these drugs also have cardiotoxicity that limited their applicability. A new group of anthraquinone-based analogs, LCC, has less toxicity on the heart, and its effects and dosage is investigated on in vitro and in vivo glioma models.
Methods:
Human glioma tumor cell line U87MG was seeded in 96-well plates and treated with LCC (1, 10 and 100 μM) for 24 and 48 hours. The cell viability was analyzed by SRB assay. Apoptotic cell numbers were detected by flow cytometry. In vivo study, we used Subcutaneous Xenograft Tumor Models. U87MG was injected subcutaneously and treated with 100μM LCC for 24 hours in 0.1 ml of the cell suspension (1×10^6 cells). Then, nude mice were sacrificed and tumor volume were measured.
Results:
In our study, based on the results of SRB assay and flow cytometry analysis, we observed that cell survival was decreased and cell numbers in sub G1 phase increased after drug treatment. In addition, the tumor volume significantly reduced after LCC treatment in the Subcutaneous Xenograft Tumor Model.
Conclusion:
In conclusion, cell proliferation activity of U87MG was inhibited by LCC both in vitro and in vivo. Lastly, LCC may be a potential candidate for glioma treatment in the future.

Disclosures: K. Chen: None. **S. Kang:** None. **Y. Chiang:** None. **J. Wu:** None. **H. Huang:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.03/F41

Topic: B.14. Neuro-Oncology

Support: MOST 104-2320-B-006-007-MY3

Title: Inhibition of sonic hedgehog signaling suppresses glioma stem-like cells through inducing autophagic cell death

Authors: *P.-W. GEAN[1], H.-C. HUNG[2];
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Abstract: Glioma stem cells (GSCs) are thought to contribute to tumorigenesis and recurrence. In this study, we isolated GSCs from human glioblastoma U87 cell line by Magnetic-activated cell sorting (MACS) using CD133 as a marker. These $CD133^+$ glioma cells exhibit self-renew property and stem cell markers. We inoculated $CD133^+$ or $CD133^-$ cells (1×10^4) subcutaneously into the nude mice and 10 days later, tumors were observed in $CD133^+$ cells but not $CD133^-$ cells inoculated mice. Western blotting analysis showed that Sonic hedgehog (Shh) expression is higher in $CD133^+$ cells than in $CD133^-$ cells. Immunocytochemical analysis revealed that over 90% of $CD133^+$ cells were positive for Shh. Application of Shh inhibitors LDE225 induced cell death and reduced the number of neurospheres. In LDE225-treated cells, appearance of large membranous vacuoles in the cytoplasm which is a characteristic feature of cells undergoing autophagy was observed. Immunoblotting revealed the conversion of LC3-I to LC3-II. In addition, p62 increased at 2 h and returned to baseline within 12 h after the treatment of LDE225. Finally, LDE225-induced cell death was reversed by autophagy inhibitor 3-methyladenine (3-MA) suggesting that autophagy plays a critical role in LDE225-induced cell death. Lenti-shRNA-*Shh* inhibited tumor growth in intracranial glioma xenograft model. These results suggest that the Shh inhibitors may be developed as a new therapeutic strategy against malignant gliomas.

Disclosures: P. Gean: None. **H. Hung:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.04/F42

Topic: B.14. Neuro-Oncology

Title: Sevoflurane may increase glioma cell invasion via CD44

Authors: *Z. ZUO, R. LAI, W. SHAN;
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Abstract: Patients with cancer often require surgery or procedure under general anesthesia. Studies have shown that anesthetics may affect cancer cell biological characteristics. Whether sevoflurane, a commonly used anesthetic in current practice, has any effects on glioma cells is still unknown. Here, we show that sevoflurane dose-dependently increased the invasion of human glioma cells. Sevoflurane also increased the expression of CD44 and calpain activity. Silencing the expression of CD44 attenuated sevoflurane-induced increase of glioma invasion and calpain activity. We conclude that CD44 mediates sevoflurane-increased invasion of human glioma cells and that a downstream event includes calpain activation.

Disclosures: Z. Zuo: None. **R. Lai:** None. **W. Shan:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

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Agence Nantional de Recherche project DENANORNA

Title: Downregulation of TLX induces TET3 expression and inhibits glioblastoma stem cell self-renewal and tumorigenesis

Authors: *Q. CUI[1], S. YANG[1], P. YE[2], E. TIAN[2], G. SUN[2], J. ZHOU[3], G. SUN[4], X. LIU[10], C. CHEN[10], K. MURAI[2], L. YANG[5], X. WU[5], M. D'APUZZO[6], C. BROWN[7], B. BADIE[8], L. PENG[10], A. D. RIGGS[4], J. J. ROSSI[9], Y. SHI[2];
[1]Developmental and Stem Cell Biology; Irell and Manella Grad. Sch. of Biol. Sci., [2]Developmental and Stem Cell Biol., [3]Dept. of Mol. and Cell. Biol., [4]Dept. of Diabetes and Metabolic Dis. Res., [5]Integrative Genomics Core, [6]Dept. of Pathology, [7]Dept. of Hematology and Hematopoietic Cell Transplantation, [8]Dept. of Surgery, [9]Dept. of Mol. and Cell. Biology; Irell and Manella Grad. Sch. of Biol. Sci., Beckman Res. Inst. City of Hope, Duarte, CA; [10]Ctr. Interdisciplinaire de Nanoscience de Marseille, Aix-Marseille Universite´, Marseille, France

Abstract: Glioblastoma is the most common and lethal type of brain tumor in adults. The discovery of glioblastoma stem cell (GSC) showed that the GSCs are able to repopulate the tumors and this side population is more resistant to current therapy than bulk tumor cells. Therefore, GSC targeted therapeutics is urgently needed for treatment of glioblastoma. TLX is an orphan nuclear receptor that plays important roles in maintaining normal neural stem cells at the self-renewable state. TLX has also been shown to be involved in tumorigenesis and progression of glioblastoma in mouse models. Here, we show that TLX is critical to promote the growth, self-renewal and tumorigenesis of GSCs derived from patient samples of multiple glioblastoma subtypes. TLX, as a transcription factor, regulates the self-renewal of GSC through suppressing the expression of downstream target genes such as the DNA hydroxylase Ten eleven translocation 3 (TET3), an important epigenetic regulator. Potential therapeutics including both viral vector delivered shRNA and nanovector delivered siRNA have been developed to target TLX in GSCs in patient derived xenograft models. Using DNA microarray analysis, we identified a set of novel TLX downstream target genes/pathways. How TLX regulates these downstream genes/pathways to regulate GSC self-renewal and tumorgenesis is under investigation. In addition, we are developing new ways to deliver TLX siRNAs into GSCs in order to establish more effective therapies for glioblastoma.

Disclosures: Q. Cui: None. **S. Yang:** None. **P. Ye:** None. **E. Tian:** None. **G. Sun:** None. **J. Zhou:** None. **G. Sun:** None. **X. Liu:** None. **C. Chen:** None. **K. Murai:** None. **L. Yang:** None. **X. Wu:** None. **M. D'Apuzzo:** None. **C. Brown:** None. **B. Badie:** None. **L. Peng:** None. **A.D. Riggs:** None. **J.J. Rossi:** None. **Y. Shi:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

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Topic: B.14. Neuro-Oncology

Support: The study was supported by the Ministry of Science and Technological Development of the Republic of Serbia (grant number 173053 and 41025). Ljubica Harhaji-Trajkovic is a recipient of the UNESCO L'OREAL national scholarship program "For Women in Scie

Title: *In vitro* antiglioma action of indomethacin is mediated via AMPK/mTOR signaling pathway

Authors: *A. PANTOVIC[1], A. PANTOVIC[2];
[1]Neurol., Military Med. Acad., Belgrade, Serbia; [2]Military Med. Acad. and Inst. for microbiology and immunology Sch. of Med. Univ. of Belgrade, Belgrade, Serbia

Abstract: We investigated the role of the intracellular energy-sensing AMP-activated protein kinase (AMPK)/ mammalian target of rapamycin (mTOR) pathway in the in vitro antiglioma effect of the cyclooxygenase inhibitor indomethacin. The toxicity of indomethacin towards U251 human glioma cell line, determined by crystal violet/MTT assay, was associated with oxidative stress, caspase activation, and the induction of apoptosis, as determined by flow cytometry. An immunoblot analysis revealed that indomethacine increased the phosphorylation of AMPK and its targets Raptor and acetyl-CoA carboxylase (ACC), while it reduced the phosphorylation of mTOR and its substrate p70S6 kinase (S6K). The phosphorylation of the upstream mTOR activator Akt was increased by indomethacine, thus excluding its involvement in the drug-induced mTOR suppression. AMPK knockdown by RNA interference, as well as the treatment with the mTOR activator leucine, prevented indomethacin-mediated mTOR inhibition and proapoptotic action. Indomethacin failed to increase beclin-1 expression, intracellular acidification, and autophagic flux, and genetic or pharmacological inhibition of autophagy had no effect on its antiglioma activity, arguing against the involvement of autophagy in the observed effect. AMPK activation by indomethacin correlated with intracellular ATP depletion, and was apparently independent of cyclooxygenase inhibition or the increase in intracellular calcium. Finally, the toxicity of indomethacin towards glioma cell lines established from glioblastoma multiforme patients was also associated with the activation of AMPK/Raptor and subsequent suppression of mTOR/S6K signaling. By demonstrating the involvement of AMPK/mTOR pathway in the antiproliferative/proapoptotic action of indomethacin in glioma cells, our results support its further exploration in glioma therapy.

Disclosures: **A. Pantovic:** Other; The study was supported by the Ministry of Science and Technological Development of the Republic of Serbia (grant number 173053 and 41025).

Ljubica Harhaji-Trajkovic is a recipient of the UNESCO L'ORE. **A. Pantovic:** Other; The study was supported by the Ministry of Science and Technological Development of the Republic of Serbia (grant number 173053 and 41025). Ljubica Harhaji-Trajkovic is a recipient of the UNESCO L'ORE.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.07/F45

Topic: B.14. Neuro-Oncology

Support: DFG GRK1657

Title: The impact of ionotropic glutamate receptors on glioblastoma stem-like cells upon ionizing radiation

Authors: H. LUTZ[1], *H. A. BRAUN[2], B. LAUBE[1];
[1]TU Darmstadt, Darmstadt, Germany; [2]Univ. Marburg, Marburg D-35037, Germany

Abstract: Ionizing radiation, combined with surgical resection and chemotherapeutics, is the standard therapy for people suffering from glioblastoma mulitforme. Despite this aggressive therapy the median survival of patients is in the range of 15 months due to recurrence of the cancer in almost all patients. This therapeutic resistance is probably promoted by a currently discussed and highly tumorigenic subpopulation of glioblastoma stem cells (GSC), which are in focus of many new therapeutic approaches. It is proposed that the migration of glioblastoma stem cells is induced by sub curative doses of ionizing radiation as well as glutamate. Additionally glutamate increases the viability and proliferation of glioblastoma cells, while it induces excitotoxicity in the normal neural tissue. These abilities of GSCs to resist therapy and to migrate in the surrounding tissue might promote the recurrence of the glioblastoma in close proximity to the primary tumor. Our group was able to show the functional expression of NMDA and AMPA receptors in the glioblastoma cell line LN229 by immunofluorescence staining and patch clamp technique. We used the NMDA receptor antagonist Ifenprodil and the AMPA receptor antagonist Gyki-52466 to reduce the migration of these cells significantly. In LN229 cells MK-801 and NBQX delay the repair of DNA double-strand breaks, indicated by γH2AX and 53BP1 foci, after irradiation. Latest results show that this effect is even more prominent in $CD133^+$ glioblastoma stem cells. This observation leads to the question of the role of glutamatergic signaling in glioblastoma stem cell, the underlying mechanisms and the applicability for tumor treatment.

Disclosures: **H. Lutz:** None. **H.A. Braun:** None. **B. Laube:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.08/F46

Topic: B.14. Neuro-Oncology

Support: California Institute for Regenerative Medicine

Title: Heterogeneous glioblastoma cell responses to TNF depend on molecular subtype.

Authors: ***M. E. BARISH**[1], A. MIZES[1], B. BREWSTER[1], N. BAGHDADCHI[1], C. E. BROWN[2];
[1]Neurosciences, [2]Immuno-Oncology, Beckman Res. Inst. City of Hope, Duarte, CA

Abstract: Glioblastoma multiforme (GBM), one of the most aggressive of human cancers, displays wide variation between patient tumors. To impose an orderly classification, patient tumors have been stratified, and while the tumor subtype classifications that have emerged vary, there is a consensus that two ends of the spectrum are anchored by the proneural (PN) and mesenchymal (Mes) subtypes. PN tumors are associated with preferential expression of neural stem and progenitor cell markers, and Mes tumors with mesenchymal markers and markers of epithelial-to-mesenchymal transition (EMT). Regions within a tumor may vary in subtype, and for GBM up to one-third of total cells may be microglia and tumor associated macrophages. Overall, the interplay of intrinsic molecular subtypes with the tumor microenvironment is complex.
We examined how GBM molecular subtype and differentiation status influence invasive behaviors. The microglia/macrophages associated with GBM secrete a number of proinflammatory cytokines including tumor necrosis factor (TNF). We have reported that in vitro, TNF up-regulates GBM cell expression of the adhesion molecule VCAM-1 (Mahadev et al., PLoS One 9: e95123; 2014). To compare GBM cells of varying molecular subtype and differentiation status, we utilized a cohort of patient-derived primary glioma cell lines characterized by TCGA signature gene expression as PN or Mes (Brown et al., PLoS One 8: e7769; 2013), grown in culture with stem-like or differentiated cell properties (Brown et al., Cancer Res 69: 8886-8893; 2009). We used a “dot migration assay” that recapitulates in vivo brain tumor dissemination along blood vessels and associated ECM (Baghdadchi et al., in preparation), and measured spatial dependence of tumor cell morphology and physical interactions with other tumor cells, migration, and expression of tumor adhesion molecules and other markers, by immunofluorescence.

We examined responses to TNF by proneural (PBT003) and mesenchymal (PBT017) GBM cell lines over a period of six days (as with chronic exposure in vivo). Our most striking observation is that the (PN) PBT003 and (Mes) PNT017 cells differ markedly in their VCAM-1 expression responses to TNF. Specifically, VCAM-1 immunoreactivity is initially low on (PN) PBT003 cells and is markedly enhanced by exposure to TNF (10 ng/ml). In contrast, VCAM-1 immunoreactivity is initially higher on (Mes) PBT017 cells and is decreased on chronic exposure to TNF.
While this study is on-going, these observations suggest that patient tumor responses to activated microglia and macrophages may be regionally disparate depending on local molecular subtype.

Disclosures: **M.E. Barish:** None. **A. Mizes:** None. **B. Brewster:** None. **N. Baghdadchi:** None. **C.E. Brown:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.09/F47

Topic: B.14. Neuro-Oncology

Support: NIH Grant DA014486

Title: Antineoplastic activity of cannabidiol combined with DNA-damaging agents in glioma cells

Authors: ***L. DENG**[1], L. NG[1], T. OZAWA[2,3], E. C. HOLLAND[2,3], N. STELLA[1];
[1]Dept. of Pharmacol., [2]Dept. of Neurosurg. and Alvord Brain Tumor Ctr., Univ. of Washington, Seattle, WA; [3]Div. of Human Biol. and Solid Tumor Translational Res., Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: DNA-damaging agents remain the main standard care treatment for patients diagnosed with gliomas. Evidence suggests that the non-psychotropic cannabis-derived compound, cannabidiol (CBD), has antineoplastic activity in multiple subtypes of cancers, including gliomas. Here we studied the anti-proliferative response and the effects on cell viability triggered by CBD alone and in combination with three DNA-damaging agents (e.g., temozolomide, carmustine and cisplatin) in glioma cells and healthy neuroprogenitor cells in culture. Cell proliferation is assessed using the bromodeoxyuridine (BrdU) cell proliferation assay whereas cell viability is measured using water soluble tetra-zolium salt (wst-1) reagent. We found that CBD induced a sharp, dose-dependent reduction on both proliferation and viability of glioma and neuroprogenitor cells with similar potency (2-5 μM). Co-treatment analyses showed that CBD

produced synergistic responses with DNA-damaging agents over a limited range of concentrations in most glioma cells tested as well as in neuroprogenitor cells. However, antagonistic responses were also observed under certain concentration conditions in selected glioma cells, including mouse glioma cells with amplified PDGF signaling. Our study suggests limited synergistic activity when combining CBD with DNA-damaging in gliomas, along with no therapeutic window when taking into account of its effects on healthy neuroprogenitor cells. Supported by NIH (DA014486 to NS).

Disclosures: **L. Deng:** None. **L. Ng:** None. **T. Ozawa:** None. **E.C. Holland:** None. **N. Stella:** A. Employment/Salary (full or part-time): Stella Therapeutics.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.10/F48

Topic: B.14. Neuro-Oncology

Support: NSF Grant 1547693

Title: Synthesis and characterization of a novel copper-containing nano-biocomposite for potential drug delivery and imaging in brain

Authors: *M. A. DECOSTER[1,2], D. MILAM[2], A. KARAN[2], M. DELAHOUSSAYE[2]; [2]Biomed. Engin., [1]Louisiana Tech. Univ., Ruston, LA

Abstract: Nanomaterials and nanoparticles (NPs) offer opportunities for tools in neuroscience, for example, to cross the blood brain barrier, thereby targeting and delivering therapeutics to different stages of brain tumors. While comparing the relative toxicities of iron and copper NPs towards brain tumor (glioma) cells in culture (using the cell line CRL-2303 from ATCC), we discovered that over the long term (82 hours), copper NPs transformed from individual particulates ranging from 25-100 nm in diameter, to larger clumps in the micron range, and finally to large clusters with high-aspect ratio structures (HARS) similar to “urchin” like structures. Under identical conditions and concentrations iron NPs were largely inert and non-toxic. To investigate the chemical mechanism behind this transformation, we stepwise subtracted components from the glioma cell cultures, while retaining physiological conditions. We discovered by this subtractive process that a component of the cell culture media, the amino acid cystine, was responsible for the HARS development. Remarkably, we have now simplified and accelerated the process, so that copper HARS can be formed biochemically, without cells or cell culture proteins, but with only key components including cystine, water, and slightly basic

conditions using NaOH to solubilize the amino acid. Self-assembly synthesis can now be accomplished in under 6 hours. We have discovered a second synthesis route, by providing the copper source from copper sulfate. This gives a cleaner yield of HARS, as copper sulfate is completely soluble in water. Both transmission and scanning electron microscopy proved that these synthesized HARS scale from the nano- to the micro- in diameter, with lengths of a few microns to hundreds of microns. Cystine, the dimer of the amino acid cysteine, has a disulfide bond, and many sulfur-containing molecules are known to bind copper and to serve as chelators and antioxidants. We have purified and stabilized these novel copper HARS from the liquid synthesis so that they can be dried and produced in milligram quantities. Using these purified HARS we have added them back to brain tumor cells as well as brain microglia. The copper HARS are less toxic on a mass/volume level than pure copper NPS (tested at 10-200 ug/ml), and distinct from a number of other developed nanomaterials, these novel copper HARs are slowly biodegradable, being broken down and phagocytosed by brain microglia and glioma cells in culture over 2-3 days, in comparison with 8-12 hours for pure copper NPs. These properties of a novel copper containing nanocomposite may provide benefits for brain cell imaging and therapeutics, such as drug delivery.

Disclosures: **M.A. DeCoster:** None. **D. Milam:** None. **A. Karan:** None. **M. Delahoussaye:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.11/F49

Topic: B.14. Neuro-Oncology

Title: Microglial properties of glioblastoma as potential therapeutic targets

Authors: ***G. D. MANOCHA**[1], J. A. KULAS[1], T. N. SEYFRIED[2], C. K. COMBS[1];
[1]Basic Sci., Univ. of North Dakota, Grand Forks, ND; [2]Biol., Boston Col., Boston, MA

Abstract: Glioblastoma multiforme (GBM) is a highly invasive brain cancer associated with poor prognosis. It is typically characterized by heterogeneity including microglia/macrophage phenotype cells that appear to contribute to the poor outcomes. In order to better understand the contribution of microglial/macrophage-like cells to disease a highly invasive *in vivo* GBM model was developed from a spontaneous brain tumor (VM-M3). As seen in human GBM, the VM-M3 tumor cells invade throughout the brain using the “Secondary Structures of Scherer”. Additionally, the VM-M3 cells express properties similar to those of macrophages including phagocytosis and expression of a number of macrophage/microglia associated genes. We

hypothesize that the macrophage/microglia-like properties of the invasive VM-M3 tumors can be targeted therapeutically with strategies typically employed to limit microglial activation. In order to test this idea, cells grown from the VM-M3 tumors were further examined *in vitro*. As previously shown, these cells demonstrated many properties of microglia including immunoreactivity for macrophage/microglial specific proteins such CD68, CD11b, and F4/80. The cells also exhibited phagocytic uptake of fluorescently labeled β-amyloid peptide. LPS stimulation of the VM cells increased TNF-α and IL-6 secretion and activated a number of cell signaling pathways including the tyrosine kinases Lyn and Src and the MAP kinase, JNK. More importantly, the brain penetrant Src/Abl inhibitor, dasatinib, was able to inhibit proliferation of these cells. These data support the idea that strategies targeting microglial activation may be efficacious at attenuating the microglial/macrophage-like GBM cells to affect disease progression.

Disclosures: G.D. Manocha: None. **J.A. Kulas:** None. **T.N. Seyfried:** None. **C.K. Combs:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

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Program#/Poster#: 304.12/F50

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: CEP5003, a novel compound targeting glioblastoma cancer stemcells

Authors: C. SHARMA[1,2], M. JANI[2], N. AMEZCUA[1], M. SHARMA[1], P. NARAYANAN[1], M. NAVEL[1], D. STANTON[1], S. SHARMA[1], D. FOSTER[1,3], J. COLLINS[4], S. SHARMA[3], J. JANI[1], *J. SHARMA[1,4];
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Abstract: Glioblastoma multiforme (GBM) is a genomically complex and aggressive primary adult brain tumor, with a median survival of 12 - 14 months. The heterogeneous nature of this disease has made the clinical treatment difficult. Using Patient Derived Xenograft (PDX) models of untreated GBM patients we have generated a model (and evaluated the efficacy of various compounds for GBM therapeutic treatments. We also generated a 3D GBM culture system for screening potential drug compounds for personalized clinical applications. The 3D GBM culture system of patient's survival model was constructed and validated on independent data sets that demonstrated an excellent prediction of GBM patients survival (log-rank test: p = 0.0001). Using

a multivariate Cox proportional hazards model we identified that our 3D GBM model is distinct from other known GBM criteria (age at diagnosis, extent of surgical resection, post-operative Karnofsky Performance (KPS) score, treatment with temozolomide (TMZ) chemoradiation, and methylation of the MGMT gene). A five -fold cross-validation of our PDX model generation procedure confirmed validation of our 3D GBM model system. The model was further validated on an independent set of compounds such as CEP5003 that is capable of crossing the blood brain barrier and selectively targeting GBM Cancer Stem Cell (CSCs). Our data demonstrate that CEP5003 selectively targets GBM CSCs and we hypothesize that the clinical use of this class of compounds will improve quality of life and overall survival of GBM patients.

Disclosures: **C. Sharma:** None. **M. Jani:** None. **N. Amezcua:** None. **M. Sharma:** None. **P. Narayanan:** None. **M. Navel:** None. **D. Stanton:** None. **S. Sharma:** None. **D. Foster:** None. **J. Collins:** None. **S. Sharma:** None. **J. Jani:** None. **J. Sharma:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.13/F51

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant 1K23NR014902-01A1

Title: Brain structural changes after treatment of cerebellar tumors in children

Authors: J. TANEDO[1,2], D. SACCHETTO[1], F. YEPES[1], J. COLOIGNER[1], M. DESCOTEAUX[4], M. D. NELSON, Jr[1,3], N. LEPORE[1,3], *M. BARON NELSON[1,3]; [1]Children's Hosp. Los Angeles, Los Angeles, CA; [2]Sch. of Engin., [3]Keck Sch. of Med., USC, Los Angeles, CA; [4]Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: Introduction
Chemotherapy is a frontline treatment for brain tumors in very young children. However, there is evidence that chemotherapy leads to long-term brain tissue damage and resulting neurocognitive deficits in children [1,2]. MRI techniques can be used to quantify changes in brain structure due to injury. In particular, diffusion tensor imaging measures the diffusion of water in neural structures and can be used to derive indices that reflect changes to healthy brain tissue. Mean diffusivity (MD) can be used to assess cellular and fiber injury across gray and white matter as the values reflect cell size, shape and integrity, and molecular motion across tissues. Fractional anisotropy (FA) values can be used to assess axonal integrity in white matter tissue as these values reflect the degree of alignment within fiber tracts [3].

Methods
DTI was obtained as part of routine brain MRI scans for 6 subjects with posterior fossa brain tumors treated with surgery and chemotherapy at least one year ago (age at scan: 11.5 years +/- 4.5, 2M 4F) as compared to 7 age- and gender- matched healthy sibling controls (age at scan: 10 years +/-3, 3M 4F).
Imaging was acquired with a 3T Philips Achieva scanner using a novel fast DTI sequence, with parameters: 70 axial slices (1 mm thick), FOV = 256 mm x 256 mm x 140 mm, TR/TE 8657/86 ms, no gap, with a 128x126 acquisition matrix, 28 gradient images were collected with b-value=1500 . T1 images were also acquired with a voxel size of 1.0 x 1.0 x 1.0 mm with the parameters TR 9.9 ms; TE 4.6 ms; 240 x 231 matrix; FOV 24 cm; slice thickness 1 mm.
After standard preprocessing steps, all DTI images were registered to a control subject most closely related to the mean age of all subjects (mean age = 11.8, age of template subject = 12). Subsequently, statistical analysis of FA and MD were performed using AFNI's 3dttest and visualized using AFNI.
Results
Figure 1: Axial view of statistically significant changes in the FA (left image) and MD (right image) values between children with cerebellar brain tumors treated with chemotherapy versus age- and gender- matched healthy sibling controls. Blue indicates significantly decreased values whereas red indicates significantly increased values.
Children with cerebellar brain tumors had significantly decreased FA in areas of the corpus callosum, the right anterior internal capsule and the right thalamus and significantly elevated MD in the right head of the caudate as compared to controls.
Conclusion
Evidence of microstructural injury to midline grey and white matter structures may have implications for cognitive deficits in children with cerebellar tumors treated with chemotherapy.

Disclosures: J. Tanedo: None. **D. Sacchetto:** None. **F. Yepes:** None. **J. Coloigner:** None. **M. Descoteaux:** None. **M.D. Nelson:** None. **N. Lepore:** None. **M. Baron Nelson:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.14/F52

Topic: B.14. Neuro-Oncology

Title: The impact of surgical stress, immune stimulation, and native immune cells on brain metasatasis

Authors: *A. BENBENISHTY[1], L. SHAASHUA[1], A. GLASNER[2], S. BEN-ELIYAHU[1], P. BLINDER[1];
[1]Tel Aviv Univ., Tel Aviv-Yafo, Israel; [2]The Hebrew Univ. Hadassah, Jerusalem, Israel

Abstract: Surgical stress responses have been shown to promote metastasis in peripheral organs through their immune-suppressive effects and through direct effects on the malignant tissue and host physiology. However, brain metastases have not been studied in these respects, and the unique brain immune milieu, blood supply, and blood-brain barrier, may react differently to such neuroendocrine challenges. Thus, we studied the effect of surgery and the consequent systemic stress response, immune-stimulation, and the role of NK cells and resident microglia, in early and late stages of brain and peripheral metastasis. Tumor cells were injected using a novel intra-carotid inoculation approach we have developed, which generates metastases with minimal injection-related interferences to brain blood flow, with better targeting to the brain. Employing this inoculation approach in C57BL/J6 mice we studied the effects of surgery on tumor retention and growth, as measured using radioactive-labeled cells and bioluminescent imaging. Various pharmacological agents were used to study the effects of systemic neuroendocrine factors secreted under physiological stress, including corticosterone, adrenaline, and prostaglandin. Next, we studied the effects of immune stimulation, using CpG-C ODN - a TLR-9 agonist having minimal adverse effects in humans. To study the role of NK cells and resident microglia in vivo, we specifically depleted these cells populations using NK1.1 antibody and a novel colony-stimulating factor 1 receptor antagonist, respectively. We followed the various steps of the metastatic process in CX3CR1-GFP mice using awake two-photon imaging, and analyzed the effects of immune-stimulation and the interaction between microglia and tumor cells in the brain. Complementary primary culture assays were used to further study the interaction between microglia and tumor cells. Our findings indicate that surgical stress results in elevation in metastases in the periphery and in the brain, while this phenomenon is mediated by systemic secretion of endocrinal factors in the periphery but not in the brain. Furthermore, CpG-C results in a profound decrease in metastases in both the periphery and brain. Interestingly, metastasis progression and the effects of immune-stimulation were mediated by NK cells in the periphery, while in the brain, microglia, but not NK cells, were found to play a key role. Our work implies surgery is a significant risk factor for brain metastases and that microglia cells are potential targets for immune-therapy, such as CpG-C treatment, which may be used prophylactically in cancer patients.

Disclosures: A. Benbenishty: None. **L. Shaashua:** None. **A. Glasner:** None. **S. Ben-Eliyahu:** None. **P. Blinder:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.15/F53

Topic: B.14. Neuro-Oncology

Support: NIH Grant DC011534

Title: Examination of FOXJ1 as a modulator of proliferation in glioblastoma

Authors: *E. M. PARONETT[1], E. P. MCCORMACK[1], T. M. MAYNARD[1], J. H. SHERMAN[2];
[2]Dept. of Neurolog. Surgery, [1]George Washington Univ., Washington, DC

Abstract: Glioblastoma tumors, derived from astroglial cells, carry a prognosis of 12-18 months. Current treatment is limited to tumor resection followed by radiation and chemotherapy. FOXJ1, a forkhead transcription factor, is required for astrocyte and radial glia differentiation in addition to neurogenesis, and therefore may be a candidate for mediating proliferation and invasiveness of glioblastoma cells. Overexpression and knockdown of FOXJ1 have demonstrated utility in predicting prognosis and progression of several types of cancer. Thus, it is possible that FOXJ1 may modulate aspects of proliferation or invasion in glioblastoma cells. The goal of our experiments was to provide evidence for the expression of FOXJ1 in glioblastoma samples and its utility as a transcription factor in neural/glial differentiation. The glioblastoma cell line U87 is known not to express FOXJ1. We transfected a FOXJ1-tdTomato plasmid into U87 cells in order to examine its influence on proliferation. In addition, a preliminary assessment of FOXJ1 expression was made via qPCR on 6 glioblastoma and 2 lower grade glioma samples from the GWU Tissue Tumor Bank. Our results demonstrate that FOXJ1 is expressed variably in both the nucleus and cytoplasm and in a subset population of glioblastoma neoplasms, which is consistent with tumor samples from a publically accessible database. Future work will assess the effect of FOXJ1 overexpression on glioblastoma proliferation as well as its effect on resistance to such treatments as radiation and chemotherapy. Understanding the role of FOXJ1 in glioblastoma may suggest new therapies and give patients with glioblastoma an alternative to the current standard of care.

Disclosures: E.M. Paronett: None. **E.P. McCormack:** None. **T.M. Maynard:** None. **J.H. Sherman:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.16/G1

Topic: B.14. Neuro-Oncology

Support: Institutional grant of Human virology

Title: HIV-associated brain Lymphoma activates NAMPT/SIRT3/PGC-1α signaling

Authors: T. K. MAKAR[1,2], *S. RAY[1], D. D. PATEL[1], P. R. GUDA[1], J. L. BRYANT[3]; [1]Neurol., Univ. of Maryland Baltimore, Baltimore, MD; [2]VA Med. Ctr., Baltimore, MD; [3]Inst. of Human Virology, Baltimore, MD

Abstract: Primary central nervous system lymphoma (PCNSL) of the central nervous system (CNS) is an aggressive malignancy which is associated with HIV patients. PCNSL is a rare form of non-Hodgkin lymphoma. This type of lymphoma is mainly involves brain. Histological analysis suggests that HIV-associated lymphoma (HBL) is one type of the PCNSL. The involvement of critical brain regions are both pathologic and diagnostic challenges. Neurocognetic dysfunction is associated with HBL. HIV transgenic Tg26 mice show some of the complicacy associated with HIV infection. 15 % of these transgenic mice population develop PCNSL in brains. The aim of this study was to analyze the relationship between metabolic profile and pathological outcome in the hippocampus of PCNSL associated Tg26 mice.SIRT3 (sirtuin3) is the main deacetylase utilizing NAD+ within the mitochondrial matrix which induces aerobic metabolism regulates reactive oxygen species (ROS). There is controversy as to whether SIRT3 is involved in oncogene or a tumor suppressor and here we investigated its role in PCNSL associated hippocampal changes. We found a significantly higher expression of SIRT3 in those hippocampus regions of Tg26 mice compared to wild type (WT). In the same tissues we also determined the expression of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the synthesis of NAD+. Here also we found a significant increase of NAMPT expression. Earlier it has been reported that the expression of NAMPT was generally high in the more aggressive malignant lymphoma (Olesen et.al 2011 APMIS 119: 296-303). The NAMPT inhibitor, APO866, is currently in clinical phase of trials in lymphomas. SIRT3 induces deacetylation of PGC1α. It has been reported that PGC1α reprograms cancer cell metabolism and stimulates mitochondrial biogenesis via regulating several mitochondrial genes (Vazquez et.al 2013 Cancer cell 23:287-301). Our study furthermore, showed that in the hippocampus of those mice PGC1α upregulated. Taken together these finding suggested NAMPT/SIRT3/PGC1α signaling are activated in the hippocampus of PCNSL associated with the brain of TG26 mice. In conclusion our study suggests in PCNSL associated with HIV infection, inhibition of NAMPT/SIRT3/PGC1α pathway might represent a novel therapeutic approach.

Disclosures: **T.K. Makar:** None. **S. Ray:** None. **D.D. Patel:** None. **P.R. Guda:** None. **J.L. Bryant:** None.

Poster

304. Neuro-Oncology: Signaling and Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 304.17/G2

Topic: B.14. Neuro-Oncology

Support: NIH Grant R01CA200624-01

Title: Implication of mRNA binding protein HuR in PD-L1 up regulation in brain tumor.

Authors: ***N. FILIPPOVA**, X. YANG, L. B. NABORS;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: PD-L1, a programmed death ligand 1, is a transmembrane protein that is responsible for the immune escape of different types of tumors due to inhibition of T lymphocytes and B cells activation during the anti-tumor immune response. PD-L1 ligand is up regulated across several tumor types and interacts with PD1 and B7.1 receptors on T lymphocytes and B cells, therefore inhibiting their proliferation. In our current report, we provide a detailed analysis of PD-L1 expression at the mRNA and protein levels in normal and brain tumor samples as well as on glioma cell lines and describe several new mechanisms which could account for the PD-L1 up regulation. We performed a comparison of PD-L1 protein expression in array of brain tumor samples (WHO i-iv) and normal tissue samples. Overall, the PD-L1 exhibits heterogeneous distribution across the tumor samples (from 1-2 % to up to 50-60 % cells per tumor mass) with significantly stronger intensity compared to normal tissues. We confirmed almost uniformly higher total PD-L1 protein level in tumor samples compared to normal tissue by Western blot data normalized to the actin in samples content. The analysis of total PD-L1 mRNA expression (by using inventory Taqman probe against PD-L1 coding region) in 10 GBM samples versus 5 normal brain tissue samples revealed that PD-L1 mRNA level significantly exceeds averaged normal PD-L1 mRNA level in two GBM samples and significantly below of averaged normal level in three GBM samples. The sporadic PD-L1 distribution and intensity in most GBM samples suggest the persistence of the inductive nature of PD-L1 up-regulation in tumor. The analysis of PD-L1 gene revealed that the mRNA binding protein HuR may be involved in PD-L1 up regulation through the direct 3‘-UTR stabilization or indirectly through the enhancement of PD-L1 promoter function via up regulation of the transcriptional factor HIF1-alpha. We confirmed both pathways of HuR dependent PD-L1 up regulation in U251 cells by using several assays including, but not limited to: HuR overexpression assay, PD-L1 reporter assay and by

mimicking hypoxic/or metabolic HIF1-alpha potentiation via CoCl2 cells treatment. We conclude that the mRNA binding protein HuR may contribute to the PD-L1 up regulation and therefore tumor immune escape in glioma.

Disclosures: **N. Filippova:** None. **X. Yang:** None. **L.B. Nabors:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.01/G3

Topic: B.14. Neuro-Oncology

Support: CCN holds a Canada Research Chair

Title: Modelling human glioma using 3D bioprinting

Authors: ***C. C. NAUS**[1], K. HARADA[2], W. SIN[1], D. SONG[2], N. KITA[2];
[1]Dept of Cell. & Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada; [2]Cyfuse Biomed. K.K., Tokyo, Japan

Abstract: Malignant glioma is an aggressive cancer originating in the central nervous system with very poor prognosis and a median survival of about one year. The current standard for care is surgical resection, adjuvant chemotherapy and radiation therapy. Most cancer treatments aim to kill tumor cells by taking advantage of their accelerated growth. However, gliomas almost always return due to survival of invading glioma cells, often arising in a region proximal to the primary tumor site. Approaches to study human gliomas vary from in vitro culture conditions to establishing tumors in vivo in animal models. We have previously demonstrated that the loss of connexin43-mediated intercellular communication in U118 human glioma spheroids increased their invasiveness on a fibronectin substrate (Aftab et al., 2015, Oncotarget). In order to better understand how loss of connexin43 will affect glioma growth, invasion and recurrence in a multicellular environment, we used a bioprinting system (Regenova Bio-3D Printer, Cyfuse Biomedical KK, Tokyo, Japan) to generate human neural organoids consisting of iPSC-derived neurospheres printed with the "Kenzan" method. After culturing the neurospheres in chambers for several weeks, individual cellular spheroids merged to simulate an in vivo neural microenvironment. Human glioma cells grown as spheroids were implanted into this organoid. Tumor growth can be readily assessed in this system, as well as quantification of glioma cell invasion into the surrounding neural tissue, as detailed in our previous study (Sin et al., 2016, Oncogene). This platform provides a unique tool to manipulate the glioma microenvironment and assess the efficacy of various therapeutics.

Disclosures: **C.C. Naus:** None. **K. Harada:** A. Employment/Salary (full or part-time): Cyfuse Biomedical K.K.. **W. Sin:** None. **D. Song:** A. Employment/Salary (full or part-time): Cyfuse Biomedical K.K. **N. Kita:** A. Employment/Salary (full or part-time): Cyfuse Biomedical K.K..

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.02/G4

Topic: B.14. Neuro-Oncology

Support: Grant SAF2013-45178-P

Grant SAF2010-21274

Title: 3D visualisation of Collagen IV vasculature in human GBM tissue blocks: morphometric parameters relate to tumor severity and suggest mechanisms of angiogenesis

Authors: G. P. CRIBARO, E. SAAVEDRA-LÓPEZ, P. V. CASANOVA, *C. BARCIA; Univ. Autonoma De Barcelona, Barcelona, Spain

Abstract: Revisiting the basic microarchitecture of glioma vasculature is opportune given the heterogeneity of angiogenic and vasculogenic processes within the tumor, as well as the importance of understanding the blood brain barrier (BBB) state for an effective therapy. Since routine histopathological examinations are done in 5 um-thin sections, the visualization of blood vessel structure is insufficient. Here, we analyze three-dimensionally (3D) the microvasculature in 60-um thick, free floating tissue blocks from human glioma patients, visualizing the Collagen IV^+ components of the tumor microenvironment (TME) and making comparisons with normal tissue organization and the degree of tumorigenicity. We have measured morphometric parameters of micro-vessels *in situ* and identified patterns of the primary tumor vascularization and angiogenesis (cooption, looping, intussusception [splitting] and silent corollary vessels). After detection of Collagen IV^+ basement lamina in combination with $GFAP^+$ tumor cells and DAPI counterstaining, immunoflourescent-stained tissue blocks were imaged in 3D with a laser scanning confocal microscope and the resulting 3D stacks were analyzed with image analysis software (Imaris and Fiji) to quantify individual vascular units, vessel caliber, Collagen IV^+ vascular basement lamina area, branching and continuity (as an indirect measure of vessel wall disruption). These parameters were correlated with tumorigenicity levels, such as cellularity and $GFAP^+$ tumor cell density. Our analysis shows that Collagen IV^+ vascular basement lamina area, vessel caliber range and variability increase positively with tumor severity. Collagen IV^+ vessels show increased ramification in severe tumors and basement lamina disruption also increases with

tumorigenicity. In addition, all samples show abundant silent collateral vessels. Our data shows that the human glioma environment utilizes multiple strategies of vasculogenesis that correlate with the severity and the fatal outcome of the patients. Thus, an extensive understanding of the multifaceted nature of tumor neovascularization starting with an accurate 3D topography and a detailed *in situ* characterization of regional cell populations in the TME will contribute positively to more successful antiangiogenic treatment strategies.

Disclosures: G.P. Cribaro: None. **E. Saavedra-López:** None. **P.V. Casanova:** None. **C. Barcia:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.03/G5

Topic: B.14. Neuro-Oncology

Support: Field Neurosciences Institute

Title: Use of *In vivo* imaging for stem cell therapy in a model of glioblastoma in rodents

Authors: K. IDYLE[1,2,5,3], K. COPELY[1,4,3], A. STEWART[1,5,2,3], L. HUFFMAN[1], L. KNIGHT[1], M. JEAKLE[1,2,5,3], L. SIEGEL[1,2,5,3], A. ANTCLIFF[1,2,4,5,3], D. DUES[1], K. FINK[1,6], M. LU[3,2], U. HOCHGESCHWENDER[4,2,3], G. DUNBAR[1,2,5,7,3], *J. ROSSIGNOL[1,4,2,3,5];
[1]Field Neurosciences Inst. Lab., Mount Pleasant, MI; [2]Program in Neurosci., [4]Col. of Med., [3]Central Michigan Univ., Mount Pleasant, MI; [5]Dept. of Psychology at Central Michigan Univ., Mount Pleasant, MI; [6]Univ. of California, Davis, CA; [7]Field Neurosciences Inst., Saginaw, MI

Abstract: Glioblastoma multiforme (GBM) is one of the most devastating forms of human brain cancer. Even with treatment patients are expected to only live a few months. In the Field Neurosciences Institute laboratory at CMU, great advances are being made utilizing the release of anti-inflammatory factors from mesenchymal stem cells (MSCs), for treating a variety of neurological diseases including GBM. However, while this treatment has significant potential for treating GBM, much work is needed to more effectively shrink the volume of the tumor. To address this need, this project was based on a premise that an ideal ratio of transplanted stem cells to tumor cells needs to be determined in order to improve treatment outcome. In order to better match MSCs to GBM cells, studies examining the growth kinetics of the GBM tumor cells within the animal brain are needed. To this end, the goal of this *in vivo* study, was to investigate the proliferation rate of the GBM cells using a nuclear marker bromodeoxyuridine (BrdU). GBM cells were first transplanted into 20 brains of rats that were divided in three groups and

euthanized at different time point: one, two or three weeks after the GBM transplantation in the brain. Live images of tumor growth *in vivo* were taken at days 11, 17, 25 and 31 in a different set of animals; post-fixation immunohistochemistry was also completed for tumor growth. Results showed an increase in tumor size over the 3 weeks which was confirmed by live imaging. Ultimately, the results of this study will help to determine the optimal time for intervening with MSCs in order to reduce the proliferation of GBM cells in the brain.
Support for this study was provided by the Office of Research and Sponsored Programs at CMU, the College of Medicine, and the Field Neurosciences Institute and John G. Kulhavi Professorship

Disclosures: **K. Idyle:** None. **K. Copely:** None. **A. Stewart:** None. **L. Huffman:** None. **L. Knight:** None. **M. Jeakle:** None. **L. Siegel:** None. **A. Antcliff:** None. **D. Dues:** None. **K. Fink:** None. **M. Lu:** None. **U. Hochgeschwender:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.04/G6

Topic: B.14. Neuro-Oncology

Support: NIH Grants P01-DK049210 and R01-NS084965

Title: Lipid droplet accumulation in glioblastoma multiforme

Authors: ***B. TAIB**, R. AHIMA;
Dept of Med, Div. of Endo, Diabetes and Metabolism, & the Inst. for Diab, Obesity & Metabolism, Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA

Abstract: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults. The standard treatment for GBM is surgical resection followed by adjuvant radiotherapy and chemotherapy, but overall survival is usually less than 12 months. Analysis of brain tissues from GBM patients show that lipid droplets (LD) are highly enriched in tumor tissues but not detectable in normal brain tissues. However, the nature and functions of lipid species in GBM are not well understood. In the present study, we demonstrate that a human GBM cell line (U138) treated with oleic acid accumulate Perilipin 2-coated LD and triglycerides enriched in C18:1, C18:2 and C20:1 fatty acids. Phospholipid extracted from oleic acid treated GBM cells has higher levels of C16:0, C18:0 and C18:1 fatty acids. Oleic acid treatment increases mRNA expression of carnitine acyltransferase1 (CPT1), diacylglycerol O-acyl-transferase (DGAT)1, stimulates fatty acid oxidation and glucose utilization, and promotes

proliferation but not migration of GBM cells. Conversely, pharmacological inhibition of DGAT decreases LD and triglycerides and blunts proliferation of GBM cells. Oleic acid treatment increases phosphorylation of AKT, and this effect is reversed by inhibition of DGAT. Our findings reveal a novel LD-mediated lipid signaling pathway that provides a rational metabolic therapy for GBM.

Disclosures: **B. Taib:** None. **R. Ahima:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

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Program#/Poster#: 305.05/G7

Topic: B.14. Neuro-Oncology

Support: NIH Grant P20GM103408

CSHL RNA-Seq for the Next Generation

Title: Differential gene expression profiles of adherent and neurosphere-like GL261 cells

Authors: J. L. V. MONTEIRO DE BARROS, *R. L. DANIELS;
Biol., Col. of Idaho, Caldwell, ID

Abstract: Glioblastoma Multiforme is an extremely aggressive cancer with poor prognosis, comprising 15% of all primary intracranial cancers. The GL261 murine cell line is a widely utilized model system for investigating high-grade glioma pathobiology. These cells can be cultured adherently or as free-floating aggregates (neurospheres) in vitro when exposed to epidermal growth factor (EGF), fibroblast growth factor (FGF) and B-27. Additionally, in vivo studies have shown that the neurosphere phenotype forms malignant tumors that display higher infiltration rates, lethality, as well as greater similarity to cancer stem-like cells when implanted into mice, thus suggesting a different gene expression profile in comparison to the adherent phenotype. However, the fundamental differences between cells cultured under each condition and the particular changes in gene expression that lead to increased tumorigenicity in the neurosphere phenotype still remains unclear. A more thorough understanding of gene expression under both phenotypic conditions would allow better interpretation of experimental results in the GL261 literature. To address this, we have used whole transcriptome shotgun sequencing (RNA-Seq) to examine differential gene expression between GL261 cells cultured adherently or as neurospheres. A total of 538 genes were shown to be upregulated by at least a factor of 2, while 331 genes were observed to display at least halved expression levels in comparison to the

adherent phenotype. Gene ontology analyses determined that neurosphere-like Gl261 cells displayed an over-expression of genes related to cell-cell adhesion, inflammatory response, and regulation of adaptive immunity. Downregulated genes included those that have functional roles in cell cycle control and angiogenesis. A complete characterization of the gene expression profiles of both the adherent and neurosphere GL261 phenotypes will contribute to our understanding of this model system and will further our knowledge regarding the changes in gene expression that permit tumorigenesis and tumor growth. The present study provides important data regarding gene products and processes that could be targeted in drug discovery efforts. Lastly, this is the first report to compare gene expression between the two GL261 phenotypes.

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Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.06/G8

Topic: B.14. Neuro-Oncology

Title: longitudinal functional alterations of peritumoral neurons in a murine glioma model

Authors: *E. TANTILLO[1], C. CERRI[2], F. OLIMPICO[1], S. FRANCESCHI[3], E. VANNINI[2], P. ARETINI[3], M. MENICAGLI[3], C. MAZZANTI[3], M. CALEO[2];
[1]Scuola Normale Superiore, Pisa, Italy; [2]Inst. of Neurosci., Italian Natl. Res. Council CNR, Pisa, Italy; [3]Pisa Sci. Foundation-ONLUS, Pisa, Italy

Abstract: Introduction Gliomas are the most common primary brain tumors of the central nervous system, with glioblastoma multiforme (GBM) being the most malignant form of this disease. A better understanding of the bidirectional interactions between glioma cells and surrounding brain tissue is required for the development of novel therapies. A successful brain tumor treatment should indeed aim at halting tumor growth and at the same time protecting neuronal cells to prevent functional deficits and cognitive deterioration, which have a strong impact on the patients' quality of life. However, currently very little is known about the functional status of peritumoral tissues during glioma progression. **Methods** in this study, we induced glioma through transplant of GL261 cells into the primary visual cortex of syngenic C57JBl6 adult mice. A chronic bipolar electrode was implanted in the same area to record local field potentials (LFPs). We performed longitudinal recordings of neural activity in awake, head-fixed animals to monitor the effect of glioma on neural tissues during its development. The visual cortex was chosen as a recording site since it can be easily activated by physiological

stimuli, and for the presence of many well established parameters to evaluate its functionality. A detailed analysis of visual evoked potentials (VEPs) and baseline LFPs was performed in order to follow changes induced by tumor growth. **Results and Discussions** In control, naive mice, field potential responses were consistently evoked by photic stimulation. We observed a progressive increase in the VEP amplitude over the recording session, indicative of stimulus-dependent response potentiation (SRP). On the contrary, in glioma-bearing mice, an initial phase of SRP was followed by rapid decay and deterioration of visual responses, that completely disappeared by day 25-28 after GL261 cell transplant. Single unit recordings showed that peritumoral neurons showed several physiological alterations including enhanced spontaneous firing, reduced visual responsiveness over a range of stimulus contrasts and reduced response reliability. Baseline LFPs recordings revealed the occurrence of seizures in a subset of the recorded animals. **Conclusions:** Our findings indicate the feasibility of longitudinal monitoring neural activity during glioma progression to better understand the interaction of tumor cells with the brain microenvironment. These data provide a set of quantitative parameters to measure dysfunction in peritumoral regions during glioma growth, and assess the impact of radiotherapy, chemotherapy, and antiepileptic drugs on neuronal activity in glioma-bearing animals.

Disclosures: **E. Tantillo:** None. **C. Cerri:** None. **F. Olimpico:** None. **S. Franceschi:** None. **E. Vannini:** None. **P. Aretini:** None. **M. Menicagli:** None. **C. Mazzanti:** None. **M. Caleo:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.07/G9

Topic: B.14. Neuro-Oncology

Support: NIH-R01-NS078223

Title: The effects of glioma growth on resting state networks

Authors: ***I. E. ORUKARI**, A. Q. BAUER, G. A. BAXTER, J. B. RUBIN, J. P. CULVER; Washington Univ. In St Louis, St Louis, MO

Abstract: Background: Gliomas are known to cause significant changes in normal brain function that lead to cognitive deficits. Disruptions in resting state networks (RSNs) are thought to underlie these changes. However, investigating the effects of glioma growth on RSNs in humans is complicated by the heterogeneity in lesion size, type, and location across subjects. In this study, we evaluated the effects of tumor growth on RSNs over time in a mouse model of glioma where glioma properties can be controlled. **Methods:** Glioma cells ($5x10^4$-10^5 U87s)

were stereotactically injected into the forepaw somatosensory cortex of adult nude mice (n=5). Disruptions in RSNs were evaluated weekly with functional connectivity optical intrinsic signal imaging (fcOIS), in conjunction with bioluminescence imaging (BLI) to monitor tumor growth. In order to characterize how tumor growth affected different RSNs over time, we calculated functional connectivity (fc) between homologous (bilateral) brain regions and the spatial similarity of regional fc maps for all brain pixels within our field of view. **Results:** Deficits in fc initiate near the lesion, and over a period of several weeks, extend more globally (Fig 1A). We observe a focal decrease in bilateral fc near the tumor injection site at week 5. This focal decrease in bilateral fc became more global at week 6 (Fig 1B). We also observed a focal decrease in the similarity of regional fc maps near the tumor injection site at week 5, which became more pronounced over the brain by week 6 (Fig 1B). These decrements in spatial similarity were found to strongly correlate with the BLI signal in each mouse and across all time points investigated (Fig 1C) indicating that increased tumor size is associated with increased RSN disruption. **Conclusions:** We have shown that fcOIS and BLI are capable of detecting alterations in mouse RSNs due to brain tumor growth. A better understanding of how RSN disruption contributes to the development of cognitive deficits in brain tumor patients may lead to better patient risk stratification and consequently improved cognitive outcomes.

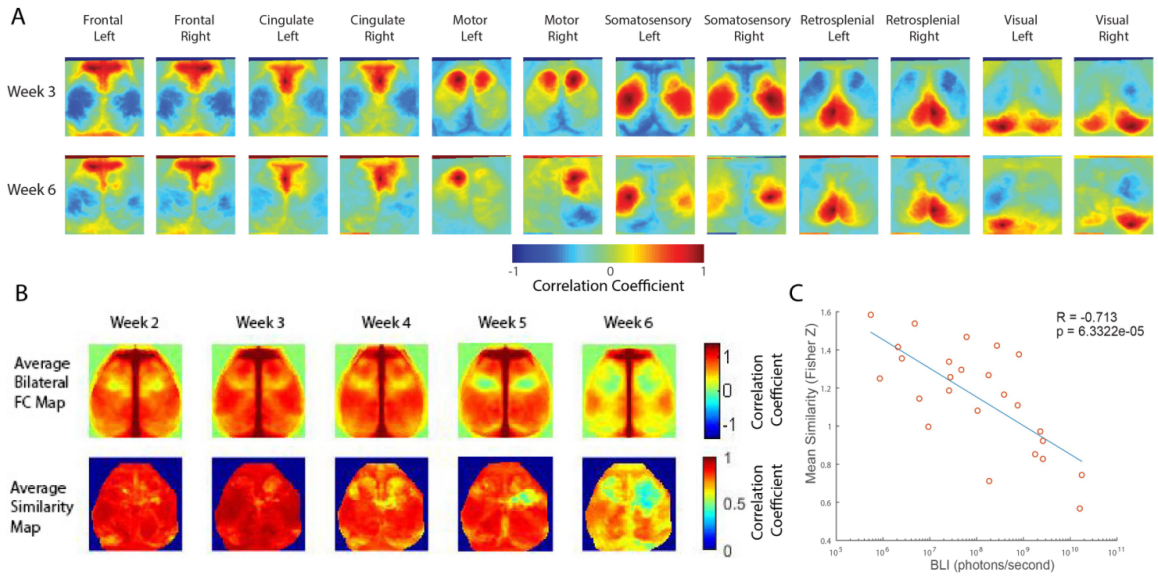


Fig 1. Alterations in functional connectivity due to glioma growth. Tumors were injected in the forepaw region of the right somatosensory cortex. A) Averge functional connectivity (fc) maps from 5 mice for 12 networks at week 3 and week 6 post injection. B) Average bilateral fc map and average similarity map for 5 mice at 5 different weeks. C) Mean similarity score across the whole brain for individual mice plotted against corresponding BLI signal.

Disclosures: I.E. Orukari: None. **A.Q. Bauer:** None. **G.A. Baxter:** None. **J.B. Rubin:** None. **J.P. Culver:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.08/G10

Topic: B.14. Neuro-Oncology

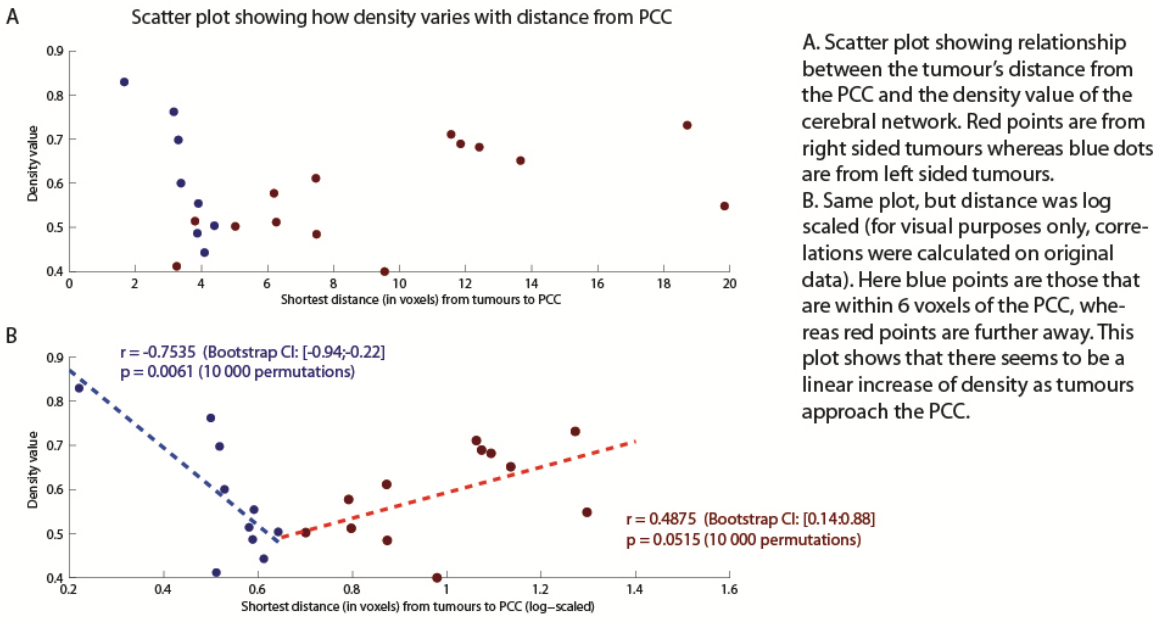
Support: Bourse aux études supérieures de la Faculté de médecine et des sciences de la santé de l'Université de Sherbrooke

Fonds de recherche santé Québec

Title: Brain tumours near the posterior cingulate cortex have a larger effect on overall cerebral connectivity

Authors: *S. GHUMMAN[1], D. FORTIN[2], M. NOEL-LAMY[3], S. CUNNANE[4], K. WHITTINGSTALL[3];
[1]Dept. of nuclear medicine and radiobiology, [2]Dept. of surgery, [3]Dept. of diagnostic radiology, [4]Dept. of medicine, Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: Introduction : Graph theory is a method of studying cerebral networks that provides information on overall connectivity in the brain rather than probing specific networks. The goal of this study was to describe how tumours affect modularity and density of the cerebral network. Moreover, we also sought to investigate whether tumours near certain regions of the brain had larger effects on density. Material and methods: T1 MPRAGE and fMRI scans were taken in 67 patients with brain tumours and 23 healthy aging patients. fMRI data was pre-processed in accordance with modern guidelines for resting state connectivity studies. 50 ROIs were chosen by parcelling the brain with a spatially constrained spectral clustering technique. Graph theory metrics were calculated using the MATLAB toolbox "brain connectivity toolbox". Results: We found that graph theory metrics were only significantly different from healthy controls in patients with tumours in the occipital/parietal (posterior) regions of the brain. These patients had significantly decreased modularity and significantly increased density. Moreover, we also found that graph density was negatively correlated to distance from PCC for tumours within 18 mm of the PCC. Beyond this distance, density seems positively correlated with distance, although this correlation is weaker and statistically insignificant. Conclusion: Decreased modularity and increased density in patients with posterior brain tumours could reflect a plasticity response to the lesions. In fact, posteriorly located tumours are closer to the PCC, which is a known hub in the brain; cerebral hyper-connectivity (increased density) may be a reaction to tumour-induced damage to this hub. Our distance analysis supports this conclusion as tumours located nearer the PCC have a larger effect on density values.



A. Scatter plot showing relationship between the tumour's distance from the PCC and the density value of the cerebral network. Red points are from right sided tumours whereas blue dots are from left sided tumours.
B. Same plot, but distance was log scaled (for visual purposes only, correlations were calculated on original data). Here blue points are those that are within 6 voxels of the PCC, whereas red points are further away. This plot shows that there seems to be a linear increase of density as tumours approach the PCC.

Disclosures: **S. Ghumman:** None. **D. Fortin:** None. **M. Noel-Lamy:** None. **S. Cunnane:** None. **K. Whittingstall:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.09/G11

Topic: B.14. Neuro-Oncology

Support: Childhood Brain Tumor Foundation Diffuse Intrinsic Pontine Glioma award

Title: Mechanisms of diffuse intrinsic pontine glioma invasion of the subventricular zone

Authors: ***E. Y. QIN**[1], D. COOPER[1], S. NAGARAJA[1], A. MACKAY[2], C. JONES[2], M. MONJE[1];
[1]Neurol., Stanford Univ. Sch. of Med., Stanford, CA; [2]The Inst. of Cancer Res., Sutton, United Kingdom

Abstract: Diffuse intrinsic pontine glioma (DIPG) is a devastating childhood brain cancer characterized by its diffusely infiltrating nature. Morbidity and ultimately mortality result in part from this invasive pattern of growth, which reaches far beyond the pons during the course of the

disease. One of the major sites of DIPG spread is the subventricular zone of the lateral ventricles (LV SVZ), a stem/precursor cell niche in the postnatal brain. DIPG spread to a stem/precursor cell niche is crucial to address, as this may serve as a reservoir of tumor cells and play a role in disease recurrence. We have cultured DIPG cells from both the pons and the LV SVZ metastatic tumor from the same individual. Tumor cells from both sites exhibit the H3.3K27M mutation, but diverge in terms of other genomic aberrations and gene expression patterns central to invasive capacity, such as matrix metalloproteinase expression. DIPG cells cultured from the LV SVZ metastatic site exhibit increased invasive behavior *in vitro* and *in vivo*, with a propensity to metastasize to the cerebrum when orthotopically xenografted to the pons. Using *in vitro* invasion assays, we found that DIPG cells cultured from the LV SVZ are more invasive toward media conditioned by LV SVZ neural precursor cells than towards media conditioned by other neural precursor cell populations. In contrast, exposure to fourth ventricular zone (4VZ) neural precursor cell conditioned media increases the overall invasive behavior of DIPG cells non-directionally. Together, these results suggest that molecules secreted by 4VZ neural precursor cells increase the invasive ability of DIPG cells, which spread widely through the entire brain and have a preference for invasion toward the LV SVZ stem cell niche due to a chemoattractant effect of molecule(s) secreted by LV SVZ neural precursor cells. Proteomic analysis of LV SVZ and 4VZ neural precursor cell conditioned media revealed several candidate DIPG chemoattractants and invasion-promoting factors secreted by neural precursor cells. RNA-Seq analysis of LV SVZ metastatic DIPG cells after exposure to LV SVZ or 4VZ neural precursor cell conditioned media also revealed several upregulated gene expression pathways that may contribute to invasion. Ongoing studies investigating the necessity and sufficiency of these candidate secreted proteins and pathways may elucidate mechanisms of DIPG invasion to the supratentorial LV SVZ, which may provide targets for future therapies aimed at limiting DIPG spread.

Disclosures: **E.Y. Qin:** None. **D. Cooper:** None. **S. Nagaraja:** None. **A. Mackay:** None. **C. Jones:** None. **M. Monje:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.10/G12

Topic: B.14. Neuro-Oncology

Support: R01HD062484

Title: Obesity as a potential attribute for vincristine induced peripheral neuropathy

Authors: F. BOYLE[1], K. FORAN[1], *T. J. SAJDYK[2], E. SMITH[3], R. HO[4], E. WELLS[5], J. RENBARGER[1];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Pediatrics - Hematology/Oncology, Indiana Univ. Sch. Med., Indianapolis, IN; [3]Univ. of Michigan, Ann Arbor, MI; [4]Vanderbilt Univ., Nashville, TN; [5]Children's Children Res. Inst., Washington, DC, DC

Abstract: In pediatric oncology, vincristine is a primary chemotherapeutic agent used in the treatment of acute lymphoblastic leukemia (ALL), as well as a variety of other curable pediatric malignancies. Children exposed to vincristine during treatment are at increased risk for development of highly variable cumulative dose-dependent neurotoxicity known as vincristine-induced peripheral neuropathy (VIPN). VIPN is characterized by painful numbness and tingling of the hands and feet, severe jaw pain, orthostatic hypotension, constipation, and muscle weakness that often require chemotherapy dose reduction, thereby potentially compromising effectiveness and cure rates. Our recent studies show that 78% of children receiving vincristine as part of their chemotherapy treatment develop VIPN. The objective of the current study was to evaluate the association between obesity and VIPN severity. We hypothesized that ALL patients who fall into the obese weight category will have a more severe neurotoxicity score than the ALL patients who fall into the healthy weight category. Pediatric patients between the ages of two and eighteen diagnosed with pre B-cell ALL and receiving vincristine as part of their primary chemotherapy treatment plan were enrolled prior to day 8 of initiating vincristine. Neuropathy exams were performed prospectively to assess each patient's VIPN using The Total Neuropathy Score (TNS). Weight categories were assigned to each patient based on his/her BMI percentile. An unpaired t test was done to evaluate the difference in maximum TNS-5 item score between obese and healthy patients. We found that obese pediatric ALL patients had a significantly higher maximum TNS score than pediatric ALL patients of healthy weights ($p= 0.03$). Future studies investigating healthy and obese ALL patients' recovery times from VIPN are needed to assess obesity as a risk factor not only for severity of VIPN but also chronicity of this life-altering toxicity.

Disclosures: F. Boyle: None. **K. Foran:** None. **T.J. Sajdyk:** None. **E. Smith:** None. **R. Ho:** None. **E. Wells:** None. **J. Renbarger:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.11/G13

Topic: B.14. Neuro-Oncology

Support: NIH Grant 5R01HD062484-04

Title: Potential biomarkers for early detection of neuropathy in pediatric ALL patients

Authors: *S. E. ROSS[1], E. SMITH[2], R. HO[3], E. WELLS[4], T. SAJDYK[1], J. THEN[1], L. LI[1], J. RENBARGER[1];
[1]Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Univ. of Michigan, Ann Arbor, MI; [3]Vanderbilt Univ., Nashville, TN; [4]Children's Natl. Med. Ctr., Rockville, MD

Abstract: Sixty percent of all individuals diagnosed with acute lymphoblastic leukemia (ALL) are children. Fortunately, advances in cancer treatment since 1975 have increased the 5-year survival rate from 57% to 90%. A major contributor to the increase in survival is the use of vincristine, one of the core drugs used in the treatment of ALL. Seventy-eight percent of children receiving vincristine as part of their chemotherapy developed vincristine-induced peripheral neuropathy (VIPN), the major dose-limiting toxicity. VIPN can have an impact on the cancer patient in two major ways: 1) it can induce long-term peripheral neuropathy which leads to a decreased quality of life well into adulthood and 2) it can cause the physician to alter the use of vincristine during treatment thus potentially reducing the overall effectiveness of the cancer therapy. In the current study we evaluated 27 different cytokines in pediatric patients with ALL as potential biomarkers for the development of severe peripheral neuropathy. Whole blood was collected from 72 patients from one of four sites (Indiana University, George Washington University, University of Michigan, or Vanderbilt University) while undergoing vincristine treatment. Neuropathy scores were collected from patients during their treatments including their total neuropathy score (TNS), total reflex score, NCI common terminology criteria for adverse events (CTC) motor neuropathy score, and CTC sensory neuropathy score. Plasma was isolated and analyzed for cytokine concentrations. Multivariate logistic regression was utilized to evaluate the association between cytokine concentrations, university site, and/or patient age and neuropathy score (any of the four scores listed above). Higher interleukin-13 (IL-13) expression and older age both correlated with higher TNS ($p=0.0490$ and $p=0.0278$, respectively) for these patients. Age was also directly associated with higher CTC motor ($p=0.0111$) and sensory scores ($p=0.0210$). Platelet-derived growth factor-BB (PDGF-BB) expression was inversely associated with CTC sensory scores ($p=0.0313$). Biomarkers may allow early prediction of neuropathy in pediatric ALL patients. Most importantly, biomarkers provide a method to identify VIPN sensitive infants and toddlers - a population in whom neuropathy evaluations are not feasible.

Disclosures: **S.E. Ross:** None. **E. Smith:** None. **R. Ho:** None. **E. Wells:** None. **T. Sajdyk:** None. **J. Then:** None. **L. Li:** None. **J. Renbarger:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.12/G14

Topic: B.14. Neuro-Oncology

Support: NIH 1R21NS0931099-01A1

UCLA ARC 3R's Award

Title: Brain-mimetic hydrogels to study development of glioblastoma resistance to EGFR inhibition

Authors: *W. XIAO[1,3], R. ZHANG[4], S. SUN[3], A. EHSNAIPOUR[4], C. WALTHERS[4], J. LIANG[4], L. TA[2], D. NATHANSON[2], S. SEIDLITS[1];
[1]Bioengineering, [2]Dept. of Mol. and Med. Pharmacol., UCLA, Los Angeles, CA; [3]Dept. of Bioengineering, [4]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Glioblastoma Multiforme (GBM) is an aggressive cancer that is minimally responsive to current treatments. Pharmacological inhibition of the epidermal growth factor receptor (EGFR) has shown promise as a clinical treatment; however, tumors invariably acquire resistance to continued treatment. As hyaluronic acid (HA) - a major component of the unique brain extracellular matrix (ECM) - and its corresponding membrane receptors are highly upregulated in the GBM microenvironment, we posit their interactions are key mediators of acquired resistance to EGFR inhibition. To characterize effects of the tumor microenvironment on acquired resistance in GBM, we have developed 3D culture systems that mimic the native brain microenvironment and maintain many clinical features of patient-derived GBM cells. We have successfully cultured multiple human primary GBM cell lines in HA-based hydrogels with independently varied levels of compressive modulus, HA content, and adhesive peptides. GBM cells derived from individual patients demonstrated exceptional erlotinib resistance in high HA content hydrogels compared to those with low HA content or in standard neurosphere culture (**Fig. 1A,B**). We also observed a shift in GBM-cells deposited ECM after erlotinib treatment. Integrin-binding peptides, such as RGD, conjugation to the hydrogels significantly reduced apoptosis after 3 days of treatment. In luciferase-based assay of transcription factor activity, we found that erlotinib treatment reduced the activity of P53, which increases with apoptosis, and increased activity of oncogenic transcription factors such as cMyc. Finally, we report that only HA based hydrogels maintained phosphorylation of EGFRvIII under 1 μM erlotinib treatment (**Fig. 1C**). We report development of biomimetic, modularly tunable culture platforms that maintain clinically relevant physiology of patient-derived GBM cells *ex vivo*. We have demonstrated the utility of these biomaterial platforms for mechanistic investigations of the role of the unique brain microenvironment in therapeutic resistance of GBM.

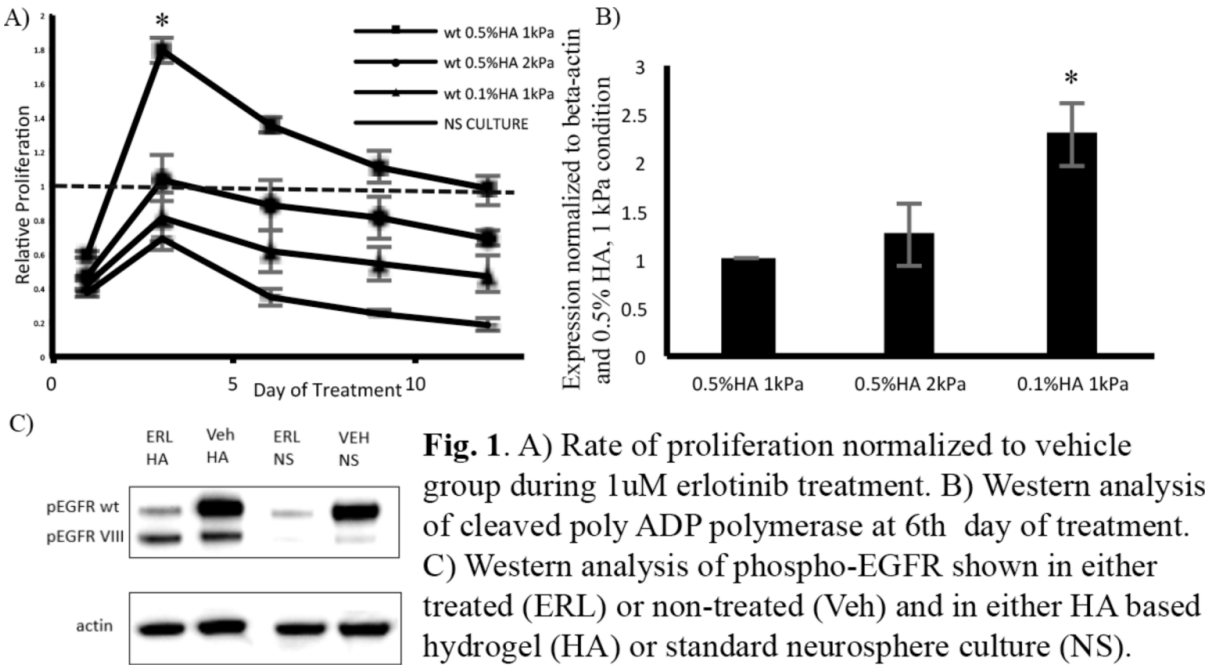


Fig. 1. A) Rate of proliferation normalized to vehicle group during 1uM erlotinib treatment. B) Western analysis of cleaved poly ADP polymerase at 6th day of treatment. C) Western analysis of phospho-EGFR shown in either treated (ERL) or non-treated (Veh) and in either HA based hydrogel (HA) or standard neurosphere culture (NS).

Disclosures: W. Xiao: None. **R. Zhang:** None. **S. Sun:** None. **A. Ehsnaipour:** None. **C. Walthers:** None. **J. Liang:** None. **L. Ta:** None. **D. Nathanson:** None. **S. Seidlits:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.13/G15

Topic: B.14. Neuro-Oncology

Title: Increased phosphorylation of the mitochondrial fission protein DRP1 in p75 neurotrophin receptor ($p75^{NTR}$)-induced glioma

Authors: *Y. AHN[1], N. SALEM[2], B. AHN[3], S. M. ROBBINS[3], D. SENGER[3], J. M. RHO[1]; [1]Pediatrics, Alberta Children'S Hosp. Res. Inst., Calgary, AB, Canada; [2]Biomed. Hlth. Sci., [3]Oncology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Glioblastoma (GBM) is the most prevalent primary brain tumor and has a median survival of only 12-18 months after diagnosis, thus ranking it among the most lethal of human cancers. Like most cancers, glioblastomas exhibit a metabolic shift toward glycolysis, a phenomenon known as the Warburg effect. This fundamental shift in bioenergetics invokes both substrates and organelles responsible for metabolic homeostasis. In this light, mitochondria are

well known to generate energy from oxidation of nutrients, and they are also highly dynamic organelles that undergo fusion, fission, trafficking and mitophagy. Each of these dynamic processes is critical to maintenance of a healthy mitochondrial population. In addition, mitochondrial dynamics have been linked to multiple functions including respiratory chain activity. Using previously established human GBM cell lines, invasive GBM (U87p75$^{NTR)}$) cells showed lower oxidative phosphorylation (OXPHOS) and cellular glycolytic activity than non-invasive (U87pcDNA) GBM cells. (Basal respiration: 194 vs. 112 pMoles/mim, maximal respiration: 474 vs. 159 pMoles/min, glycolysis: 24 vs. 13 mpH/min, and glycolytic capacity: 30 vs. 17 mpH/min, respectively). Because mitochondrial bioenergetics and dynamics influence each other in a reciprocal fashion, we further assessed mitochondrial morphology using the Tom20 antibody and mitotracker red (MTR) under immunofluorescence. We found that mitochondria in invasive GBM cells were more fragmented relative to non-invasive GBM cells (Approx. 70% vs. 50%, respectively, n=80 cells per group), suggesting that invasive GBM cells increase mitochondrial fission. Moreover, the essential mediator of mitochondrial fission, dynamin-related protein 1 (DRP1), was actively phosphorylated in invasive GBM cells. Utilizing flow cytometry we determined that mitochondrial content was higher in the invasive GBM cells (1.83 vs 1.42). This increase was confirmed with western blot analysis of VDAC protein expression (1.88 vs 1.22). No difference was found in mitochondrial membrane potential. Thus, these results suggest that the dynamic properties of mitochondria represent an important mechanism critical to brain metabolism, and we anticipate that mediators of fission and fusion (e.g., Mdivi-1, an inhibitor of fission) may provide novel molecular targets for therapeutic interventions aimed at highly invasive glioma.

Disclosures: Y. Ahn: None. **N. Salem:** None. **B. Ahn:** None. **S.M. Robbins:** None. **D. Senger:** None. **J.M. Rho:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.14/G16

Topic: B.14. Neuro-Oncology

Support: NIH Supplement of the MSM/TU/UABCCC U54 Partnership grant 3U54-CA118948-09S1

Title: PAD expression and citrullination profiles in malignant glioma

Authors: E. A. SUSWAM[1], J. JYOTI[2], G. Y. GILLESPIE[3], C. P. LANGFORD[3], U. MANNE[1], *A. P. NICHOLAS[4];

[1]Pathology, [2]Microbiology, [3]Neurosurg., [4]Dept Neurol, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Background: Citrullination (or deimination) is a post-translational modification in which protein-bound arginine amino acids are converted to citrullines by a family of peptidyl arginine deiminase (PAD) enzymes. Several PAD isoforms (PAD 1-4 and 6) have been reported and demonstrate tissue specificity. Citrullination results in loss of target protein structure and has been associated with progression of various diseases, including rheumatoid arthritis and several neurological disorders. Recent studies suggest an increasing role of citrullination in cancer; increased epithelial-mesenchymal transition and breast cancer metastasis and alterations in chemotherapeutic responses in prostate cancer have been linked to GSK3β citrullination and PAD inhibition, respectively. No studies have been done to assess the contribution of citrullination to brain cancer, although PAD expression has been demonstrated in the brain. Our preliminary studies indicate high reactivity of astrocytic tumors to an anti-peptidyl citrulline antibody; hence, we analyzed malignant glioma cells and glioblastoma (GBM) tumor and xenograft tissues for PAD expression to identify citrullinated proteins in brain cancers.
Hypothesis: We hypothesize that PAD expression and citrullination are upregulated in brain cancer and modulate tumor progression and the malignant phenotype.
Methods: PAD expression was quantified from total RNA from five malignant glioma cell lines (U251, U373, JX10, LN229, D456) and normal human astrocytes by qRT-PCR. Whole cell extracts were analyzed for presence of citrullinated proteins by Western blotting using an anti-citrullination monoclonal antibody (F95). Subcellular localization was investigated by immunocytochemistry. To begin to establish the molecular identity of citrullinated proteins, the extracts were immunoprecipitated with the anti-citrullination and other antibodies. Protein citrullination in brain tumor and control tissues was investigated by immunohistochemistry.
Results: There was differential expression of PAD isoforms and a high prevalence of citrullinated proteins (MW 15-170 kDa) in brain cancer cells. Strong immunoreactivity in both nuclear and cytoplasmic compartments was detected by immunocytochemistry. Immunohistochemistry of GBM and control tissues showed higher citrullination in tumors, with both nuclear and cyoplasmic staining.
Conclusions: We conclude that PAD expression and protein citrullination are increased in brain cancer and may modulate disease progression. Further work to characterize citrullinated proteins and establish a functional role for deimination in brain cancer is on-going.

Disclosures: E.A. Suswam: None. **J. Jyoti:** None. **G.Y. Gillespie:** None. **C.P. Langford:** None. **U. Manne:** None. **A.P. Nicholas:** 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; Cynapus, Michael J Fox Foundation, Adamas. F. Consulting Fees (e.g., advisory boards); UCB, US WorldMeds, Ipsen, Acadia, Lundbeck.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

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Program#/Poster#: 305.15/G17

Topic: B.14. Neuro-Oncology

Support: Scott and White foundation foundation and Texas A & M Health Science Center

Title: The potential impact of pro-inflammatory B cells and gamma delta T cells on the progression of glioblastoma multiforme (GBM) in a syngeneic mouse model of GBM.

Authors: *S. MUKHERJEE[1], C. PEDDABOINA[2], L. DAO[2], S. HENDERSON[3], J. KAIN[2], D. LITTLE[4], M. NEWELL ROGERS[2,3];
[1]Surgery, Texas A & M, Temple, TX; [2]Surgery, Texas A and M Hlth. Sci. Ctr., Temple, TX; [3]Surgery, Baylor Scott and White, Temple, TX; [4]Psychiatry, Baylor Col. of Med., Houston, TX

Abstract: Background and Rationale. Glioblastoma multiforme (GBM) is a life threatening brain tumor with an average life expectancy of approximately 14 months. For many cancers, there has been a major effort to target the immune system, to provoke an anti-tumoral response and to prevent immunosuppression by cells including Tregulatory cells (Tregs) and myeloid suppressor cells (MDSCs). While the brain is considered an immune privileged site, GBM has been shown to increase permeability of the blood brain barrier (BBB). This suggests that immune cells could infiltrate the brain, but GBM progresses in spite of both BBB permeability and the potential entry of immune cells into the brain. Recent studies demonstrate a negative correlation between the frequency of Tregs in GBM and a poor prognosis. **Hypothesis**. Our work is aimed at testing the hypothesis that selective permeability of the BBB in GBM allows regulatory cells to protect the tumor from an anti-tumoral response and that this protective strategy can be reversed by the selective expansion of pro-inflammatory cells, including a subset of activated B cells that express the Class II invariant peptides (CLIP) and certain γδT cells that can elicit an anti-tumoral immune response. **Materials and Methods.** For these studies, we have used neurospheres derived from the mouse GBM cell line 261. The neurospheres were transplanted by micro-injection into the caudate nucleus. The neurospheres were injected into wild type C57Bl6 mice; into mice lacking CD74 (CD74def); into mice deficient for γδ T cells; and into wild type C57Bl6 animals treated for 10 days post tumor injection with TLR agonists. At the end of the study, tumors were dispersed into single cell suspensions, and the intra-tumoral lymphocytes were characterized by flow cytometric analysis. **Results.** For 261-injected wild type animals, the average number of days until the animals succumbed to GBM was 30 to 40 days post tumor implantation. For 261-injected CD74def mice and γδ T cell knock out mice , the average was approximately 20 to 30 days post-implantation. For wild type animals treated at day 10 post implantation with TLR modulators, there was a correlation between TLR-induced

increases in the number of CLIP+ B cells and in modulation in longevity. **Conclusions and Future Directions**. Taken together our data are consistent with the hypothesis that selective expansion of pro-inflammatory B cells and a parallel expansion of certain subsets of γδ T cells can restrict tumor progression. Our future work is aimed at understanding the molecular mechanism of this restricted growth in aid of developing novel immune-based therapies for GBM.

Disclosures: S. Mukherjee: None. **C. Peddaboina:** None. **L. Dao:** None. **S. Henderson:** None. **J. Kain:** None. **D. Little:** None. **M. Newell Rogers:** None.

Poster

305. Neuro-Oncology: Tumor Characterization and Modeling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 305.16/G18

Topic: B.14. Neuro-Oncology

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Title: Cellular origin influences glioma pathogenesis and treatment

Authors: *D. IRVIN[1], R. MCNEILL[2], M. VITUCCI[2], R. BASH[2], R. SCHMID[3], C. MILLER[2,3,4];
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Abstract: The role of cellular origin during glioma development and treatment response remains elusive. We used genetic lineage tracing and fate mapping to show that Rb (T) and Pten (P) inactivating combined with Kras (R) activating (TRP) mutations transformed two subpopulations of mouse astrocytes expressing either Gfap or Glast. Both served as cells of origin for TRP-induced gliomas, but kinetics of tumor growth and progression differed. GFAP-CreER induced recombination in ~50% of astrocytes. GFAP-TRP mice harbored 6-11-fold more TdTomato$^+$ astrocytes after 8 weeks, but Glast-targeted astrocytes were 1-4 fold lower. Moreover, GFAP astrocyte transformation induced uniformly rapid growth of low-grade tumors that frequently progressed to lethal, high-grade glioblastomas (GBM). In contrast, transformation of GLAST astrocytes induced tumors that progressed to anaplastic, non-GBM tumors over a more protracted course. In the absence of mutations, genetic lineage tracing with Glast-CreER;TdTomato mice produced recombination in 8-38% of astrocytes. Astrocyte proliferation increased 8-29-fold after 3 weeks and TdTomato$^+$ cells increased 3-7-fold by 8 weeks after TRP transformation. Ki-67 labeling showed focal clonal expansion by 16 weeks in both models. Magnetic resonance imaging of GFAP-TRP mice showed stochastic progression to contrast-enhancing GBM by 12-16 weeks. In both models, transformed astrocytes maintained expression of Gfap and Blbp, yet gained expression of the stem cell marker Nestin. Nestin was expressed in 82% of transformed, proliferating GFAP astrocytes, but only 9% of GLAST astrocytes ($P<0.0001$). These results suggest that identical TRP mutations induce distinct patterns of tumor growth due to differential effects on astrocyte de-differentiation. To explore de-differentiation further, we examined cultured GFAP$^+$ TRP astrocytes and found that they gained unlimited self-renewal and multi-lineage differentiation capacity *in vitro*. These cells harbored altered chromatin landscapes associated with downregulation of astrocyte- and upregulation of stem cell-associated genes, particularly the Hoxa locus of embryonic transcription factors. TRP astrocytes formed serially-transplantable GBM allografts that were sensitive to radiation, but were resistant to temozolomide due to Mgmt expression. Radiation induced a shift in transcriptome subtype from proneural to mesenchymal in TRP allografts, similar to the shift observed after treatment in human GBM. These findings support the use of GFAP- and GLAST-TRP as tractable models for transforming astrocyte subpopulations, studying glioma stem cells, and determining treatment efficacy.

Disclosures: D. Irvin: None. **R. McNeill:** None. **M. Vitucci:** None. **R. Bash:** None. **R. Schmid:** None. **C. Miller:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.01/G19

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant AG033649

Title: Acute insulin treatment of hippocampal neurons highlights new mechanisms of action

Authors: S. MAIMAITI, H. N. FRAZIER, *K. L. ANDERSON, L. D. BREWER, N. M. PORTER, O. THIBAULT;
Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Our lab studies the effects of different insulin formulations, including zinc-containing insulin and zinc–free insulin, on cognition. We identified that intranasal delivery of Levemir or Humalog (zinc-containing insulins) improves cognition in the aged F344 rats (Maimaiti et al., 2015). Intranasal Apidra (zinc-free insulin), however, was unable to improve cognition in the aged animal (Anderson et al., 2016). Growing evidence supports the concept that brain insulin defects in signaling or receptor numbers are associated with memory decline in Alzheimer's disease (AD) and advanced age. Although intranasal insulin enhances memory in AD patients, it also does so in young adult subjects with normal insulin signaling at baseline. While prior work has provided evidence that ion channels (AMPA, NMDA, GABA), glucose transporter-4, insulin degrading enzyme and vascular function, very little work has focused on voltage-gated calcium channels and intracellular Ca^{2+} as a target of insulin action in the brain. Ca^{2+} is a key neuronal molecular regulator of hippocampal-dependent memory and intracellular elevations in hippocampal neurons from aged animals are associated with poor spatial memory. We recently showed that insulin reduces the Ca^{2+} -dependent afterhyperpolarization (AHP) in hippocampal neurons in both young and aged animals (Maimaiti et al., 2015), however, the underlying mechanism for this reduction has not been studied in depth.
The goal of the present work is to test the hypothesis that insulin improves memory by reducing Ca^{2+} dysregulation, levels and currents. We used whole cell patch clamping and Ca^{2+} imaging techniques to measure voltage-gated Ca^{2+} currents (VGCCs) and intracellular Ca^{2+} levels in 13-17 DIV primary hippocampal neurons in culture. Apidra (10nM, rapid-acting, zinc-free insulin), boiled Apidra, reconstituted human insulin, and zinc were tested acutely for effects on VGCCs. Results show that both 10 nM Apidra and reconstituted insulin reduce Ca^{2+} currents. 10 nM Apidra did not reduce resting Ca^{2+} levels or spontaneous Ca^{2+} transient, but did reduce KCl-induced intracellular Ca^{2+} transients. These effects were attenuated by pretreatment with an insulin antibody. Together, these results indicate insulin can reduce intracellular calcium levels during neuronal depolarization, highlighting a potential mechanism for reducing the AHP and improving memory.

Disclosures: S. Maimaiti: None. **H.N. Frazier:** None. **K.L. Anderson:** None. **L.D. Brewer:** None. **N.M. Porter:** None. **O. Thibault:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.02/G20

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant AG033649

Title: Signaling and expression of a truncated, constitutively active human insulin receptor in neurons and astrocytes

Authors: *H. N. FRAZIER[1], S. MAIMAITI[1], K. L. ANDERSON[1], K. HAMPTON[1], L. D. BREWER[1], S. D. KRANER[2], C. M. NORRIS[2], R. J. CRAVEN[1], N. M. PORTER[1], O. THIBAULT[1];
[1]Pharmacol., [2]Sanders-Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: Insulin signaling is indispensable in the periphery and it is becoming clear that insulin is also important for normal brain function. Early stage clinical trials report a positive impact of intranasal insulin on memory recall in young subjects and patients with mild cognitive decline or Alzheimer's disease (AD). To address alternative strategies for enhancing insulin signaling in the brain, we have conducted a series of experiments using a constitutively active human insulin receptor (IR). Distribution and functional characteristics were evaluated using rat pheochromocytoma (PC12) and primary mixed hippocampal cultures.
Cells were transfected with either a mammalian expression plasmid encoding a red fluorescence protein (ires-dsRed), or a construct containing the truncated human IR beta subunit (HA-IRβ-ires-dsRed), via electroporation or a targeted lentiviral delivery system. The expression of IRβ receptor in PC12 cells was corroborated by the expression of the red fluorescent protein. A silencing site (RE1) was used to restrict expression to neurons. Photomicrographs of mixed primary hippocampal cultures confirmed expression of the lentiviral plasmid in neurons and astrocytes. The expression level and effect of IRβ overexpression on insulin signaling was confirmed in both PC12 cells and hippocampal cultures by performing Western immunoblots using antibody against HA-tagged IRβ and measuring pAkt/Akt ratio. Glucose uptake and utilization rates were measured in both neurons and astrocytes using 2-NBDG imaging. Calcium imaging (Fura-2) was also used to test for a link between glucose utilization and calcium levels both at rest and during insulin stimulation in transfected neurons and astrocytes.
Western blots of both transfected PC12 cells and mixed hippocampal cultures provide evidence that transfection with the truncated IRβ plasmid confers a greater response to insulin treatment. Lentiviral infection of mixed primary hippocampal cultures was successful for all of our constructs suggesting that this approach is viable for enhancing insulin signaling. Glucose uptake and utilization measures, together with calcium level quantification in neurons and astrocytes,

validated cellular health and sensitivity to insulin. This initial characterization provides insights into future intervention approaches to combat cognitive decline in AD and/or aging using molecular methods to enhance insulin signaling.

Disclosures: **H.N. Frazier:** None. **S. Maimaiti:** None. **K.L. Anderson:** None. **K. Hampton:** None. **L.D. Brewer:** None. **S.D. Kraner:** None. **C.M. Norris:** None. **R.J. Craven:** None. **N.M. Porter:** None. **O. Thibault:** None.

Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.03/G21

Topic: C.01. Brain Wellness and Aging

Support: 5R01AG033649-07

Title: Insulin phosphosignaling in the hippocampus of young and aged animals

Authors: K. K. HAMPTON, H. FRAZIER, K. ANDERSON, O. THIBAULT, *R. CRAVEN; Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Insulin binding is reduced in the brains of aged, compared to young-adult rats. Furthermore, on post-mortem analyses, hippocampal tissues from AD and MCI patients display a reduction in insulin sensitivity compared to younger or non-demented patients. The hippocampus along with the olfactory bulb are two brain areas showing greatest insulin receptor density, suggesting intranasal delivery as an approach to combat cognitive decline. Surprisingly, both young healthy subjects and AD patients appear to benefit equally from intranasal insulin. One explanation is that adding the ligand to the brain by bypassing the blood brain barrier may compensate for the reduced insulin signaling in AD. However, these data also suggest insulin signaling may be working differently in young *vs.* aged brains. Clearly, the mechanisms involved in insulin receptor signaling across aging are critical for the advancement of insulin enhancing therapies in the treatment of cognitive decline in aging and/or AD. We have compared insulin sensitivity in acute hippocampal slices of young and aged F344 rats, where the endpoints are 24 species of phospho-proteins broadly associated with MAPK/ERK signaling. Our model is that young and aged brain tissues may employ alternate modes of insulin signaling. Slices were incubated for 5-30 minutes with 1,10, or 100 nM insulin. Different insulin formulations were tested including zinc-free Apidra, reconstituted human insulin, and humalog. In the hippocampus of young animals, insulin stimulated the phosphorylation of ERK1, JNK, p38beta and p53. When compared to young animals, the hippocampus of aged rats had increased phosphorylation of

JNK1 and JNK2 and p53, while p38alpha was diminished. For some proteins, insulin signaling was similar in young versus aged animals, for example, ERK1 phosphorylation was induced 2.1 and 1.5-fold in young and aged animals, respectively. However, insulin signaling was distinct for other proteins, particularly p38beta (4-fold induction vs. 2-fold suppression, respectively) and JNK2 (3.7-fold induction vs. no change, respectively). These results suggest a heretofore unappreciated complexity in insulin signaling in the young and aged brain. Once insulin is past the blood brain barrier, even in aged animals, it is capable of signaling robustly through intermediates such as ERK. However, our results also support a model in which some downstream effectors of insulin cannot be readily manipulated in the aged brain. Our work lends further support to continue therapeutic efforts attempting to maintain insulin signaling in the brain of the elderly population, in order to offset cognitive decline with age or with AD.

Disclosures: K.K. Hampton: None. **H. Frazier:** None. **K. Anderson:** None. **O. Thibault:** None. **R. Craven:** None.

Poster

306. Brain Energetics in Health and Disease

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Topic: C.01. Brain Wellness and Aging

Support: CIHR grant # MOP-130282

Research Manitoba

Alzheimer Society of Manitoba

NSERC

St. Boniface Research Foundation

Title: Altered mitochondrial function and succinate-dependent H_2O_2 production in the cortex of type 1 diabetic rodents

Authors: *S. ROY CHOWDHURY, J. DJORDJEVIC, E. THOMSON, D. SMITH, B. C. ALBENSI, P. FERNYHOUGH;
Div. of Neurodegenerative Disorders, St. Boniface Hosp. Res. Ctr., Winnipeg, MB, Canada

Abstract: ***Rationale & Hypothesis.*** Mitochondrial dysfunction and altered levels of reactive oxygen species have been implicated in diabetic neuropathy. Abnormalities in mitochondrial

function under diabetic conditions can lead to detrimental consequences on the function of brain cells and subsequently have a pivotal role in the pathogenesis of neurodegenerative disorders. ***Objectives.*** This comprehensive study was aimed at evaluating mitochondrial function in cortical tissues and included the simultaneous assessment of mitochondrial respiration rates and membrane potential (mtMP) or H_2O_2 generation, mitochondrial enzymatic activities and proteins involved in mitochondrial complexes, antioxidant enzymes, the AMPK/SIRT/PGC1α pathway and mitochondrial dynamics. ***Methodology.*** Freshly isolated mitochondria from cortex of 5 month old streptozotocin (STZ)-induced diabetic rats or mice, a model of type 1 diabetes, and aged-match controls were used for simultaneous measurements of mitochondrial respiration rates and mtMP or H_2O_2 level (performed by Oxygraph-2K- Fluorescence LED2 module, OROBOROS instruments). Enzymatic activities were measured by a spectrophotometer. Protein levels in cortical mitochondria and homogenates were determined by Western blotting. ***Results.*** Simultaneous measurement of oxygen consumption rates (OCR) and mtMP revealed that mitochondrial coupled respiration rates in the presence of glutamate, pyruvate, malate and succinate and FCCP- titrated uncoupled respiration rates were significantly decreased in cortical mitochondria from 5-month old STZ-diabetic rats compared to controls. The mtMP was significantly depolarized in the diabetic cortical mitochondria in the presence of ADP; however, FCCP-titrated polarization status in diabetic rats was not significantly changed compared to controls. The simultaneous measurement of OCR and H_2O_2 level showed that succinate-dependent respiration rates and H_2O_2 production were significantly decreased in STZ-diabetic cortical mitochondria from rats and mice, which was accompanied by a decrease in expression of antioxidant enzymes, CuZn- and Mn-superoxide dismutases. The specific enzymatic activities of Complex I, II, and IV, protein levels of Complex I and II, mitofusion 2 (Mfn2), dynamin-related protein 1 (DRP1), phosphorylated AMPK, SIRT-2, and PGC-1α were decreased in diabetic cortical tissues. ***Conclusion.*** These results support the idea that alterations in mitochondrial function, dynamics, and antioxidant capabilities through altered AMPK/SIRT/PGC-1α pathway activity are involved in the development of neurodegenerative disease under diabetic conditions.

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Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.05/G23

Topic: C.01. Brain Wellness and Aging

Support: Austrian National Bank (Jubiläumdsfond, Grant-12316 to H. Baran)

Title: Respiratory parameters of rat brain, liver and heart mitochondria during the aging process

Authors: *H. BARAN[1,2], K. STANIEK[3], M. BERTIGNOL-SPÖRR[2], M. ATTAM[3], B. KEPPLINGER[1];
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Abstract: Background: Tryptophan metabolism is significantly altered in various organs of mammals with aging. Kynurenine metabolites differently affect bioenergetic function of brain, liver and heart mitochondria. Therefore, we investigated the respiratory parameters of brain, heart and liver mitochondria isolated from healthy rats during the aging process.
Animals and Methods: Brain, heart and liver mitochondria isolated from 3, 12 and 26 months old male Wistar rats (n=6) were incubated with respiratory substrates glutamate/malate (5 mM each) or succinate (10 mM). Study was performed according to Austrian ethical regulations. Oxygen consumption rates were measured within a DW1 oxygen electrode chamber. Differences in respiratory parameters were analyzed by ANOVA followed by Student's t-test.
Results: With regard to investigated mitochondria of 3 months old rats, we observed that heart mitochondria exert the highest oxygen consumption rates (state 2: 22.4 ± 1.16 nmol O × min^{-1} × mg^{-1}, state 3: 140.3 ± 8.7 nmol O × min^{-1} × mg^{-1}), followed by liver mitochondria (state 2: 11.2 ± 0.5 nmol O × min^{-1} × mg^{-1}, state 3: 46.9 ± 3.7 nmol O × min^{-1} × mg^{-1}) and brain mitochondria (state 2: 4.7 ± 0.3 nmol O × min^{-1} × mg^{-1}, state 3: 19.2 ± 1.6 nmol O × min^{-1} × mg^{-1}) using glutamate/malate. Succinate-respiring mitochondria showed higher oxygen consumption rates in comparison to glutamate/malate-supplied mitochondria. However, the highest respiratory activities, as in the glutamate/malate data, were found in heart mitochondria (state 2: 92.0 ± 7.8 nmol O × min^{-1} × mg^{-1}, state 3: 285.9 ± 19.5 nmol O × min^{-1} × mg^{-1}), followed by liver mitochondria (state 2: 26.3 ± 1.9 nmol O × min^{-1} × mg^{-1}, state 3: 103.7 ± 6.7 nmol O × min^{-1} × mg^{-1}) and brain mitochondria (state 2: 8.8 ± 0.6 nmol O × min^{-1} × mg^{-1}, state 3: 30.5 ± 1.9 nmol O × min^{-1} × mg^{-1}). The highest respiratory control (RC) values were detected in glutamate/malate-respiring heart mitochondria (6.3 ± 0.2), while other RC values were in the range of 3.2-4.3. The aging process was accompanied by a significant increase of RC values in heart mitochondria incubated with glutamate/malate or succinate. RC values of liver mitochondria exposed to glutamate/malate were significantly increased during aging. The highest RC values of succinate-respiring brain mitochondria were obtained from 12 months old rats. The ADP/oxygen ratios were moderately but not significantly changed in brain, heart or liver mitochondria during aging.
Conclusion: The energy-conserving capacity of rat heart, liver and brain mitochondria was not impaired, but on the contrary, partially improved during the aging process.
Support: Austrian National Bank (Jubiläumsfonds, Grant-12316 to H. Baran).

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Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.06/G24

Topic: C.01. Brain Wellness and Aging

Support: BUAP-VIEP (DIFA-NAT 16-I)

PRODEP (DSA/103.5/15/7449)

Title: Metabolic syndrome impairs spatial memory & dendritic morphology of rat hippocampal neurons

Authors: *A. D. DIAZ[1], P. AGUILAR-ALONSO[1], A. MORENO-RODRIGUEZ[1], U. PEÑA-ROSAS[1], G. LÓPEZ-LÓPEZ[1], E. BRAMBILA[1], R. A. VAZQUEZ-ROQUE[2], J. GUEVARA[3], G. FLORES[2], S. TREVIÑO[1];
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Abstract: A high calorie intake can to induce the metabolic syndrome (MS) appearance, which is a serious public health problem, this is characterized by impaired on glucose & triglycerides levels in blood. Recently, it has been suggested that MS can cause brain complications, since chronic hyperglycemia with insulin resistance are risk factors for neurodegeneration. The hippocampal region is sensitive of deterioration, which is reflecting in cognitive functions as well as in processes of plasticity, however, mechanism is not clear. In this study, we evaluated the spatial learning & memory, as well as their effects on neuronal morphology of hippocampus in rats with MS. Our results demonstrated that 90 days of eating a high-calorie diet, altered the main energy metabolism markers, indicating the development of MS. In the same way was observed an impairment in spatial memory, which was evaluated by testing recognition of novel objects (NORT). Therefore, the brains of animals were exposed to the solution of Golgi-cox & coronal sections was performed by Sholl analysis to develop the morphology of the regions of the hippocampus: CA1, CA3 & dentate gyrus (DG). The results of the group with MS shown a significant difference in the dendritic order, total dendritic length, & in the density of dendritic spines of the three brain regions analyzed (CA1, CA3 & DG) compared to the control group. Additionally, the immunoreactivity of sirtuin-1 & synaptophysin (markers of neuronal plasticity) decreased in the group with MS compared to the control group. Results suggest that the MS contributes to development of a neurodegenerative process & cognitive failure. In this regard, is important to understand the relationship between MS & neuronal damage, with the purpose of prevent the onset of neurodegenerative disorders. Furthermore, this work open up the possibility of researching treatments to counteract or prevent the brain damage occurrence, caused by metabolic disorders in an excessive calorie intake.

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Poster

306. Brain Energetics in Health and Disease

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Topic: C.01. Brain Wellness and Aging

Support: National Institute on Aging Intramural Research Program

Title: Metabolic fuel switch from glucose to ketones regulatesSIRT3 in the brain

Authors: ***K. MAROSI**, S. KIM, M. P. MATTSON, R. CUTLER, S. CAMANDOLA; NIH, Baltimore, MD

Abstract: SIRT3 is a member of the Sirtuin family of NAD^+-dependent deacetylases and plays a critical role in metabolic regulation. Previously we found that neurons lacking the mitochondrial deacetylase SIRT3 are more vulnerable to dysfunction and degeneration in mouse models of epilepsy and Huntington's disease. We also showed that exercise and synaptic activity induce hippocampal SIRT3 expression to modulate mitochondrial protein acetylation and bolster neuronal resistance to oxidative stress and apoptosis. In our current study we investigated how physiological and metabolic changes resulting from dietary interventions and exercise affect SIRT3 levels and activity in the brain. We found that alternate day fasting and exercise promote a metabolic fuel switch involving elevated ketone levels and reduced blood glucose levels in C57BL/6J mice. Elevated plasma ketone levels were correlated with enhanced SIRT3 protein levels in the brain of the mice that were subjected to alternate day fasting and exercise compared to the sedentary controls. We then investigated how the ketone 3β-hydroxybutyrate (3OHB) regulates SIRT3 expression in cultured primary rat cortical neurons. 3OHB increased SIRT3 protein levels and activity in a low glucose condition, but not in a high glucose condition indicating that changes in the substrate utilization regulate SIRT3. We found that 3OHB induces ATP and NAD^+ production by bypassing glycolysis and promoting mitochondrial respiration in the neurons, which might play a role in the modulation of SIRT3 activity. We are currently employing SIRT3-/- mice to determine whether SIRT3 mediates neuroprotective effects of fasting in models of brain injury and neurodegenerative disorders. Supported by the National Institute on Aging Intramural Research Program.

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Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.08/G26

Topic: C.01. Brain Wellness and Aging

Support: Minnesota Women's Healthy Aging Project, University of Minnesota Foundation

American Legion Brain Sciences Chair, Univeristy of Minnesota

Title: Nutrition and healthy brain functioning across the lifespan

Authors: ***J. HEATH MATHISON**[1], L. M. JAMES[1], A. LEUTHOLD[1], A. GEORGOPOULOS[1], A. GEORGOPOULOS[1], H. HOOVER[2];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Minneapolis VA Med. Ctr., Minneapolis, MN

Abstract: The Healthy Brain Project (HBP; http://brain.umn.edu/brp/index.shtml) is a unique study at the Brain Sciences Center at the Minneapolis VA Medical Center and the University of Minnesota that integrates neuroimaging (magnetoencephalography, structural and functional MRI, diffusion weighted MR imaging, MR spectroscopy), genetics (apolipoprotein E genotype), cognitive, and lifestyle data to identify characteristics associated with healthy brain aging. Participants are cognitively healthy (MoCA score > 25) adults (primarily women veterans), 30-100+ years old who are being studied annually, thus enabling the search for associations among study variables cross-sectionally and their validation longitudinally. We have recently extended this rich data set to include detailed nutritional information, since nutritional status can have a major effect on brain functioning. In addition, collecting such data from a healthy population could allow for more detailed identification of dietary habits associated with healthy brain function across the lifespan. Specifically, participants are asked to prepare food journals by recording food and beverage intake for three days, two weekdays and one weekend day. The items are then entered into Nutritionist Pro Version 6 software for further analysis. Using this software, we are able to quantify 115 macro and micro-nutrients, including amino acids, trace minerals, and lipids and associate the amount of intake to neural and other information above. We have collected, up to now, such nutritional data from 100+ participants. Preliminary analyses have revealed large variety in participants' intake of several nutrients, which allows for a rigorous, ongoing, exploration of associations with other information (neural functioning,

genetics, lifestyle, etc.). Since all our participants are cognitively healthy, we expect that these analyses will yield valuable insights into the dietary habits that underlie healthy brain aging.

Disclosures: J. Heath Mathison: None. **L.M. James:** None. **A. Leuthold:** None. **A. Georgopoulos:** None. **A. Georgopoulos:** None. **H. Hoover:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.09/G27

Topic: C.01. Brain Wellness and Aging

Title: Hippocampal damage in the offspring exposed to perinatal high sucrose diet

Authors: *I. ZARCO DE CORONADO[1], S. MOSSO-MENDOZA[2], M. A. HERRERA[3]; [2]Fisiologia, [3]Biologia Celular, [1]UNAM, Mexico DF, Mexico

Abstract: In addition to its metabolic disturbances in offspring exposed to perinatal high sucrose diet (HSD), the male subjects showed deficit in spatial learning. This study determined the influence of maternal HSD on hippocampal neurons of infantile rats. Dams were fed, since 14 gestation day to the end of lactation period, a HSD (20 % sucrose solution) or control diet. Brains were removed after lethal anesthesia, from infantile offspring. Fixed in formaldehyde 3.7%. Freeze sliced to 20 µm using Leica CM Criostat1510S. Stained Cresyl violet, Kluver Barrera and HE. Nikon Eclipse 80i microscope. Nikon DS-Fi1c digital camera, Software NIS-Elements F; Image Pro Plus 7.0 analysis. Decreased number of pale pyramidal cells in CA3 (66% in males and 61 % in females). There were vascular dilatation and increased number of glial cells. The results suggest that perinatal HSD induce previously reported obesity, dyslipemia and spatial learning acquisition deficit in male offspring associated with altered hippocampal proliferative and inflammatory activity.

Disclosures: I. Zarco de Coronado: A. Employment/Salary (full or part-time): A. **S. Mosso-Mendoza:** Other; voluntary. **M.A. Herrera:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.10/G28

Topic: C.01. Brain Wellness and Aging

Title: Oxidative stress underlies amyloid-beta toxicity and mitochondrial dysfunction in Alzheimer's disease

Authors: *L. ADLER, W. REGENOLD, S. DODDI;
Psychiatry (Geriatric), Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Background: The amyloid cascade theory hypothesizes overproduction and/or reduced clearance of Amyloid-Beta (Aβ) are etiologic in late-onset Alzheimer's Disease (LOAD). Unanswered questions persist. (1) Are these processes downstream biologic events? (2) What is the physiologic role of soluble Aβ monomers? (3) What is the physical chemistry in of Aβ aggregation? (4) Are biological factors converging with Aβ aggregation in the genesis for AD? (5) Is there a vicious cycle where disrupted energy transduction facilitates toxic Aβ aggregation and aggregation further disrupts mitochondrial energy transduction?**Objective:** Review of risk factors for LOAD which converge to dysregulate mitochondrial energy transduction.
Methods: PubMed, EMBASE, and Medline were searched using (Alzheimer's -risk factors - inflammation- mitochondria- vascular-bioenergetics- Aβ). Relevant manuscripts were reviewed to generated a coherent hypothesis of converging risk factors leading to disruption of mitochondrial function. **Results:** Age is the primary risk factor. Vascular risk factors include hypertension, hyperlipidemia, diabetes and ApoE4. Recurrent depressive episodes and their association with cortisol dysregulation confer increased risk.
Vascular burden is associated with sub-cortical CNS ischemic changes. Recurrent depressive episodes are associated with microvascular changes and with cortisol dysregulation which is neurotoxic. These events promote increases in inflammatory mediators. Reduction in proton-motive force and inability of mitochondria to buffer against reactive oxygen species (ROS) result. Mitochondrial DNA (mtDNA) is damaged by close proximity to ROS at the site of electron transport. There is insufficient energy for clearance of soluble Aβ into the CSF and in to the glymphatic system. At nanomolar concentrations. aggregated Aβ results in activate microglia- mediated inflammation. Tau hyperphosphoryllation and deposition of amyloid result, perhaps through up-regulated GSK-3β. Activation of caspases leads to neuronal apoptysis. Soluble Aβ monomers at picomolar concentrations are implicated in neurogenesis and synaptic protection.The biophysics of conversion of helical Aβ to pleated sheet Aβ aggregates requires further study; the role of chaperones needs further elucidation. **Conclusion:** Deposition of amyloid and tau are likely down-stream events resulting convergence of vascular factors,

disruption of mitochondrial energetics, subcellular and physical chemical events influencing aggregation and changes in physiology related to decrease in pulsatile energy in small arteries promoting glymphatic clearance.

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Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.11/G29

Topic: C.01. Brain Wellness and Aging

Title: Neuroprotective effect of blackberry juice in rats under a hypercaloric diet

Authors: *B. PÉREZ GRIJALVA[1,2], R. MORA ESCOBEDO[2], R. GUZMAN GERONIMO[3], A. DIAZ[4], S. TREVIÑO[4], C. PEREZ CRUZ[1];
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Abstract: Recent studies have shown that hyperglycemia and hyperinsulinemia conditions can produce neuroinflammation and increase the oxidative stress, resulting in neuronal damage and cognitive impairment[1,2.]. Anthocyanins and other phenolic compounds poses important antioxidant properties and can prevent Aβ aggregation and prevent the cognitive deficits in AD-like mice[4]. Blackberries are fruits rich in anthocyanin content with important anti-hyperglycemic[5], anti-inflammatory, antiproliferative and chemopreventive activities[6,7]. So far, there are no reports about blackberry fruit effect in brain health in rats under a hypercaloric diet. The objective of this study was to evaluate the neuroprotective effects of blackberry juice in rats fed a hypercaloric diet (HCD). Male Wistar rats (100 ± 20 g) were divided in 2 groups: Control group (LabDiet 5001, n=14) and HCD group (MX/E/2013/047377, n=14), and kept on this diets during 3 months. At this stage, metabolic parameters were already altered in HCD group, accompanied by cognitive deficits. Thereafter, each group as divided into 2 sub-groups: one received blackberry juice (50 mg/kg/day of anthocyanins, adjusted weekly for weight changes), and the other plain water during 21 days (n=7, each). Cognitive assessment was done before sacrifice and brain samples were taken, hippocampus and cortex were dissected for immunohistochemistry and diolistic labelling studies. Our results indicates that HCD causes neuroinflammation in hippocampus, increases oxidative stress in the cortex, and reduces the

number of dendritic spines in the CA1 region of the hippocampus, resulting in memory impairments. The blackberry juice supplementation, however, modified this neuroanatomical alterations and the behavioural performance in HCD rats. Thus, our results suggest that blackberry juice can be a potential therapeutic strategy against metabolic and cognitive deficits induced by high-caloric diets. Referencias: [1] Farr et al., 2008; [2] Treviño et al., 2015; [4] Wang et al., 2015;[5] Stefanut et al., 2013; [6] Hassimotto and Lajolo, 2010, [7] Dai et al., 2007.

Disclosures: **B. Pérez Grijalva:** None. **R. Mora Escobedo:** None. **R. Guzman Geronimo:** None. **A. Diaz:** None. **S. Treviño:** None. **C. Perez Cruz:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.12/G30

Topic: C.01. Brain Wellness and Aging

Support: Indian Council of Medical Research

International Brain Research Organisation

International Society for Neurochemistry

Title: Weakened neurotrophic support and aberrant levels of neurometabolites in the brain underlie reduced lifespan of WNIN/Ob obese rats.

Authors: *J. K. SINHA[1], S. GHOSH[1], V. TIWARI[2], A. B. PATEL[2], M. RAGHUNATH[1]; [1]Natl. Inst. of Nutr. (NIN), Hyderabad, India; [2]Ctr. for Cell. and Mol. Biol., Hyderabad, India

Abstract: Aim: Wistar NIN obese (WNIN/Ob) rats developed at the National Institute of Nutrition are the heaviest inbred rat strain in the world. These rats are hyperphagic, hyperinsulinemic, hyperleptinemic and have reduced longevity (an average lifespan of 15-18 months in contrast to 36 months in normal Wistar rats). The major objective of the current study was to delineate the factors responsible for reduced longevity in the WNIN/Ob rats. **Methods:** Neurotrophic factors are responsible for the survival of developing neurons and the maintenance of mature neurons. The levels of key neurotrophic factors was estimated using BioPlex assay. The astrocytic infiltration was monitored using immunohistochemistry in different brain regions of these rats. As glutamate (Glu) and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the matured mammalian CNS, and are responsible for the majority of energy metabolism in brain. The brain energy metabolism associated with Glu and GABA pathways was measured in different brain regions of WNIN/Ob rats and their age

matched normal rats using ^{13}C Magnetic Resonance Spectroscopy (MRS) in conjunction with infusion of ^{13}C labeled [1-^{13}C]glucose. In addition, volumetric differences in the brain of WNIN/Ob rats was measured using Magnetic Resonance Imaging (MRI). **Results:** Our findings show that the levels of key neurotrophic factors were significantly altered *e.g.* decreased BDNF and increased IGF-1 in the WNIN/Ob rats which could cause degeneration of neural tissue and macromolecular damage in the brain. We have previously reported this in the cerebral cortex and hippocampus of the obese rats. There were no significant volumetric changes in the brain of the WNIN/Ob rats when compared to controls. ^{13}C MRS data indicate hypo-metabolism in the brain of WNIN/Ob rats when compared with age matched controls which shows insufficient supply of energy needs of brain at an early age. Such decreased brain metabolism could contribute to the accelerated ageing observed in the WNIN/Ob rats. **Conclusion:** To conclude we can say altered levels of neurotrophic factors and aberration in neurometabolism in the brain underlie decreased longevity of WNIN/Ob rats.

Disclosures: J.K. Sinha: None. **S. Ghosh:** None. **V. Tiwari:** None. **A.B. Patel:** None. **M. Raghunath:** None.

Poster

306. Brain Energetics in Health and Disease

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Topic: C.01. Brain Wellness and Aging

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Title: Mitochondrial complex-1 assembly into supercomplexes determines mitochondrial reactive oxygen species production in neurons and astrocytes

Authors: *J. P. BOLANOS[1], I. LOPEZ-FABUEL[1], J. LE DOUCE[2], G. BONVENTO[2], A. M. JAMES[3], M. P. MURPHY[3], A. ALMEIDA[4];

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Abstract: Neurons tightly depend on oxidative phosphorylation for energy generation, whereas astrocytes do not, a distinctive feature that is essential for neurotransmission and neuronal survival. However, whether these metabolic differences are dictated by cell-specific structural organization of the mitochondrial respiratory chain is unknown. Here, we aimed to investigate this issue and found that in neurons, mitochondrial complex I is predominantly assembled into supercomplexes, whereas in astrocytes it is mainly present as an individual complex. In consequence of this, astrocytes produce reactive oxygen species (ROS) by complex I several-fold faster than neurons. Using a complexomics approach, we identified complex I subunit, NDUFS1, to be more abundant in neurons than in astrocytes. Interestingly, NDUFS1 knockdown in neurons limited the association of complex I with supercomplexes, leading to impaired mitochondrial oxygen consumption and increased mitochondrial ROS. Conversely, over-expression of NDUFS1 in astrocytes promoted complex I incorporation into supercomplexes leading to decreased ROS. Thus, complex I assembly into supercomplexes regulates ROS production and contributes to the bioenergetic shapes of neurons and astrocytes.

Disclosures: J.P. Bolanos: None. **I. Lopez-Fabuel:** None. **J. Le Douce:** None. **G. Bonvento:** None. **A.M. James:** None. **M.P. Murphy:** None. **A. Almeida:** None.

Poster

306. Brain Energetics in Health and Disease

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Topic: C.01. Brain Wellness and Aging

Support: NSERC Grant 355803

Scottish Rite Charitable Foundation Grant11103

CCNA Grant 137794

Title: Aerobic glycolysis in the frontal cortex correlates with memory performance in wild-type but not APP/PS1 mice: implications for metabolic intervention in Alzheimer's disease

Authors: R. A. HARRIS[1], S. L. MACAULEY[4], D. M. HOLTZMAN[4], R. BARTHA[2], *R. C. CUMMING[3];

[1]Biol., [2]Robarts Res. Inst., [3]Dept. of Biol., Univ. of Western Ontario, London, ON, Canada; [4]Dept. of Neurol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: The majority of glucose consumed by the adult brain is fully oxidized in the mitochondria of neurons to supply the large amounts of ATP required for synaptic transmission. However, a certain percentage of glucose in the brain is exclusively metabolized by glycolysis to generate lactate, even when oxygen is not rate limiting. This form of metabolism is known as aerobic glycolysis. Emerging evidence now suggests that aerobic glycolysis in the brain plays a critical role in generating biosynthetic metabolites during early CNS development and persists in certain regions of the adult brain to support synaptic plasticity, learning and memory. However, aerobic glycolysis steadily declines with age and virtually disappears in the elderly. Our lab has recently demonstrated that a metabolic shift to aerobic glycolysis confers nerve cells with a survival advantage against the toxic effects of amyloid beta, a key pathogenic peptide in Alzheimer's disease (AD). However, the beneficial effect of aerobic glycolysis in the AD brain remains to be fully elucidated.

Here we examined the relationship between cerebral lactate levels and memory performance over a 12 month period in the APP/PS1 mouse model of AD which progressively accumulates amyloid-β (Aβ). *In vivo* ^{1}H-magnetic resonance spectroscopy and western blot analysis of brain tissue revealed a progressive decline in aerobic glycolysis in the frontal cortex of the mouse brain with age. In 12 month old wild type mice we observed that increased expression of lactate producing enzymes in the frontal cortex correlated with better memory performance in the Morris water maze, whereas expression of LDHB, a lactate consuming enzyme, correlated with poorer memory. Interestingly, in 12 month old APP/PS1 mice the opposite effect was observed where increased expression of lactate producing enzymes, and overall lactate levels, correlated with poorer memory performance. In addition, immunofluorescent staining of brain sections revealed expression of the aerobic glycolysis enzymes PDK1 and LDHA primarily in neurons in control mice, as well as in astrocytes surrounding amyloid plaques in APP/PS1 mice. These observations collectively indicate that the production of lactate through aerobic glycolysis is beneficial for memory function in healthy subjects but declines naturally with age. In contrast, elevated lactate levels in APP/PS1 mice indicate perturbed lactate processing; a factor that may contribute to AD pathogenesis. Moreover, these findings suggest that metabolic intervention may offer a new therapeutic strategy for the treatment of AD.

Disclosures: **R.A. Harris:** None. **S.L. Macauley:** None. **D.M. Holtzman:** None. **R. Bartha:** None. **R.C. Cumming:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.15/G33

Topic: C.01. Brain Wellness and Aging

Title: Functional food restores SIRT1 levels and reverses dendritic spine loss in medial prefrontal cortex of obese rats

Authors: *L. PÉREZ JIMÉNEZ[1], T. BEGUM S[1], A. RAMIREZ-MIRAFUENTES[2], M. SANCHÉZ-TAPIA[2], N. TORRES-TORRES[2], C. PEREZ-CRUZ[1];
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Abstract: Mild-life obesity has been associated with alterations in gene expression, decreased metabolic expenditure and subsequently learning and memory deficits. Sirtuin 1 (SIRT1) is a NAD- dependent class III deacetylase that function as cellular energy sensor. Diet-induce obesity (DIO) reduces SIRT1 expression in brain and liver, while SIRT1 activation reduces body weight and increases life-span. Recent investigations shows a permissive role of SIRT1 in memory consolidation and dendritic out-growth [1,2]. One therapeutic strategy against obesity-induced metabolic alterations is the use of functional food (FF). FF enhances the expression of enzymes and proteins related to energetic metabolism, and has shown neuroprotective effects in obese rats [3] . The objective of this study was to evaluate the effects of a combination of FF (5% dehydrated cactus, 20% soybean oil, 3% chia and 1% turmeric) in SIRT1 and spine density in medial prefrontal cortex (mPFC) of obese rats. Male Wistar rats weighing 250 ± 20 g were divided in two groups: Controls (fed control diet AIN-93, n=10) and High-fat-diet group (HFD, plus 5% sucrose in water, n=20). Animals were kept on this diets during 4 months. Once animals in HFD-S reached significant metabolic alterations (i.e. overweight, high plasma levels of triglycerides, cholesterol, LDL and glucose intolerance) cognitive performance was evaluated (T-maze and novel object recognition, NOR). Thereafter, HFD-S rats were divided in two groups: one has been supplemented with FF and the other remained solely as HFD-S during 3 more months. Cognitive assessment was done before sacrifice. Immunofluorescence for SIRT1 and diolistic labelling for spine quantification was performed in the right mPFC. Our results shows that obesity decreased SIRT1 protein in HFD+S compared to AIN (**$p < 0.01$) along with a reduction in mean spine density (*$p<0.05$). However, addition of FF was able to recover SIRT1 levels (HFD-S+PD vs HFD-S, ***$p<0.001$) and modify the morphology of spines by increasing the number of mushroom type-spines (HFD-S+PD vs HFD-S,*$p<0.05$), resulting in cognitive improvements. Thus, brain alterations induced by high-fat-diet affects mPFC and behavior, while FF was able to abate this alterations. The use of FF can be a therapeutic strategy to treat metabolic and cognitive alterations in obese subjects. 1. Codocedo et al.,2012. 2. Gao, J. *et al.*

2010 3. LEONHARDT-AVALOS, L. *et al.* Neuroprotective effect of functional food in an animal model of metabolic syndrome. in *Society of Neuroscience Annual Meeting* 144.15/V27 (2014).

Disclosures: **L. Pérez Jiménez:** None. **T. Begum S:** None. **A. Ramirez-Mirafuentes:** None. **M. Sanchéz-Tapia:** None. **N. Torres-Torres:** None. **C. Perez-Cruz:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

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Program#/Poster#: 306.16/G34

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG34103

Title: Neural effects of high-fat diet are exacerbated by aging and improved by testosterone in male brown Norway rats

Authors: *C. J. PIKE[1], V. MOSER[2], A. CHRISTENSEN[3];
[1]USC Leonard Davis Sch. of Gerontology, [2]Neurosci. Grad. Program, [3]Leonard Davis Sch. of Gerontology, USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is a multifactorial disease for which both genetic and environmental risk factors have been identified. Two such environmental risk factors are midlife obesity and age-related testosterone loss in men. Because these two factors often coexist in middle aged men, obesity and loss of testosterone may coincide to cooperatively promote AD pathogenesis. Consistent with this possibility, our recent work in young adult male rodents has shown an interaction between these risk factors, demonstrating that animals with both low testosterone and diet-induced obesity exhibit exacerbated AD-related outcomes. Moreover, we and others have shown that testosterone protects against both obesity and cognitive decline. Unclear is how obesity and testosterone interact across the lifespan. The goal of the current study was to compare interactions between these two factors in young adult, middle aged, and aged brown Norway rats, which display age-related testosterone loss. Male rats were maintained on normal (10% fat) or high fat (60% fat) diet for a 3-month period beginning at age 3, 13, or 23 months. At the onset of diet manipulations and middle-aged (13 mo) and aged (23 mo) rats were implanted subcutaneously with silastic capsules containing testosterone or vehicle. Endpoints included metabolic indices, learning and memory performance, inflammation, and neural health outcomes. Results show that high fat diet was associated with increases in body weight and leptin levels across all ages, and impaired glucose metabolism in young and middle aged rats.

Learning and memory were evaluated using the Barnes Maze task. Though aged rats were slower to learn the task, all groups demonstrated learning by the end of training. High fat diet had no significant effect on memory in young rats, but increased memory errors in middle-aged and aged animals. Testosterone protected against the effects of diet on memory. Furthermore, we found effects of age and testosterone on neuroinflammation, such that testosterone protected against age-associated increases in inflammatory markers. Overall, results show interactive effects of testosterone and high fat diet on metabolic, behavioral, and neural health outcomes that vary across age. Understanding the interactions between obesity, testosterone, and aging will provide significant insight into the both the development and prevention of age-related cognitive decline and AD.

Disclosures: C.J. Pike: None. **V. Moser:** None. **A. Christensen:** None.

Poster

306. Brain Energetics in Health and Disease

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Topic: C.01. Brain Wellness and Aging

Support: NIGMS 1SC3GM086323

CTSC UL-RR024996

G12RR003037

Title: The relationship between metabolic impairment and proteasome activity the nervous system of *Drosophila*

Authors: *T. SCHMIDT-GLENEWINKEL, M. JANSEN, S. BENNETT, A. KLEIN, A. RASHID, J. NETHERCOTT;
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Abstract: Mitochondrial dysfunction and proteasome impairment are associated with many distinct neurodegenerative disorders. To better understand the relationship between metabolic impairment and proteasome function we use the the binary inducible GeneSwitch system to permanently disrupt the activity of ATP synthase in fruit flies. We show that 5uM of the steroid inducer RU486 is sufficient to significantly decrease the lifespan of both male and female flies. Furthermore we observe a 10-15% decline in ATP levels after flies are fed 5uM RU486 for 13 days or longer. This decline in ATP is accompanied by a significant reduction in 26S proteasome activity. Although the proteasome is functionally dependent on ATP, it is unclear how a 10-15%

decline in ATP levels can dramatically alter its function. In our studies we find that lactic acid levels are increased 2-4 fold when flies are fed 5uM RU486 for 13 days. The elevated levels of lactate observed in our study suggests that glycolysis may be elevated to compensate for a decline in mitochondrial efficiency. Glycolysis produces methylglyoxal (MG) as a toxic byproduct. MG is a powerful glycating agent that can modify proteins, lipids, and nucleic acids leading to the formation of advanced glycation end products (AGE). AGE has been implicated in the pathology of diabetes mellitus, aging, and neurodegeneration. Furthermore MG has been shown to covalently modify the catalytic pros beta5 subunit of the proteasome suggesting that it may directly inhibit the proteasome. We hypothesize that MG levels will be elevated in Act5C-GeneSwitch/UAS-ATP5B RNAi flies when they are exposed to 5uM of the steroid inducer RU486. Elevated levels of MG may indicate elevated levels of oxidative stress, which would increase the abundance of oxidatively damaged proteins, and promote a decline in proteasome function, and subsequently increases in protein aggregates.

Disclosures: **T. Schmidt-Glenewinkel:** None. **M. Jansen:** None. **S. Bennett:** None. **A. Klein:** None. **A. Rashid:** None. **J. Nethercott:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.18/G36

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Grants-in-Aid for Science Research (A) 26250003

JSPS Research Fellowships for Young Scientists (DC1) 12435

Title: A vascular niche for highly active neurons

Authors: ***T. MIYAWAKI**[1], S. YAMAGUCHI[2], Y. IKEGAYA[1];
[1]The Univ. of Tokyo, Hongo, Japan; [2]Gifu Univ., Gifu, Japan

Abstract: Energy constrains a system's processing capacity (Laughlin, 2001). To maximize its yield of the available energy resource, the brain has developed an elaborated supply chain, the cerebral vascular network. Just like "the Smart Grid", the cerebral vascular network can tightly match the demand with the supply, by locally increasing the amount of blood flow where the amount of synaptic activity has increased (Logothetis et al., 2001).
Despite numerous studies on the coupling to the change in metabolic demand, little work has investigated the coupling to the basal heterogeneity in metabolic demand. A recent study

reported that firing rates of individual neuron showed a preconfigured and skewed distribution, suggesting that the metabolic demand is constituently heterogeneous, even within a single brain region (Mizuseki and Buzsáki, 2014).
To examine whether there would be a mechanism to couple this basal heterogeneity, we investigated the spatial relationship between highly active neurons and blood vessels. We labeled a fraction of highly active neurons using Arc-dVenus transgenic mice (Eguchi and Yamaguchi, 2009), and measured the distances to the blood vessels in 3D using the CUBIC protocol (Susaki et al., 2014). We have found that, in the neocortex, the highly active neurons resided significantly nearer the blood vessel compared to the surrogate.
Given that oxygen and other nutrients show higher concentrations in the vicinity of blood vessels, our result implies a previously unexplored coupling mechanism of the metabolic supply to the basal heterogeneity of the metabolic demand.

Disclosures: **T. Miyawaki:** None. **S. Yamaguchi:** None. **Y. Ikegaya:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.19/G37

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant (U01 MH105941)

ADA Grant (1-15-JF-52)

Title: Effects of recurrent hypoglycemia on growth hormone releasing hormone (GHRH) neurons

Authors: ***M. BAYNE**, A. ALVARSSON, S. A. STANLEY;
Diabetes, Obesity and Metabolism Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Maintenance of normal blood glucose levels is an intricate process coordinated between the central nervous system (CNS) and peripheral organs. In disease states like diabetes, recurrent hypoglycemia is a common complication that can cause cognitive and metabolic defects. While the brain monitors and adjusts blood glucose levels constantly, the neuronal adaptations to repeated hypoglycemic episodes are poorly understood. There are several counter-regulatory responses to hypoglycemia including increased growth hormone. Those neurons expressing growth hormone releasing hormone are highly responsive to low glucose. Therefore, this hypothalamic population of neurons may serve as a model to study how glucose-responsive

neurons respond and adapt to repeated hypoglycemia. We first show that plasma growth hormone levels are modulated by acute and repeated hypoglycemia in mice. Immunohistochemical examination was used to assess morphological changes in response to hypoglycemia and quantification of the neuronal activity markers, c-fos and phosphoS6, was used to evaluate changes in GHRH neural activation after single or repeated episodes of hypoglycemia. We measured changes in gene expression in GHRH-expressing neurons subjected to single or recurrent hypoglycemia. These experiments present possible adaptations to recurrent hypoglycemic episodes.

Disclosures: M. Bayne: None. **A. Alvarsson:** None. **S.A. Stanley:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.20/G38

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Natural Sciences and Engineering Council of Canada

Title: Dynamics of lactate and glucose in the extracellular compartment of the motor cortex during running: Impact of intraperitoneal glucose, fructose, and insulin

Authors: *C. MESSIER[1], A. BELAND[2], P. SHUKLA[2], J. LARCHER[2];
[1]Sch. of Psychology, [2]Psychology, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: As most textbooks state, glucose is the main fuel of the brain. The Neuron-to-Astrocyte-Lactate Shuttle (NALS) suggests that the lactate is a by-product product of neuronal glucose. Conversely, a competing hypothesis by Pellerin and Magistretti, the Astrocyte-to-Neuron-Lactate Shuttle (ANLS) hypothesis proposes that peripheral glucose is taken up by astrocytic end feet, converted to lactate and released in the extracellular space and used by neurons as a source of metabolic fuel. Within this context, we examine the changes in lactate and glucose in the extracellular fluid of the motor cortex during running in a running wheel following intraperitoneal (ip) injections of saline or 2g/kg of glucose, fructose, or 1.2 IU / kg insulin. Electrochemical electrodes (Pinnacle Tech.) that detect glucose or lactate were inserted in the left or right motor cortex of CD-1 male mice so that glucose and lactate could be measured simultaneously. Glucose and lactate levels were measured before and after the initiation of running and results were expressed as a percentage of pre-running values. First, we determined that small minute movements produced a sharp decrease in motor cortex extracellular lactate and to a lesser extend glucose as been shown previously with discrete brain stimulation. This result

suggests that both lactate and glucose are transferred to brain cells following neuronal activation. Sustained running in a running wheel was associated with a gradual increase in lactate (up to 140%) and a smaller (-10%) but sustained decrease in glucose. These were specific to the motor cortex limb area because sustained running did not produce any changes in the extracellular glucose and lactate in the visual cortex. In many instances, the slope of lactate increase after the onset of running appeared to predict the length of a running bout - the sharper lactate rises were associated with the longest bouts. A strong and positive correlation ($r=0.53, p < .001$) was found for lactate but not for glucose ($r=0.08, p =.69$) between individual rate of increase and their associated running length. When mice were running after receiving an injection of glucose or fructose, the rise in extracellular lactate was significantly reduced while the extracellular glucose decrease was similar to that without injection. While insulin generally reduced extracellular glucose and lactate, running still produced a small increase in extracellular lactate but no change in extracellular glucose. These results show that complex interactions influence the extracellular levels of glucose and lactate that depends on neuronal activity and blood levels of nutrients.

Disclosures: **C. Messier:** None. **A. Beland:** None. **P. Shukla:** None. **J. Larcher:** None.

Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.21/G39

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: SNSF 310030B_148169

Title: PTG is a central regulator of glycogen synthesis in astrocytes

Authors: *I. ALLAMAN[1], E. RUCHTI[1,2], P. J. ROACH[3], A. A. DEPAOLI-ROACH[3], P. J. MAGISTRETTI[4,1,2];
[1]EPFL/Brain Mind Inst., Lausanne, Switzerland; [2]Dept. de Psychiatrie, Ctr. de Neurosciences Psychiatriques (CHUV), Prilly/Lausanne, Switzerland; [3]Dept. of Biochem. and Mol. Biol., Indiana Univ. Sch. of Med., Indianapolis, IN; [4]Div. of Biol. and Envrn. Sci. and Engin., King Abdullah Univ. of Sci. and Technol. (KAUST), Thuwal, Saudi Arabia

Abstract: The storage and use of glycogen, which is the main energy reserve in the brain, is a metabolic feature of astrocytes. Protein targeting to glycogen (PTG) is a member of specific glycogen-binding subunits of protein phosphatase-1 (PP-1) which positively regulates glycogen synthesis through de-phosphorylation of both glycogen synthase (activation) and glycogen phosphorylase (inactivation). To gain further insight into the role of PTG in the regulation of

astrocytic glycogen, its levels of expression were manipulated in primary cultures of mouse cortical astrocytes using adenovirus-mediated overexpression of tagged-PTG or siRNA to downregulate its expression. Infection of astrocytes with adenovirus led to a strong up-regulation of PTG expression and was associated with massive glycogen accumulation (more than 100 fold), demonstrating that increased PTG expression is sufficient to induce glycogen synthesis and accumulation. In contrast, siRNA-mediated downregulation of PTG resulted in a 1.8 fold decrease of glycogen basal values. Interestingly, PTG downregulation was also shown to significantly impair long-term astrocytic glycogen synthesis induced by insulin or noradrenaline, while mobilization of residual glycogen by short term treatment of noradrenaline was not affected. Finally, these effects of PTG down-regulation on glycogen metabolism could also be observed in cultured astrocytes isolated from PTG-KO mice. Collectively, these observations point to a major role of PTG in the regulation of glycogen synthesis in astrocytes and indicate that conditions leading to changes of PTG expression will directly impact glycogen levels in this cell type.

Disclosures: I. Allaman: None. **E. Ruchti:** None. **P.J. Roach:** None. **A.A. DePaoli-Roach:** None. **P.J. Magistretti:** None.

Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.22/G40

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: HHMI

JPB Foundation

Title: Dorsal raphe nucleus: A locus for energy homeostasis

Authors: *B. FIELD, A. NECTOW, Y. LIANG, J. FRIEDMAN;
Rockefeller Univ., New York, NY

Abstract: The balance of food intake and energy expenditure, both from exercise and the baseline metabolic rate, determine an animal's weight gain, loss, or maintenance. In the mammalian brain, the anorexigenic hormone leptin principally works on hypothalamic neural subpopulations to control appetite. Here we investigate the role of the dorsal raphe (DRN), an extrahypothalamic structure, in behaviors modulating energy balance in wildtype and leptin-deficient mice (ob/ob). By manipulating its glutamatergic and GABAergic populations, we find

that this brainstem region is capable of exerting control over feeding-related behaviors. Via whole-brain projection mapping, we have shown that these neural populations are anatomically and molecularly distinct and have direct interaction with the hypothalamus. These data show that the dorsal raphe nucleus is a functionally significant brain region in energy homeostasis.

Disclosures: B. Field: None. **A. Nectow:** None. **Y. Liang:** None. **J. Friedman:** None.

Poster

306. Brain Energetics in Health and Disease

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Program#/Poster#: 306.23/G41

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: UC Leaf Award National Science Foundation

Title: Estradiol in the medial amygdala prevents ovariectomized induced metabolic responses in female rats

Authors: *C. ESTRADA[1], V. GHISAYS[2], E. T. NGUYEN[3], J. CALDWELL[3], J. STREICHER[3], M. B. SOLOMON[3];
[2]Psychology, [3]Psychiatry and Behavioral Neurosci., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: The posterodorsal medial amygdala (MeA) modulates energy homeostasis in rodents. For example, MeA lesions increase body weight, adiposity and circulating insulin concentrations. Notably, this metabolic phenotype is exaggerated in females relative to males. In female rodents, bilateral removal of the ovaries (ovariectomy) induces a metabolic phenotype characterized by increased body weight and visceral adiposity, elevated glucose and insulin concentrations, and decreased activity. Of note, systemic (17-β-Estradiol-(E2)) administration prevents and/or reverses many of these metabolic endpoints in ovariectomized females. The metabolically beneficial effects of systemic E2 are in part due to binding via estrogen receptors in brain regions regulating energy homeostasis. Given the prominent role of the posterodorsal medial amygdala in energy homeostasis and the abundance of estrogen receptors within this region, we tested the hypothesis that E2 administration in the MeA prevents ovariectomy-induced metabolic sequela. To test this hypothesis, adult ovariectomized female rats were implanted with bilateral E2 or cholesterol micropellets aimed at the posterodorsal MeA. In Experiment 1, females with confirmed MeA-E2 hits lost body weight relative to their cholesterol treated counterparts. When food intake was normalized to body weight there was no difference between females treated with E2 in the MeA and cholesterol treated controls suggesting that the E2-mediated effects on body weight were due to increased energy expenditure. Notably, E2

administration in the MeA decreased visceral adiposity, but had no effect on subcutaneous fat. This finding indicates a adipose depot specific effect of E2 treatment. In Experiment 2 we tested the hypothesis that E2 administration in the MeA prevents glucose intolerance in ovariectomized females. Consistent with our hypothesis, females treated with E2 in the MeA had decreased glucose concentrations in response to an oral glucose challenge. Studies are currently underway to determine the mechanisms mediating improved glucose tolerance in ovariectomized females treated with the E2 in the MeA. In addition we plan to delve further into the role of ERα signaling within the MeA on energy homeostasis in different rodent models of obesity. Taken together, the findings highlight a prominent role of the MeA as a critical node in regulating metabolic responses in ovariectomized females.

Disclosures: **C. Estrada:** None. **V. Ghisays:** None. **E.T. Nguyen:** None. **J. Caldwell:** None. **J. Streicher:** None. **M.B. Solomon:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.24/G42

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Genetically dissecting a hypothalamic neural circuit controlling glucose homeostasis

Authors: *C. S. LOW, X. YI, C. BARTOLOME, J. XU, P. WANG, D. KONG;
Neurosci., Tufts Univ., Boston, MA

Abstract: Growing evidence has recently shown that neurons in the hypothalamus play important roles in regulating glucose homeostasis, and their dysfunctions contribute to the pathogenesis of diabetes. However, the complexity of the neural system and difficulties in probing neuronal function *in vivo* has left us with an incomplete understanding of both the molecular mechanism and neuro-circuitry of neuron-mediated blood glucose regulation. Fortunately, we have recently perturbed a major group of glucose-sensing neurons, arcuate hypothalamic Pro-opiomelancortin (POMC) neurons. We have validated that these neurons play a significant physiological role in regulating whole body glucose homeostasis. Improving their glucose-sensing capacity has been shown to dramatically attenuate the hyperglycemia in mouse models of both Type I and Type II diabetes. Thus, our recent work has focused on POMC neurons and elucidating their control of whole body glucose homeostasis by combining a battery of genetic approaches. Briefly, we first used a pharmacogenetic tool to acutely manipulate the firing of POMC neurons and assessed the causal effects in blood glucose control. By combining the cutting-edge genomic editing method, clustered regularly interspaced short palindromic

repeats (CRISPR), and viral tools, we went further to identify the glucose-lowering neurotransmitter released from POMC neurons. The pathological contributions to induced Type I and Type II diabetes has also been explored. Our studies demonstrate that POMC neurons in the arcuate nucleus play an essential role in whole body glucose regulation. The elucidation of the molecular mechanisms and factors in these neurons will also assist in the identification of novel therapeutic targets for treating diabetes.

Disclosures: C.S. Low: None. **X. Yi:** None. **C. Bartolome:** None. **J. Xu:** None. **P. Wang:** None. **D. Kong:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.25/G43

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Physiological characterization of the hypoxia tolerant heart and brain of the naked mole rat

Authors: *D. T. APPLEATE[1], J. LARSON[2], T. PARK[2];
[1]Neurosci., Univ. of Illinois at Chicago Dept. of Neurosurg., Chicago, IL; [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Hypoxic brain damage, as occurs in stroke or after heart attacks, can lead to permanent disability and/or death. Ischemic strokes or other interruptions to cerebral oxygen supply lead to neuronal cell death that begins within a matter of minutes. The unpredictability of cerebral ischemic events and their rapidly-developing consequences make prevention and treatment of stroke-induced brain damage particularly difficult. Study of model animals that are naturally resistant to hypoxia may provide clues to the treatment of hypoxic brain injury. One organism exhibiting remarkable hypoxia tolerance is the naked mole-rat, which survives underground in a chronically oxygen-depleted environment. Previous studies have shown that hippocampal neurons in naked mole-rat brain are extremely tolerant to hypoxia in vitro. While the physiological mechanisms underlying brain hypoxia tolerance are poorly understood, insights can be gained by monitoring whole body responses to environmental oxygen depletion. To characterize the hypoxia tolerance of naked mole-rats, we asked the following questions: (1) What are the effects of hypoxia on cardiac activity? (2) How does brain activity respond to low levels of oxygen? To measure the effects of hypoxia on physiological activity of the naked mole rat, electrocardiogram and electroencephalography were used. We observed that upon exposure to hypoxia, the naked mole rat experienced a significant drop in heart rate and a suppression of brain electroencephalographic activity. These states were maintained for many minutes in a low

oxygen environment and recovered to baseline levels upon replacement of the ambient oxygen. These findings characterize the physiological response of naked mole rats to oxygen depletion and provide preliminary insights into the mechanisms underlying neuroprotection of the hypoxia tolerant brain.

Disclosures: D.T. Appleate: None. **J. Larson:** None. **T. Park:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.26/G44

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Natural Sciences and Engineering Council of Canada

Title: Alternate fuels for the brain: impact of intraperitoneal glucose, fructose, galactose, lactate, pyruvate, beta-hydroxybutyrate and insulin on glucose and lactate levels in the brains' antechamber.

Authors: *A. BELAND[1], J. COURTEMANCHE[2], J. LARCHER[2], T. YUAN[2], C. MESSIER[2]; [1]Univ. of Ottawa, Dunvegan, ON, Canada; [2]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: As most textbooks state, glucose is the main fuel of the brain. The Neuron-to-Astrocyte-Lactate Shuttle (NALS) suggests that the lactate is a by-product product of neuronal glucose. Conversely, a competing hypothesis by Pellerin and Magistretti, the Astrocyte-to-Neuron-Lactate Shuttle (ANLS) hypothesis proposes that peripheral glucose is taken up by astrocytic end feet, converted to lactate and released in the extracellular space and used by neurons as a source of metabolic fuel. Within this context, we examine the changes in lactate and glucose in the extracellular fluid of the motor cortex following intraperitoneal (ip) injections of glucose, fructose, galactose, lactate, pyruvate, beta-hydroxybutyrate and insulin. Electrochemical electrodes (Pinnacle Tech.) that detect glucose or lactate were inserted in the left or right motor cortex of CD-1 male mice so that glucose and lactate could be measured simultaneously. In separate experiments, we also measured glucose and lactate levels in tail blood using portable glucose and lactate meters. Glucose and lactate levels were measured before and after intraperitoneal injections and results were expressed as a percentage of pre-injection values. Intraperitoneal 2 g/kg glucose, fructose, lactate, pyruvate, beta-hydroxybutyrate and to a much lesser extent, galactose all raised cortical extracellular glucose levels (200%) while intraperitoneal insulin reduced cortex extracellular glucose and lactate equally. As expected, ip glucose raised and ip insulin reduced blood glucose. All ip injections raised blood lactate levels.

In contrast, extracellular cortical lactate levels remained unchanged except for a transitory raise following ip lactate and a 40% decrease following ip insulin. The present methodology does not allow determining the origin of the extracellular changes in glucose and lactating. However, the data indicates that blood increases of many potential metabolic substrates lead to increases in brain extracellular glucose but only the ip injection of lactate produced a significant but transient brain extracellular lactate increase. The observation that the ip injection of all substrates increased blood lactate raise the possibility that the extracellular glucose increase in the brain is due to brain metabolism of circulating lactate. However, the ip injection of 2g/kg of lactate does not lead to greater brain extracellular glucose. Interestingly, the extracellular glucose increase following the ip injection of glucose or lactate is almost immediate whereas the brain extracellular glucose increase is delayed following the ip injection of the other substrates.

Disclosures: **A. Beland:** None. **J. Courtemanche:** None. **J. Larcher:** None. **T. Yuan:** None. **C. Messier:** A. Employment/Salary (full or part-time): University of Ottawa.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.27/G45

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

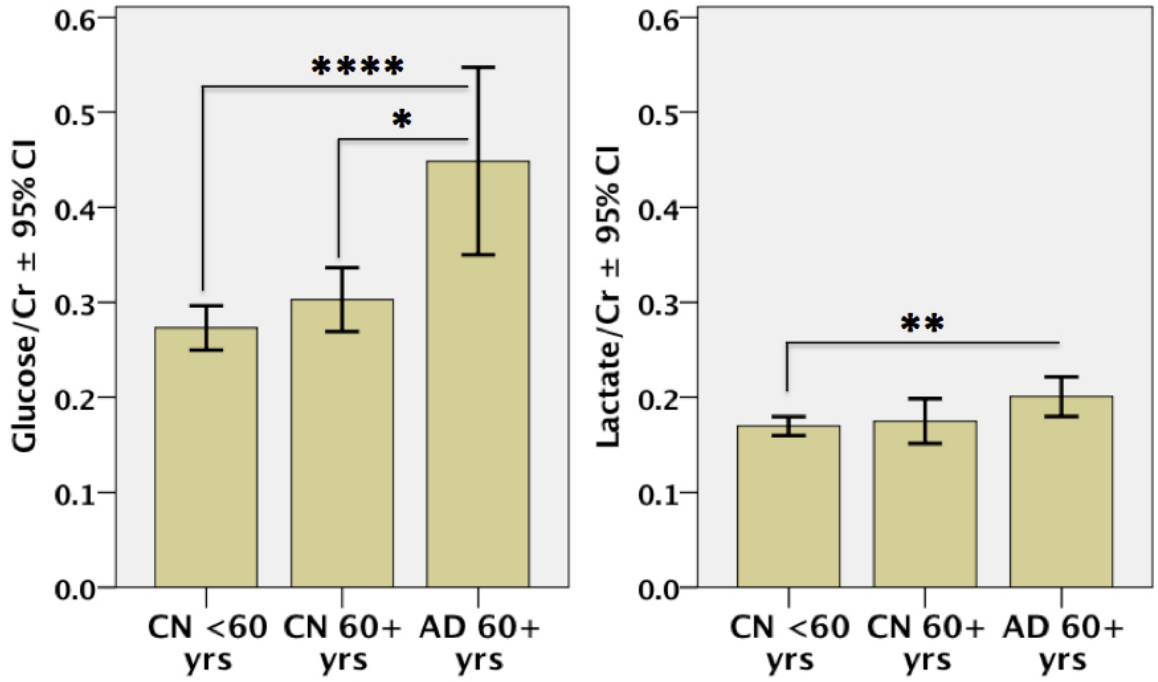
Support: Intramural Research Program of the NIH, National institute on Aging

Title: Low glucose utilization and high lactate production in the Alzheimer's disease brain

Authors: *R. J. MULLINS[1], D. REITER[2], D. KAPOGIANNIS[2];
[1]Lab. of Neurosci., Natl. Inst. On Aging, Baltimore, MD; [2]Natl. Inst. on Aging, Baltimore, MD

Abstract: Two defining neuropathological characteristics of Alzheimer’s disease (AD) are the presence of Amyloid-beta (Aβ) plaques and abnormalities in glucose metabolism. Brain regions with prominent Aβ deposition also have a higher metabolic reliance on glycolysis. The posterior cingulate/precuneus region shows early Aβ deposition and lower glucose utilization in AD. We hypothesized that AD patients show higher concentrations of glucose (due to decreased utilization) and lactate (due to greater production) in the posterior cingulate/precuneus. We studied 44 cognitively normal (CN) younger controls (42 ± 10 years old), 14 CN older controls (69 ± 8 years old), and 22 patients with high probability AD (based on low CSF Aβ, high CSF tau and/or p-tau) and a clinical dementia rating (CDR) of 0.5 or 1 (74 ± 8 years old). In exploratory models, patients with CDR 0.5 or 1 had no differences and were therefore combined into one group. Glucose and lactate levels were obtained using localized 2D J-resolved

spectroscopy (jPRESS) within a 25x18x20 mm posterior cingulate/precuneus voxel. Spectroscopy data were analyzed using ProFit to quantify metabolite concentrations normalized to creatine. We found that AD patients had higher glucose concentrations than both younger ($p < 0.0001$) and older ($p = 0.024$) CN participants. AD patients also had higher lactate concentrations than younger CN participants ($p = 0.008$). These findings suggest that metabolic abnormalities in AD result in higher glycolytic activity generating lactate while a greater portion of available glucose remains unused. We propose that glucose and lactate levels in posterior cingulate/precuneus can serve as novel biomarkers of abnormal brain metabolism in AD. Recruitment of older (60+) CN participants is ongoing. This research was supported entirely by the Intramural Research Program of the NIH, National institute on Aging.



*Tukey's post-hoc, * = p < 0.05, ** = p < 0.01, **** = p < 0.0001*

Disclosures: **R.J. Mullins:** None. **D. Reiter:** None. **D. Kapogiannis:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.28/G46

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: DFG DE 231/24-2

Title: Imaging cytosolic metabolites and pH in mouse astrocytes

Authors: *J. W. DEITMER[1], J. SCHMÄLZLE[1], T. WEBER[1], I. RUMINOT[2], S. M. THEPARAMBIL[1];
[1]Univ. Kaiserslautern, D-67653, Germany; [2]CECs, Valdivia, Chile

Abstract: The high energy requirement of the mammalian brain is primarily fueled by the degradation of blood-derived glucose. Astrocytes, the major glial cells in the brain, take-up the larger portion of blood glucose, and by aerobic glycolysis they preferably produce more lactate. There is strong evidence that glucose consumption and glycolysis is highly dependent on cytosolic pH, but that it can also be stimulated by an increase in the intracellular [HCO_3^-]. Since pH and HCO_3^- shift concomitantly at constant CO_2 level, and both can be potential signals for modulating glucose metabolism, the question arises, whether the enhanced glucose consumption and glycolytic rate are mediated by proton or bicarbonate. We have asked, whether pH and the [HCO_3^-] must both rise in mouse cortical astrocytes, or if it suffices when either pH *or* HCO_3^- increases, to enhance glucose metabolism in mammalian astrocytes. We have recorded intracellular pH, using the pH-sensitive dye BCECF, and cytosolic glucose and lactate, using a FRET-based nanosensors, while imposing different intracellular pH and [CO_2]/[HCO_3^-], in cultured cortical astrocytes and in astrocytes of organotypic hippocampal brain slices. Glucose consumption and glycolytic rate were augmented by an increase in cytosolic pH, irrespective of a concomitant rise or fall in intracellular HCO_3^-. The transport of HCO_3^- into and out of astrocytes by the electrogenic sodium-bicarbonate cotransporter (NBCe1) played a crucial role for the changes in intracellular pH and [HCO_3^-], but was not obligatory for the pH-dependent changes in glucose metabolism. Our results suggest that the glycolytic rate in astrocytes is primarily augmented by the fall in $[H^+]_i$ not by a rise in $[HCO_3^-]_i$. *Supported by the Deutsche Forschungsgemeinschaft, DE 231/24-2.*

Disclosures: J.W. Deitmer: None. **J. Schmälzle:** None. **T. Weber:** None. **I. Ruminot:** None. **S.M. Theparambil:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 306.29/G47

Topic: C.01. Brain Wellness and Aging

Support: Swedish research council

Petrus and Agustas Hedlunds foundation

Title: Cell specific expression of alpha amylase 1 and 2A in hippocampus of non demented elders and patients with Alzheimers's disease

Authors: *E. K. BYMAN[1], N. H. SCHULZ[1], N. BRAIN BANK[2], M. WENNSTRÖM[1];
[1]Lund Univ., Malmö, Sweden; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Amylose can be found in at least in two different forms in the brain. Either as intracellular inclusions in astrocytes, called corpora amylacea or as a high molecular weight un-branched glucose polymer, which has been associated with Alzheimer's disease. Branched glucose polymers, such as glycogen, are usually degraded by glycogen phosphorylase and glycogen de-branching enzyme in the brain. However, these enzymes are unable to cleave amylose effectively. The un-branched glucose polymer is instead known to be degraded by alpha-amylases, which cleaves 1,4 glucosidic bonds in glucose polymers. The specific cellular expression of alpha-amylase in the brain is not yet determined. We will therefore in the current study investigate the presence of alpha-amylases in different brain cells in elder individuals and patients diagnosed with Alzheimer's disease.
Postmortem brain sections of hippocampus from patients with AD and non-demented controls were immunohistochemically stained against salivary alpha-amylase (AMY1a) and pancreatic alpha-amylase (AMY2a). Immunoreactivity of AMY1a was foremost found in pericytes and neuronal dendrites, whereas AMY2a immunoreactivity was found in neuronal cellbodies as well as activated astrocytes. We further found increased numbers of AMY1a positive inclusion resembling Hirano bodies in the hippocampal subarea CA1 of individuals with AD related pathology. Some of these inclusions co-localized with p-tau positive tangles and amyloid beta (Aβ)1-42 plaques. Some of the Aβ1-42 plaques in the CA1 of AD patients were also surrounded by AMY2a positive astrocytes. In conclusion our study demonstrates a cell specific expression of AMY1a and AMY2a and an association between these two enzymes and AD related pathological changes. We hypotheses that brain cells express alpha-amylases to degrade accumulated amylose but potentially also generate energy trough an alternative cleavage pathway of glucose polymers.

Disclosures: **E.K. Byman:** None. **N.H. Schulz:** None. **N. Brain Bank:** None. **M. Wennström:** None.

Poster

306. Brain Energetics in Health and Disease

Location: Halls B-H

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Program#/Poster#: 306.30/G48

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01DK092587

P20GM103629 (HM)

P30DK072476 (SY, HM)

Title: Role of DMH/DHA LepRb-expressing neurons in temperature-dependent neuronal circuits.

Authors: *M. FRANCOIS, S. YU, E. QUALLS-CREEKMORE, C. HUESING, C. MORRISON, H.-R. BERTHOUD, H. MUNZBERG;
Pennington Biomed. Res. Ctr., Baton Rouge, LA

Abstract: Background: Leptin receptor (LepRb) expressing neurons in the dorsomedial hypothalamus/dorsal hypothalamic area (DMH/DHA) are key actors in energy expenditure regulation. In fact, we previously showed that their chemogenetic activation increased energy expenditure and decreased body weight via both β_3-adrenoreceptor-dependent BAT thermogenesis and locomotor activity. The DMH/DHA has been proposed as downstream target of the preoptic area (POA), and both project to BAT sympathetic premotor neurons in the rostral raphe pallidus (rRPa). Even though we verified anatomically that POA-LepRb neurons project to the DMH/DHA, functional data strongly suggested that POA- and DMH/DHA-LepRb-expressing neurons act independently to modulate energy expenditure. Here, we tested if activation of DMH/DHA-LepRb neurons mediates a similar temperature-dependent change in energy expenditure as observed for POA-LepRb neurons to further indicate possible functional associations of POA and DMH/DHA-LepRb-expressing neurons. Methods: We injected a cre-dependent adeno-associated virus expressing hM3Gq-mCherry in the DMH/DHA of LepRb-cre mice, making LepRb-expressing neurons excitable by peripheral injection of Clozapine-N oxide (CNO). Oxygen consumption and locomotor activity were measured in metabolic cages with vehicle or CNO injections (1.5mg/kg ip) at different temperatures (23^{o}C, 4^{o}C and 30^{o}C). Results: Chemogenetic stimulation of DMH/DHA LepRb-expressing neurons robustly increased the oxygen consumption and locomotor activity similarly at all tested ambient temperatures. Thus, in contrast to chemogenetic activation POA-LepRb neurons, DMH/DHA-LepRb neurons regulate energy expenditure independent of ambient temperature. Conclusion: Consistent with our earlier results, these data further suggest that DMH/DHA and POA LepRb-expressing neurons act independent on thermoregulatory pathways.

Disclosures: **M. Francois:** None. **S. Yu:** None. **E. Qualls-Creekmore:** None. **C. Huesing:** None. **C. Morrison:** None. **H. Berthoud:** None. **H. Munzberg:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.01/G49

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

Support: National Institute on Aging UO1 AG047222

Kenneth T and Eileen L. Norris Foundation

Title: Allopregnanolone promotes neural stem cell differentiation to neurons and oligodendrocyte precursor cells

Authors: ***S. CHEN**[1], J. YAO[1], K. WONG[1], R. D. BRINTON[1,2];
[1]Sch. of Pharm., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci. and Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Previous studies by our group demonstrate that the endogenous neurogenic steroid Allopregnanolone promotes proliferation of both human and rodent neural stem cells *in vitro,* as well as *in vivo* in the triple transgenic mouse model of Alzheimer's disease. In this study, we investigated the impact of Allopregnanolone on neuronal differentiation. We first assessed the effect of aging and Alzheimer's-associated genotype on the differentiation capacity of adult neural stem cells in the triple transgenic mouse model. Neural stem cells from 3-, 6- and 15-month triple transgenic and non-transgenic mice were cultured and differentiation capacity was determined. We demonstrate that there is an age- and Alzheimer's gene- dependent decrease in overall neural stem cell differentiation and a shift from neuronal to glial differentiation. Allopregnanolone treatment significantly increased the ratio of MAP2-positive-neurons to GFAP-positive-astrocytes from embryonic rat and adult mouse neural stem cells. To further determine the *in vivo* efficacy of Allopregnanolone to prevent or reverse the loss of neuronal differentiative capacity, we investigated the impact of Allopregnanolone on neuronal differentiation in 5-month-old male triple transgenic mice. Flow cytometry-based analysis of new generated cells indicated that Allopregnanolone treatment increased the number of newly generated neurons as indicated by the increase in the number of BrdU positive cells and BrdU/NeuN double positive cells. Allopregnanolone enhanced expression of the neural proliferation marker, proliferation cell nuclear antigen, and the immature neuronal marker doublecortin, which was further confirmed by immunohistochemistry labeling.

Allopregnanolone treatment also increased the expression of Olig2, an oligodendrocyte precursor cell marker. Immunohistochemistry analyses showed that more Olig2 positive cells were distributed in corpus callosum area in the Allo-treated brain. The increase in neural differentiation and oligodendrocyte differentiation by Allopregnanolone treatment was paralleled by an increase in Brain-Derived Neurotrophic Factor. Collectively these findings suggest that Allopregnanolone is a regenerative therapeutic candidate to prevent or delay neurogenic deficits associated with mild cognitive impairment and Alzheimer's disease.

Disclosures: **S. Chen:** None. **J. Yao:** None. **K. Wong:** None. **R.D. Brinton:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.02/G50

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

Support: NIA U01 AG031115; NIA U01 AG047222; and UF1 AG046148 to RDB.

Title: Allopregnanolone activates LXR and PXR gene expression and signaling pathways regulating neuroinflammation, apoptosis, Aβ trafficking, and cholesterol clearance: Implications for Alzheimer's disease

Authors: *R. W. IRWIN[1], H. M. SWANSON[1], R. D. BRINTON[2,3,1];
[1]Pharmacol. and Pharmaceut. Sci., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tuscon, CA; [3]Pharmacol., Univ. of Arizona, Tuscon, AZ

Abstract: Allopregnanolone (Allo) is in early stage clinical trials as the first regenerative therapeutic for Alzheimer's disease (NCT02221622). Altered cholesterol metabolism is linked to AD pathology. Allo increases expression of two nuclear receptors, liver-X-receptor (LXR) and pregnane-X-receptor (PXR). LXR, a sensor of cholesterol levels, initiates its clearance to prevent overactivation of gamma-secretase and overproduction of amyloid-beta (Aβ). PXR activation induces CYP3A enzymes that lead to cholesterol hydroxylation and extrusion thereby decreasing Aβ and neuroinflammatory burden. Binding properties of Allo to LXR and to PXR were determined subsequent to studies of *in vivo* regulation of genes and proteins implicated in Alzheimer's pathology. Crystal structures from the Protein Data Bank for PXR/RXRα, LXRβ, and LXRα/RXRβ were used in a series of docking computations that revealed the K_i for Allo (LXRβ 0.12 μM; PXR 1.17 μM), comparable to known ligands for each receptor. The free energy of binding for Allo (LXRβ -9.44 kcal/mol; PXR -8.49 kcal/mol) signified favorable protein-ligand associations. Lipoprotein Signaling and Cholesterol Metabolism gene arrays and

protein expression immunoblots were performed for hippocampus and cortex tissue from 3xTgAD mouse. Allo increased LXRβ, LDLR, SORL1, ABCA1, Cyp46a1, and decreased NF-κB protein expression in 3xTgAD male mice. Downstream of LXR, Allo altered LDLR-associated genes: CXCL16, OLR1, LRP6, STAB2, and PCSK9. A decrease in OLR1 and PCSK9 genes leads to increased cholesterol efflux and reduced inflammation. Allo also increased gene expression of CXCL16, LRP6, HMGSC1, and Cyp51 involved in stimulation of the PI3K/Akt/Erk and Wnt pathways and cholesterol synthesis pathways. STAB2 and Idi2 gene expression also increased suggesting a reduction in neuroinflammation. Allo decreased PCSK9 and AKR1D1 genes that promote cholesterol efflux while decreased ANGPTL3 provides a mechanism for reducing plasma cholesterol levels. Our results suggest that Allo directly binds to and activates LXR and PXR, to regulate downstream gene expression and molecular signaling pathways implicated in neuroinflammation, apoptosis, Aβ trafficking, and cholesterol clearance to prevent Alzheimer's pathology. Collectively, these data further reveal Allo's systems-wide coordination and identify target systems to prevent AD pathogenesis. Early phase dose-escalating clinical studies (placebo-controlled) are underway in men and women age 55+ with MCI/earlyAD (NCT02221622).

Disclosures: **R.W. Irwin:** None. **H.M. Swanson:** None. **R.D. Brinton:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.03/H1

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

Support: NIH NIA U01AG031115 to RDB

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NIH NIA AG005142 to HCC

NIH NIA P50 AG16573 to UCI-ADRC

USC Provost Fellowing to CMS

Title: Human iPSC-based biomarker strategy to identify neuro-regenerative responders to allopregnanolone: proof of concept

Authors: *C. M. SOLINSKY[1], V. HENNES[2], J. A. PARK[4], H. C. CHUI[3], M. BLURTON-JONES[4], J. K. ICHIDA[2], R. D. BRINTON[1,5];
[1]Clin. Pharm. and Pharmaceut. Econ. & Policy, [2]Stem Cell Biol. & Regenerative Med., [3]Neurol., USC, Los Angeles, CA; [4]Neurobio. & Behavior, Univ. of California Irvine, Irvine, CA; [5]Ctr. for Innovation in Brain Sci. and Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's disease (AD) is a national and global epidemic with complex patho-etiology including compromised brain metabolic activity and decreased regenerative capacity. Allopregnanolone (Allo) is an investigational neuroregenerative therapeutic, currently in Phase 1b clinical trial for AD (NCT02221622, https://clinicaltrials.gov/ct2/show/NCT02221622?term=NCT02221622&rank=1). In rodent preclinical models, Allo promotes neural stem cell (NSC) proliferation and neural differentiation and improves mitochondrial function. To develop biomarkers to predict regenerative response to Allo, we have initiated proof of concept analyses to determine the impact of Allo on human induced pluripotent stem cells (iPSCs) and iPSC-derived neural cells. T-cells from a patient with familial AD due to the A431E presenilin-1 point mutation were reprogrammed via a non-integrating, non-viral method, to iPSCs. Additional iPSCs were provided by the University of California Irvine Alzheimer's Disease Research Center (UCI-ADRC) and the Institute for Memory Impairments and Neurological Disorders. Isogenic iPSCs were generated using CRISPR-Cas9. Using dual inhibition of SMAD signaling, iPSCs were differentiated to NSCs. Mitochondrial respiration and regenerative capacity were determined using metabolic analyzer and FACS. Mitochondrial respiration and proliferation analyses were conducted in AD-derived and healthy control iPSCs and NSCs. Initial data indicates that AD iPSCs have similar proliferation rates, but increased ATP production compared to healthy controls. Analyses were conducted to determine the regenerative and bioenergetic effect of Allo. In iPSC-derived NSCs, Allo increased basal mitochondrial respiration by 78% and maximal mitochondrial respiratory capacity by 35%. Initial data demonstrate that iPSCs from AD patients demonstrate a metabolic phenotype distinct from healthy controls and that Allo improves mitochondrial function of iPSC-derived NSCs. Going forward, this approach will be used to evaluate the effect of Allo on the regenerative capacity and metabolic phenotype of clinical trial participant iPSC-derived NSCs. These data will form the foundation for developing the first regenerative biomarker to determine and monitor response to neuro-regenerative therapeutics.

Disclosures: C.M. Solinsky: None. **V. Hennes:** None. **J.A. Park:** None. **H.C. Chui:** None. **M. Blurton-Jones:** None. **J.K. Ichida:** None. **R.D. Brinton:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.04/H2

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

Support: R01AG042890

R03AG04753701A1

Title: Increased number of neural stem cells in the hippocampus correlates with maintenance of cognitive integrity in non-demented individuals with Alzheimer's Disease neuropathology.

Authors: *D. BRILEY[1], O. ZOLOCHEVSKA[1], R. WOLTJER[2], M.-A. MICCI[1], G. TAGLIALATELA[1];
[1]Univ. of Texas Med. Br. at Galveston, Galveston, TX; [2]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Alzheimer's Disease (AD) is a debilitating neurodegenerative disease characterized by dementia, cognitive declines. The presence of amyloid beta plaques and neurofibrillary tangles in the postmortem brain of demented individuals is the conclusive diagnostic for AD. However, certain individuals evade dementia despite the presence of extensive plaque and tangle neuropathology (here referred to as Non-Demented with Alzheimer's disease Neuropathology - NDAN). NDAN subjects thus represent a unique population in which to study the cellular mechanisms underlying this extraordinary resistance to cognitive decline. We tested the hypothesis that preserved cognitive function is correlated with enhanced neurogenesis in the hippocampus, a site heavily affected by AD and also important for its role in memory. We additionally investigated the potential epigenetic regulation of neurogenesis by specific miRNAs of interest. **Methods**. AD, mild cognitively impaired (MCI), NDAN, and age-matched healthy control was stained using established immunofluorochemical techniques for SOX2 and NeuN. Cells positive for each marker, and coexpressing cells, were counted and normalized over the DAPI signal for each image. Flow cytometry of nuclei isolated from samples was used to investigate the extent of colocalization between SOX2 and NeuN in a quantitative manner. Laser Capture Microdissection was used to isolate the granular cell layer and subgranular zone of fresh frozen human hippocampus sections. RNA was isolated and probed using qPCR for miRNA known to regulate neuronal precursor proliferation and maturation. **Results.** NDAN subjects demonstrate increased expression of SOX2, compared with AD and MCI. The number of SOX2+ cells correlates positively with cognitive performance (MMSE). Intriguingly and contrary to rodents, we also found several cells co-expressing SOX2 and NeuN that also correlated with MMSE scores. The expression of all probed miRNAs was decreased in NDAN with regard to control, while MCI and AD were comparable or increased. **Conclusions.** Our data

show that the number of progenitor cells in the hippocampus of NDAN subjects is increased as compared to AD subjects, and similar to controls. Furthermore, increased number of progenitor cells in NDAN may be driven by epigenetic modulation reflecting a deregulation of the miRNAs probed, as indicated by the trend towards reduced expression. These data strongly suggest NDAN are a unique sub-set of individuals who are neither prodromal for the disease, nor simply outlying control individuals, in which sustained neurogenesis may promote maintenance of cognitive integrity in the face of substantial AD neuropathology.

Disclosures: D. Briley: None. **O. Zolochevska:** None. **R. Woltjer:** None. **M. Micci:** None. **G. Taglialatela:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.05/H3

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

Title: D-Serine mediates adult neurogenesis in mice

Authors: *R. ROYCHAUDHURI, S. H. SNYDER;
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Abstract: Neurogenesis is the formation of newborn neurons from neural precursor cells. In the adult brain, the subventricular zone and the dentate gyrus are the two major regions in the hippocampus that continue to produce newborn neurons throughout life. Postmortem human studies and investigations in mice have shown that Alzheimer's Disease (AD) is associated with enhanced neurogenesis. However, mechanisms responsible for this effect are unknown and prior studies have shown conflicting results due to differences in promoters, age of animal, age of disease onset, transgene overexpression, neurotransmitter levels and extent of overexpression of protein. The goal of our study is to explore a possible role for D-serine in adult neurogenesis. D-amino acids like D-serine and D-aspartate occur in the brain and act as co-agonists respectively at the glycine and glutamate binding sites of the glutamate-NMDA receptor (NMDAR). We used age matched WT and $SR^{-/-}$ (serine racemase; enzyme that converts L-serine to D-serine) mice to investigate the role of D-serine in adult neurogenesis. Briefly, six week old adult male WT and $SR^{-/-}$ mice (deficient in D-serine production) were injected once daily with saline and or D-serine (50 mg/kg) intraperitoneally for 7 days. Following EdU injection on day 8 (100 mg/kg ip), brains were harvested and analyzed for proliferation by immunohistochemistry. Our results show that lack of D-serine leads to significantly diminished neurogenesis. Administration of D-serine restores neurogenesis in mice deficient in D-serine ($SR^{-/-}$ mice). We hypothesize that D-serine

acts on the NMDAR and modulates neurogenesis in adult brain via an unidentified novel mechanism. These studies may provide insights into the role of D-serine in adult neurogenesis and in the pathophysiologic mechanisms of cell loss in AD.

Disclosures: R. Roychaudhuri: None. **S.H. Snyder:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.06/H4

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

Title: ProNGF/p75/sortilin: potential targets to recover neurogenesis in Alzheimer's disease

Authors: *C. FLEITAS PÉREZ[1], C. RAMPON[2], R. CURIÀ[1], E. BERNAUS[1], J. EGEA[1], C. ESPINET[1];
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Abstract: The adult neurogenesis in humans has significant implications in memory formation and cognitive functions. Adult-born neurons generated in hippocampus are able to stabilize synaptic connections and mediate neuronal plasticity. The induction of neurogenesis can reverse cognitive dysfunction in animal models of Alzheimer’s disease. Previous studies have shown that the levels of pro-NGF are increased in a stage-dependent manner in human hippocampus and enthorinal cortex affected by Alzheimer’s disease. Also the proNGF receptor, p75NTR, is expressed not only in mature neurons but also in mitotic cells from mouse hippocampus. To assess the role of these signaling molecules in the adult neurogenesis, immunofluorescence analyses were performed to detect proNGF, p75NTR, sortilin and differentiation or synaptic markers in the mouse model of Alzheimer’s disease, in the hippocampal neurospheres cultured of p75NTR deficient mice, and also in the hippocampal preparations from Alzheimer’s disease affected human brains. The results showed that adult newborn cells from both human and animal models, express increased levels of p75NTR and sortilin in Alzheimer's disease. These neurons can respond to proNGF by apoptosis and/or dedifferentiation. Consequently, increased pro-NGF in adult hippocampal brain, may be responsible for the decrease of neurogenesis. Disruption of proNGF/p75/sortilin signaling would be a relevant target in regenerative therapies for Alzheimer’s disease.

Disclosures: C. Fleitas Pérez: None. **C. Rampon:** None. **R. Curià:** None. **E. Bernaus:** None. **J. Egea:** None. **C. Espinet:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.07/H5

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

Support: FAPESP

CNPq

Title: Neural stem cell transplantation increases the neurogenesis in double transgenic mouse model of alzheimer's disease

Authors: *B. M. LONGO[1], S. A. ROMARIZ[2], D. S. PAIVA[2];
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Abstract: In view of the lack of efficient treatments for Alzheimer´s disease (AD), it has been investigated new therapies that may reduce or prevent the progression of cognitive deficits and neuronal degeneration due to deposition of Aβ plaques. In this scenario, the neural stem cell (NSC) therapy is a promising alternative. Here, we investigated the *in vivo* potential of NSC in reducing neuronal degeneration and promoting neurogenesis through intra-hippocampal transplantation in the double-transgenic mouse model (APPswe/PS1dE9). We analyzed the effects of NSC transplantation in learning and memory behaviors, as well as the levels of BDNF and neurogenesis in the hippocampus. Immunohistochemistry for GFP indicated that the NSC transplanted in AD and WT animals were able to integrate, differentiate into astrocytes and neurons and migrate to lateral ventricle and fimbria. The total number of GFP+ cells was not different between WT and AD transplanted groups (p>0,05). Doublecortin (DCX) positive neurons and BDNF levels in the hippocampus were increased in AD transplanted animals compared to WT näive animals (p= 0,0182 and p=0,005 respectively). Although transplantation of NSC increased neurogenesis in AD mice, it was not enough to promote memory recovery in these animals, only in WT translpanted mice. We suggest that, despite not having behavioral effects. NSC transpantation increased the neurotrophic levels in the hippocampus and may be responsible for other molecular modifications involved in Alzheimer´s disease. The fact that the cells mainly migrated to the fimbria may suggest an effort to restore memory, once this structure plays a role in memory formation, connecting to cholinergic neurons in basal forebrain. This study may contribute to the understanding of the role of neural stem cells in regenerative mechanisms of transgenic AD animals and therapeutic implications in aging neurodegeneration processes.

Disclosures: **B.M. Longo:** None. **S.A. Romariz:** None. **D.S. Paiva:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

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Program#/Poster#: 307.08/H6

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

Support: NIH Grant AG012694-16

NIH Grant AG000538

NIH Grant AG034667

Title: The epigenetic regulation of h3k9me3 promotes neuronal survival and functions

Authors: *L.-Q. TONG[1], S. SNIGDHA[2], C. W. COTMAN[1];
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Abstract: Epigenetic regulation of genes and proteins is fundamental to the aging process and the associated decline in learning and memory. We reported recently that pharmacological inhibition of SUV39H1 using a novel and selective inhibitor decreased levels of H3K9me3 (a repressive mark) in the hippocampus of aged mice, promoted spine formation, and improved memory performance (Snigdha et al., 2016, J Neurosci, 36(12): 3611-3622). However, the specific changes in histone methylation states in mature hippocampal neurons remains an unexplored area of research. In order to better understand the mechanisms directly in neurons, we used primary neuronal cultures where conditions are well defined and drug concentrations are precisely controlled. We evaluated the effects of H3K9me3 downregulation on neuronal structure and functions in primary cultures of hippocampal neurons using ETP69, a newly developed specific inhibitor of SUV39H1. ETP69 treatment decreased neuronal H3K9me3 levels and increased PSD-95 expression and volume of dendritic spines. Furthermore, ETP69 treatment increased phosphorylation of transcription factor CREB at Ser133. Finally, ETP69 protected neurons from glucose-oxygen deprivation-induced cell death. Collectively, these data suggest that SUV39H1 inhibition-mediated downregulation of H3K9me3 acts directly on neuronal structure and functions to promote survival and synaptic function.

Disclosures: **L. Tong:** None. **S. Snigdha:** None. **C.W. Cotman:** None.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 307.09/H7

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

Title: Discovery of novel,neuroprotective small molecules for the treatment of ER stress related toAlzheimer's disease

Authors: *K. KRAJNAK[1], F.-S. WANG[1], R. DAHL[2];
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Abstract: Alzhiemer's disease (AD) is the leading cause of neurodegenerative disease and yet still very little is known about its onset. Recent findings have linked mechanisms of cellular stress to those that are also present in a whole host of diseases, such as Type II Diabetes, Parkinsons and Cardiac disease. This cell stress induces intracellular calcium dyshomeostasis resulting in endoplasmic reticulum (ER) stress and the unfolded protein response. If this stress is excessive or prolonged, apoptotic pathways are triggered resulting in cell death and overall neuron loss. It has been established that a major cause of ER stress is a dysfunctional calcium transport ATPase (SERCA), whose sole function is to bring calcium into the ER. In this study, we have screened various SERCA modulators for their neuroprotective capabilities and potential use as pharmacological treatments for AD.
Initial screenings of compounds were done for cell viability to identify potential allosteric modulators of SERCA. The ER stress of Alzheimer's disease was simulated using the stress inducers Thapsigargin. Initial high throughput screenings of novel small molecules that would potentially reduce ER stress by restoring calcium homeostasis were performed. After narrowing the molecules down to prospective targets, the assay was utilized for dose response analysis while monitoring cell cytotoxicity.
Our studies found three compounds that showed over 70% survivability with treatment, after exposure to Thapsigargin, with a statistical significance of $p<0.001$. These compounds are further being studied in neuronally differentiated cells to further evaluate their mechanisms of action and clinical potential.

Disclosures: **K. Krajnak:** F. Consulting Fees (e.g., advisory boards); Celladon. **F. Wang:** A. Employment/Salary (full or part-time): Purdue University. **R. Dahl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodon, CEO.

Poster

307. Alzheimer's Disease: In Vitro and In Vivo Therapeutics

Location: Halls B-H

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Program#/Poster#: 307.10/H8

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

Support: Mahidol University and Thailand Research Fund (IRG5780009)

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National Institute for Health Research (NIHR)

Edmond and Lily Safra Research Foundation

Title: A genetically immortalized human stem cell line: a promising new tool for Alzheimer's disease therapy

Authors: *N. PUANGMALAI[1,2], W. THANGNIPON[1], A. SOMANI[2], C. BALLARD[2], M. BROADSTOCK[2];
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Abstract: Amyloid-β (Aβ) peptide and hyperphosphorylated tau are the main pathological hallmarks of Alzheimer's disease (AD) (Reitz et al., 2014). Given the recent failure of several large-scale clinical trials and the lack of disease-modifying pharmacological treatments, there is an urgent need to develop alternative therapies. CTX0E03 is a clinical-grade human neural stem cell line which has recently passed phase I trials in people with stroke (Hick et al., 2013). However, this line has not been investigated in other neurodegenerative disorders. This study investigates the survival of CTX0E03 neural stem cells under conditions based on the underlying AD pathology. CTX0E03 cells were seeded into laminin-precoated 96-well plates at cell density $5x10^4$ cells/ml (Pollock et al., 2006). Cells were treated with amyloid peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$) at concentrations of 0.5, 1, 5, 10, and 15 μM and okadaic acid (OA) at 0.5, 1, 5, 10, and 15 nM. Vehicle control cultures were treated with DMSO or PBS (control for OA and Aβ respectively). After 24 h incubation, the cells were examined for cell viability using PrestoBlue reagent and lactate dehydrogenase-cytotoxicity assay. Cell viability assays showed a concentration dependence of this cell line to the toxic effects of $A\beta_{1-42}$, but not $A\beta_{1-40}$, and OA. Notably, CTX0E03 cell line displayed toxicity at concentrations significantly higher than both rat neural stem cells and those previously reported for primary cultures. Our study indicates the ability of clinical grade CTX0E03 stem cell line to resist the inhospitable milieu associated with AD. Thus, CTX0E03 stem cells provide a potential candidate for cell therapy in AD patients.

Disclosures: **N. Puangmalai:** None. **W. Thangnipon:** None. **A. Somani:** None. **C. Ballard:** None. **M. Broadstock:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.01/H9

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

Support: NIH Grant R21 NS082870

NIH Grant UL1 RR025758

Title: Higher resting motor threshold associated with better cognitive function in patients with mild Alzheimer's disease

Authors: *P. J. FRIED, A. JANNATI, P. DAVILA PEREZ, V. M. CHEN, D. Z. PRESS, A. PASCUAL-LEONE;
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Abstract: Background. Nearly all applications of transcranial magnetic stimulation (TMS) begin by assessing the resting motor threshold (RMT). Conventionally defined as the minimum TMS intensity needed to elicit a positive motor response 50% of the time, the RMT serves as both a metric of cortico-motor reactivity and a means to individualize the intensity of subsequent stimulation. Under normal conditions, RMT is very stable across time, with the highest test-retest reliability of any TMS-based neurophysiological measurement. RMT can inform on structural and physiological changes in the motor system across time or in response to interventions, and group differences in RMT have been observed in diseases that impact the motor system such as focal dystonia, amyotrophic lateral sclerosis, etc. By contrast, few studies have investigated how RMT relates to non-motor measures of cognition.
Objective. The primary objective of the present study was to assess the relationship of RMT to measures of global cognitive function in patients with mild Alzheimer's disease (AD).
Methods. In a retrospective cross-cohort study, data were obtained from 30 patients with mild-AD (mean ± SD age: 69.6 ± 7.4 years, 59% female) and 26 cognitively intact controls (mean ± SD age: 62.7 ± 1.8 years, 50% female). All subjects had previously participated in research for which RMT was assessed along with measures of global cognitive function, including the Mini Mental Status Examination (MMSE) and the Cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog).

Results. The AD group performed significantly worse than controls on the MMSE (AD: 21.6 ± 2.2; controls: 29.5 ± 0.8, $p < .001$) and the ADAS-Cog (AD: 23.3 ± 10.5; controls: 4.0 ± 2.0, $p < .001$). By contrast, RMT (% of maximum stimulator output) was equivalent for the two groups (AD: 57.2 ± 2.3; controls: 59.8 ± 3.2, $p = .434$). In the AD group, there were significant correlations between RMT and both MMSE ($R_{28} = .53$, $p = .004$) and ADAS-Cog ($R_{28} = -.57$, $p = .001$). Specifically, higher RMT was associated with better cognitive function. By contrast, in controls, RMT was not significantly related to either MMSE ($R_{24} = .11$, $p = .601$) or ADAS-Cog ($R_{24} = -.07$, $p = .730$).
Conclusion. Higher RMT is associated with better global cognitive function in individuals with mild AD. This is somewhat paradoxical as AD is associated with progressive cortical atrophy, which should increase the scalp-to-brain distance and thus necessitate a stronger electromagnetic field. Nevertheless, these findings suggest a potential value in RMT as a rapid and reliable surrogate biomarker of the severity of cognitive dysfunction in dementia.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.02/H10

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

Support: NIH T32 AG00216-23

Title: Blood-based biomarkers for staging of alzheimer's disease

Authors: *C. N. WINSTON[1], E. J. GOETZL[4], J. C. AKERS[2], B. S. CARTER[2], E. M. ROCKENSTEIN[1], D. R. GALASKO[1], E. MASLIAH[3], R. A. RISSMAN[1];
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Abstract: INTRODUCTION: Levels of Alzheimer's Disease (AD)-related proteins in plasma neuronal-derived exosomes (NDEs) were quantified to identify biomarkers for prediction and staging of Mild Cognitive impairment (MCI) and AD. **METHODS:** Plasma exosomes were extracted, precipitated and enriched for neuronal source by anti-L1CAM antibody absorption. NDEs were characterized by size (Nanosight) and shape (TEM), and extracted NDE protein

biomarkers were quantified by ELISAs. Plasma NDE cargo was injected into normal mice and results were characterized by IHC to determine pathogenic potential. **RESULTS:** Plasma NDE levels of P-T181-tau, P-S396-tau, and Aβ1-42 were significantly higher, whereas those of neurogranin (NRGN) and the repressor element 1-silencing transcription factor (REST) were significantly lower in AD and MCI converting to AD (ADC) patients compared to cognitively normal controls (CNC) subjects and stable MCI patients. Mice injected with plasma NDEs from ADC patients displayed increased P-tau (PHF-1 antibody)-positive cells in the CA1 region of the hippocampus compared to plasma NDEs from CNC and stable MCI patients. **CONCLUSIONS:** Abnormal plasma NDE levels of P-tau, Aβ1-42, NRGN, and REST accurately predict conversion of MCI to AD dementia. Plasma NDEs from demented patients seeded tau aggregation and induced AD-like neuropathology in normal mouse CNS.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.03/H11

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

Title: Semantic and episodic memory impairments for faces in frontotemporal dementia and Alzheimer's disease

Authors: *J. A. COLLINS[1,2], B. C. DICKERSON[1,3];
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Abstract: The ability to recognize familiar faces is vitally important to everyday function and relies on the integrity of several neuroanatomical networks involved in perception, memory, and affect. Upon encountering someone have seen before, we immediately feel a sense of familiarity for that individual and retrieve semantic information about that person's identity and contextual details regarding previous encounters with that person. Unfortunately, this complex recognition process is often impaired in individuals with frontotemporal dementia (FTD) and Alzheimer's Disease (AD) imposing a substantial burden on quality of life.
The goal of this study was to delineate the cognitive components and neural mechanisms underlying the person recognition difficulties experienced by patients with FTD and AD.

Participants included 11 older healthy controls, 11 patients with AD, 10 patients with the semantic variant of FTD (semantic dementia or SD) and 4 patients with the behavioral variant of FTD (bvFTD). To assess semantic memory for famous faces, participants were first shown a set of 15 famous faces and 15 non-famous foils and were asked if they recognized the person (yes/no), and if so to retrieve the person's name. After a 20-minute break participants began the second portion of the task, which measured episodic memory for faces. They were shown a set of 60 faces (30 old) and on each trial are asked whether they saw the person in the previous session (yes/no), and how confident they are in their decision.
Relative to healthy controls, FTD patients (both bvFTD and SD) were significantly impaired at recognizing famous faces as being familiar, suggesting a semantic memory impairment for these individuals. Patients with SD exhibited particular difficulty naming the famous faces they had endorsed as familiar. Patients with AD exhibited preserved performance on the semantic memory portion of the task, and performed significantly better than the SD sample. Contrary to our expectations, all three patient groups were significantly impaired on the episodic memory portion of the task relative to healthy controls, with no difference in performance observed between the three patient samples. The results of a preliminary whole brain cortical thickness analysis in the FTD patient group revealed areas in frontal and temporal cortex that predicted episodic and semantic memory performance.

Disclosures: J.A. Collins: None. **B.C. Dickerson:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.04/H12

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

Support: P01AG014449

R01AG044372

R01NS082730

Title: Progression of tau oligomer pathology in cholinergic nucleus basalis neurons in mild cognitive impairment and Alzheimer's disease

Authors: *C. T. TIERNAN[1], E. J. MUFSON[4], N. M. KANAAN[1,2,5], S. E. COUNTS[1,2,5,3]; [1]Translational Sci. and Mol. Med., [2]Neurosci. Program, [3]Dept. of Family Med., Michigan State

Univ., Grand Rapids, MI; [4]Dept. of Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [5]Hauestein Neurosci. Ctr., Mercy Hlth. St. Mary's, Grand Rapids, MI

Abstract: Tau is the predominant protein found in neurofibrillary tangles (NFTs) that contribute to neuronal dysfunction and cognitive decline in Alzheimer's disease (AD) and other tauopathies. Despite not fully understanding the role of tau in neurodegenerative diseases, there is accumulating evidence that the toxic tau moiety may be soluble, oligomeric tau species. In this regard, tau oligomers appear prior to NFT formation, correlate more strongly with neuronal loss than NFTs, and exhibit neurotoxicity in preclinical models. Here, we investigated the spatiotemporal progression of oligomeric tau within cholinergic basal forebrain (CBF) neurons of the nucleus basalis (NB), a brain region selectively prone to tau pathology and neurodegeneration during the progression of AD. Tissue from Rush Religious Orders Study subjects who died with a clinical diagnosis of no cognitive impairment (NCI; $n = 12$), mild cognitive impairment (MCI; $n = 10$), or AD ($n = 12$) was immunostained with the tau oligomeric complex 1 (TOC1) antibody, as a marker of tau oligomers, and $p75^{NTR}$, as a cholinergic cell marker. Initial unbiased stereology estimates of these markers ($n = 8$ NCI, 5 MCI, and 8 AD) revealed that the number of NB neurons co-labeled with $p75^{NTR}$ and TOC1 ($p75^{NTR}$/TOC1+) were relatively sparse in NCI (1007 ± 263, mean ± SEM, or ~0.5%), whereas the number of $p75^{NTR}$/TOC1+ neurons increased incrementally in MCI (2640 ± 1466, or ~1.5%) and AD (6955 ± 4144, or ~4.5%; $p < 0.05$, AD > NCI). Initial correlation analysis revealed no association between $p75^{NTR}$/TOC1+ counts and antemortem global cognitive status via the MMSE or a global *z*-score for 17 neuropsychological tests. However, significant direct correlations were found between increasing numbers of $p75^{NTR}$/TOC1+ neurons and severity of Braak ($r = 0.6$, $p < 0.01$), NIA-Reagan ($r = 0.54$, $p < 0.05$), and CERAD ($r = 0.48$, $p < 0.05$) scores. Ongoing studies are examining the spatial progression of tau oligomer pathology through the NB subfields, and the co-localization of TOC1 with established markers of tau pathology in each of the clinical stages. Taken together, these data suggest that the progression of AD is associated with accumulating oligomeric tau pathology in the selectively vulnerable NB and may represent a target for therapy.

Disclosures: C.T. Tiernan: None. **E.J. Mufson:** None. **N.M. Kanaan:** None. **S.E. Counts:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.05/H13

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

Support: CIHR

Title: Volumetric assessment of brain areas involved in executive function deficit in Alzheimer's Disease

Authors: *F. JUNG[1], S. KAZEMIFAR[2], R. BARTHA[2], R. N. RAJAKUMAR[1];
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Abstract: Executive functional deficits are frequent findings in Alzheimer's Disease (AD) and often reported even in mild cases. Executive function comprises several cognitive abilities including working memory, behavioural flexibility, response inhibition and attention, and involves increased activity and functional connections among a number of brain areas. Current study is to determine the pattern of volume loss in these nodal brain areas during the progress of AD using the Alzheimer Disease Neuroimaging Initiative (ADNI) database. 1.5 Tesla T1-weighted MRI images acquired at baseline and at 24 months in 139 subjects with AD were selected from the ADNI database. These structural T1-weighted images were acquired using a sagittal 3D magnetization-prepared rapid acquisition with a gradient echo MP-RAGE sequence with pixel size 1mm×1mm×1.2mm; flip angle ~8°; TE ~4 ms; TR ~8 ms; matrix, 256x256; 170 slices. Regions of interest (ROI) including the caudate nucleus, dorsolateral prefrontal cortex, fusiform cortex, insula, parietal cortex and the thalamus were manually segmented from all subjects to test for associations with their neuropsychological assessment of executive functional deficit.

Disclosures: F. Jung: None. **S. Kazemifar:** None. **R. Bartha:** None. **R.N. Rajakumar:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

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Program#/Poster#: 308.06/H14

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

Support: 1-R01-AG-O36400 (PDC)

Title: Genome-wide expression and methylation profiling in Medial Temporal Gyrus reveals dysregulated genes with specific methylation associated with Alzheimer's Disease

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Abstract: We integrated the gene expression and methylation profiles of Alzheimer's Disease patients (AD) and non-demented matched controls (ND) in order to detect patterns of dysregulation in AD. Expression profiles were obtained from the Middle Temporal Gyrus (MTG) from 100 AD and 100 ND subjects using the HumanHT-12 BeadChip Array (Illumina). Methylation profiles were analyzed in a partially overlapping sample of 225 AD and 179 ND using the HM450 BeadChip array (Illumina). Data analysis was conducted using the R packages Lumi, Methylumi and Limma. P values were corrected using the False Discovery Rate (FDR) method. Expression and methylation results were combined considering common significant genes and combining P values using the Fisher's weighted Z-method (FWZ). Enrichment analysis, considering both pathways and gene ontologies, was performed using the ToppGene web tool. We detected a total of 16,947 significant DEGs (9,271 overexpressed and 7,676 underexpressed in AD). Among the top genes were: RGS4, SYT1, STMN2, CHGB and STXBP1. We performed pathway analysis using genes with Fold Change > |0.8| and identified genes associated with Synaptic Vesicle Trafficking and GABAergic transmission. The female samples were specifically enriched for the "L1CAM interaction" pathway including the CHL1 gene, a substrate for the AD-associated gene BACE1. In the methylation analysis, we detected a total of 59,602 significant differentially methylated probes (33,374 hypomethylated and 26,228 hypermethylated in AD). The top differentially methylated sites were located in: ANK1, MYO1C, TMEM104, PLAT and KIF26B. The enrichment analysis highlighted the presence of neuronal genes and, in particular, the enrichment of Rho GTPase and Rap1 pathways. The sex-specific analysis confirmed the presence of the L1CAM interaction pathway in the female sample, as detected in the gene expression results. With the combination of expression and methylation results, we detected 239 differentially expressed genes that also showed methylation changes with at least 75% of probes concordant with the methylation pattern. The top genes on the list, ordered by total number of methylation probes that were significant, are: MYT1L, RIMBP2, GABBR1, SNRPN and LRP5. The enrichment analysis confirmed the presence of neuronal and synaptic functional classes. In conclusion, we provide new information about the molecular mechanisms and genes associated with AD in a specific brain region (MTG). In particular, we explore the relationship between methylation status and gene expression, detecting new genes that potentially could have stable gene expression changes that play an important role in AD.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.07/H15

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

Support: NIH 53-5183-1046

Title: Predicting Alzheimer’s disease risk with a deep neural network model

Authors: *K. NING[1], B. CHEN[2], F. SUN[1], Z. HOBEL[1], A. ALZHEIMER'S DISEASE GENETIC CONSORTIUM[3], A. W. TOGA[1];
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Abstract: Introduction: Currently there is no effective treatment for late onset Alzheimer’s disease (AD), a form of dementia that primarily affects the elderly. Accurate AD risk prediction based on a person’s genetic information will allow for early interventional AD prevention for those at high risk. In this study, we predicted a person's risk of AD using a deep neural network model, a machine-learning tool which has demonstrated impressive power in extracting interactions from its predictors. This tool has been applied to problems like computer vision and natural language processing, but has not been used for disease prediction. **Methods:** We trained and assessed the risk prediction performance of our model using data from the Alzheimer's Disease Genetics Consortium. It includes genome-wide single nucleotide polymorphism (SNP) data for over 20,000 healthy controls (n=10,826) and AD patients (n=10,778). When building our multi-layer neural network model, we incorporated SNP-gene relationships as well as gene-pathway relationships to determine its structure. We evaluated the risk prediction performance of our model using 10-fold cross-validation, where the model was trained with 90% of the data and tested with the remaining 10%. **Results:** Our model achieves high prediction accuracy, with median area under the curve (AUC) = 0.88 in the testing data. This model includes SNPs moderately associated with AD, along with SNPs reaching genome-wide disease association significance (p-value <= 5E-8), which achieves higher prediction accuracy than a model that only includes SNPs reaching genome-wide significance. **Conclusion:** We believe our model has the best risk prediction performance reported for AD to date. Our model can also be used to

predict risk of other diseases based on genetic information, particularly those which have many moderately significant genetic risk loci.

Disclosures: **K. Ning:** None. **B. Chen:** None. **F. Sun:** None. **Z. Hobel:** None. **A. Alzheimer's Disease Genetic Consortium:** None. **A.W. Toga:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

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Program#/Poster#: 308.08/H16

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

Support: NIH P50AG05146

BrightFocus Foundation A2015332S

Title: The preclinical pathology of Alzheimer’s disease and its modulation by ApoE

Authors: O. PLETNIKOVA[1], G. L. RUDOW[1], Y. KAGEYAMA[1], K. LACLAIR[1], D. R. FOWLER[2], L. J. MARTIN[1], *J. C. TRONCOSO[1];
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Abstract: Tau and Aβ lesions are common in the cerebral cortex of older individuals and constitute the basis for the diagnosis of Alzheimer’s disease (AD). However, the extent of these pathologies in aging and preclinical AD remains unknown. Therefore, we examined cortical tau and Aβ lesions in 431 autopsy brains of subjects 30 to 65 years of age, stratified by ApoE genotype. We immunostained for tau (PHF-1) and Aβ (6E10) in hippocampus, ERC, and neocortex and measured tissue levels of Abeta 40 and Abeta 42 by ELISA.
Tau lesions in ERC/hippocampus increased steadily from 4% in subjects 30 to 34 year-old to 70% in subjects 60 to 65 year-old. These lesions consisted predominantly of subtle neuritic and perikaryal staining, neurofibrillary tangles were rare. In subjects ages 40 to 49 years, Aβ lesions were present in 15%; all of these were diffuse plaques, more frequent in neocortex than hippocampus, and not associated with local tau lesions. All subjects with Aβ deposits at this age had one or two APOE4 alleles, though many subjects with one APOE4 remained free of Aβ lesions. Over age 50, neuritic plaques could be observed, and continued to increase with age. In subjects 40 to 50 years of age, tissue levels of insoluble Abeta 42 were highest in ApoE 4/4 subjects, intermediate in ApoE 3/4 and undetectable in ApoE 3/3.

In conclusion, Aβ deposits can appear as early as 40 years of age in APOE4 allele carriers, are more abundant in neocortex than hippocampus, and are not associated with local tau changes. In our cohort, neuritic plaques first appear at age 50 and increase afterwards. These findings suggest that future AD preventive measures should consider targeting young individuals stratified by their genetic risks including ApoE genotype.

Disclosures: O. Pletnikova: None. **G.L. Rudow:** None. **Y. Kageyama:** None. **K. LaClair:** None. **D.R. Fowler:** None. **L.J. Martin:** None. **J.C. Troncoso:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.09/H17

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

Title: Neuropsychological assessment of semantic memory in alzheimer's and primary progressive aphasia patients

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Abstract: Neuropsychological assessment of semantic memory is commonly driven by using tasks requiring the active elicitation of explicitly stored information (Della Rosa et al, 2014). Nonetheless, there is a lack of instruments for Spanish speaking population suitable to detect specific impairments of semantic memory in Alzheimer Disease (AD) and Primary Progressive Aphasia (PPA) patients. Several previous studies have suggested that AD and PPA patients exhibit different profiles of semantic deterioration in the concrete-abstract dimension (Catricalà, Della Rosa, Plebani, Vigliocco, & Cappa, 2014; Hoffman, Jones, & Lambon Ralph, 2013; Hoffman & Ralph, 2011; Pobric, Lambon Ralph, & Jefferies, 2009; Yi, Moore, & Grossman, 2007). The present study analyzed the cognitive differences in the semantic deficit in the concrete-abstract dimension between AD and PPA patients. Four AD and three PPA patients, as well as a control group of seven healthy individuals participated in the study. After clinical diagnosis, participants performed two semantic memory tasks. One of the tasks consisted of 48 triplets of words, presented successively, each one in a card. Half of the triplets contained concrete words, and the other half abstract words. Stimuli were presented in random order. Participants were asked to select which pair of words in each triplet have similar meaning. The second task consisted in 48 written words, each one matched to a correct or incorrect definition.

Half of the words were concrete an half of them were abstract, and 50% of stimuli were correct, whereas 50% were incorrect. Participants were asked to verify whether or not each word is congruent with the respective definition. All of the words were controlled in lexical variables such as frequency familiarity, imageability and age of acquisition. Our results show impairment in the semantic processing of abstract words in AD patients, whilst APP patients are impaired in the processing of both types of words. We conclude that these results might be explained by the fact that processing concrete words require the activation of more contextual and associative information than the processing of abstract words (Schwanenflugel, 1991). According to this hypothesis, abstract word processing relies on an extended multimodal network over the temporal-parietal regions of the left hemisphere (Grossman et al., 2003, 2013), whereas the anterior temporal lobe atrophy, characteristic of the PPA, would be associated to a poor performance in the processing of both types of words, since this region would be a supramodal association zone, specialized in the general semantic processing.

Disclosures: J. Ferrer: None. **V. Patiño-torrealva:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

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

Support: J.L. Lévesques Foundation

FQRNT

Canadian Institute of Health Research

Title: Human brain isoprenoids and Alzheimer's disease

Authors: *S. PELLEIEUX[1], Y. S. TSANTRIZOS[2], L. LAMARRE-THÉROUX[3], D. DEA[3], J. POIRIER[4];
[1]Dr Judes Poirier's team- Perry building, Douglas Mental Hlth. Res. Inst., Verdun, QC, Canada; [2]Dept. of chemistry, McGill Univ., Montréal, QC, Canada; [3]Res. program on aging and Alzheimer's disease, Douglas Mental Hlth. Univ. Institute, McGill, Montréal, QC, Canada; [4]Res. program on aging and Alzheimer's disease, Psychiatry and medecine, McGill Univ. and Douglas Mental Hlth. Univ. Inst., Montréal, QC, Canada

Abstract: Background: The mevalonate pathway has been described to play a key role in Alzheimer's disease (AD). The present work focused on mevalonate derived non-sterol isoprenoid farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Human FPP and GGPP synthases (hFPPs and hGGPPs) lead to protein prenylation which plays a crucial role in controlling cell signalling and proliferation and which have been shown to be associated with AD. It is hypothesised that these intermediates are implicated in tau metabolism dysregulation and the progression of AD. A better understanding of the role of hFPPs and hGGPPs in the pathophysiology of AD may prove to be essential to assess the function of the isoprenoids pathway as a potential target in the treatment or prevention of AD.
Methods: mRNA levels of hFPPs and hGGPPs were assessed by RT-qPCR whereas GGPP and FPP concentrations were quantified by liquid chromatography-tandem mass spectrometry (LC/MS/MS) in control and AD brain tissues obtained from the Douglas-Bell Brain Bank (Montréal, Canada). In a second part, hFPPs and hGGPPs candidates inhibitors chemically derived from the nitrogen-containing bisphosphonates (N-BP) were tested on human SH-SY5Y neurons and their effect on the accumulation of tau and phospho-tau was measured as a function of time and drug concentrations.
Results: hFPPs and hGGPPs mRNA prevalence and FPP and GGPP concentrations are significantly increased in frontal cortex of AD-confirmed subject (Respectively $p<0.001$, $n=124$ and $p<0.05$, $n=116$) while no significant differences were found in cerebellum. We also detected that high levels of enzymes ($p<0.05$, $n=64$) and precursors are associated with an earlier age of onset of AD. The levels of mRNA for hFFPs and hGGPPs also correlate with the amount of phospho-tau protein levels ($p<0.05$, $n=38$) and neurofibrillary tangles density ($p<0.006$, $n=40$) in frontal cortex. Finally, preliminary data indicates that our novel families of FPPs inhibitors can decrease phospho-tau levels in SH-SY5Y neurons in the low micromolar range without any detectable toxicity.
Conclusions: Together, these results suggest that neuronal isoprenoids dysregulation is associated with accumulation of phospho-tau and formation of tangles in the AD brain; a phenomenon that could be alleviated by the administration of specific and potent bi-phosphonate derivatives.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.11/H19

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

Title: EEG oscillations during word processing predict MCI conversion to Alzheimer's disease

Authors: *A. MAZAHERI[1], K. SEGAERT[1], J.-C. YANG[2], Y.-Q. NIU[4], J. OLICHNEY[3], K. SHAPIRO[1], H. BOWMAN[1];
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Abstract: Mild cognitive impairment (MCI) is a syndrome characterized by cognitive decline that, although not interfering with daily life, is greater than expected given an individual's age. Approximately one-half of individuals diagnosed with MCI progress to dementia within 5 years of diagnosis. Identifying factors that can predict conversion of MCI to dementia could lead the way to early intervention. In the current study, we investigated oscillatory changes in the EEG induced by visual word processing for healthy controls and MCI patients, a subset of whom would go on to develop Alzheimer's disease within 3 years. We used a language comprehension task, in which a phrase describing a category (e.g., `a type of wood', `a breakfast food') was presented first, followed by a single target word that was either congruent (i.e., oak, pancake) or incongruent with the category established by the preceding phrase. The focus of our analyses was the oscillatory activity in theta and alpha frequency bands, given that prior studies have implicated these bands in specific aspects of language processing. In the MCI individuals that would go on to develop Alzheimer's disease (AD), theta activity during the processing of the word (i.e., retrieval of the lexical and semantic properties of these words) was significantly diminished. This theta attenuation served as an imminent conversion marker from MCI to Alzheimer, not only on the group level but could also be observed in the individual patients within the group. Looking at individual patients, 60% of MCI convertors (to AD dementia) had a smaller theta increase than the least amount of theta increase observed in the MCI non-convertors, as well as healthy, control group.MCI non-convertors, as well as MCI convertors, were distinguishable from healthy controls by a neural signature related to processing semantically congruous words (i.e., integration of the target word meaning with the semantic/linguistic context set up by the preceding phrase): an induced alpha suppression, as well as trial-by-trial cross-frequency coupling between the posterior theta increase and the alpha suppression. In sum, importantly for the prognosis of MCI patients, our findings suggest that basic anomalies in the lexical/semantic processing of single words picked up using EEG oscillations could serve as an objective neurophysiological marker for impending conversion of MCI to Alzheimer's disease. We believe that our study adds valuable insight into the subtle breakdown of neural circuits involved in semantic and lexical processing.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.12/H20

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

Support: NINDS Grant 5RO1NS023945-24

Title: Integrity of the basal forebrain cholinergic space and its relation to the hippocampus in Alzheimer's disease.

Authors: *J. L. REEP[1], B. A. ARDEKANI[3], C. E. MYERS[4,2], L. ZABORSZKY[1];
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Abstract: Neuropathological studies suggest that the basal forebrain cholinergic system (BFC) is affected in Alzheimer's disease (AD) and aging. However, much remains as to whether both its rostral (septal) and caudal or only its caudal component are affected and whether the BF process correlates with hippocampal pathology.
The *volumetric integrity*, a novel scale-invariant measure of local brain tissue density (Ardekani et al., 2016), of the cholinergic space from the ADNI database 3D T1-weighted high-resolution structural images were estimated at baseline and one-year follow-up in AD (n=120) and cognitively normal (CN= 118) subjects using the Automatic Registration Toolbox (http://www.nitrc.org/projects/art). To define the septal (Ch1-2) and the more caudal nucleus basalis compartment (Ch4), we used a postmortem 3D probabilistic map (Zaborszky et al., 2008).
The integrity of Ch1-2 compartment was significantly reduced in AD as compared to CN subjects (p=0.000). Also, the rate of reduction of Ch1-2 integrity was significantly greater in AD (p=.000). The Ch1-2 integrity and its rate of change correlated with the corresponding hippocampal values in both the AD and CN groups separately. Furthermore, the ADAS, MMSE, and CDR scores significantly correlated with both Ch1-2 integrity and its rate of decline in the AD group but not in the CN group.
The integrity of Ch4 compartment was significantly reduced in AD as compared to CN subjects (p=0.000). The rate of reduction of Ch4 integrity was also significantly greater in AD (p=.017). Ch4 integrity was correlated with the hippocampal integrity only within the patient group. Rate of decline of the Ch4 integrity was not correlated with that of hippocampus in either group. Within the AD group, the ADAS, MMSE, and CDR scores significantly correlated with Ch4 integrity, while its rate of change only correlated with the MMSE. Finally, within the AD group,

the Ch4 integrity significantly correlated with the number of APOE e4 alleles carried by the patient.
The preliminary results suggest our volumetric integrity measure is sensitive to AD pathology and cognitive deficits. Our analysis also suggests that Ch4 atrophy may occur at an earlier stage of the disease with stronger effect on APOE e4 carriers. On the other hand, at the mild to moderate AD stage, the Ch1-2 component seems to be more vulnerable to AD pathology. Further studies are needed to identify if specific integrity changes in the cholinergic space correlate with specific cognitive domains in MCI and AD patients. These studies will ascertain whether BFC integrity measurements can be used as a biomarker to select patient population at risk and lay the foundation for early intervention.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.13/H21

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

Support: U24 AG041689

Title: Nia genetics of alzheimer’s disease data storage site (niagads): 2016 update

Authors: ***L. QU**[1], A. PARTCH[1], P. GANGADHARAN[1], O. VALLADARES[1], M. CHILDRESS[1], R. CWEIBEL[1], J. MALAMON[1], H.-J. LIN[1], Y. ZHAO[1], E. GREENFEST-ALLEN[2], C. J. STOECKERT JR.[1], A. NAJ[1], G. SCHELLENBERG[1], L.-S. WANG[1];
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Abstract: Background: NIAGADS is a national genetics data repository that facilitates access of genotypic data to qualified investigators for the study of the genetics of late-onset Alzheimer's disease (AD) and other neurological diseases. Collaborations with large consortia such as the Alzheimer's Disease Genetics Consortium (ADGC) and the Alzheimer's Disease Genome Sequencing Project (ADSP) allows NIAGADS to lead the effort in managing large AD datasets that can be easily accessed by the research community and utilized to its fullest extent. NIAGADS’ position as the centralized location for the storage of AD genetic data and its collaboration with the National Cell Repository for AD (NCRAD) to make availability of data and samples makes it essential to the scientific community in order to achieve the National

Alzheimer's Project Act's (NAPA) research goal to discover therapeutic targets for the treatment and prevention of AD by the year 2020.
Methods: Since 2012, NIAGADS has been supported by National Institute on Aging (NIA) under a cooperative agreement (U24 AG041689). This agreement expanded the NIAGADS storage and data sharing capacity for large-scale sequencing projects. All data derived from NIA funded AD genetics studies is expected to be deposited at NIAGADS or another NIA approved site. NIAGADS has partnered with the database of Genotypes and Phenotypes (dbGaP) and the Sequencing Read Archive (SRA) in this effort. In order to facilitate research, secondary data, including GWAS summary statistics and imputation data, as well as deep phenotypes have expanded the existing ADGC collection. The redesigned Genomics Database is a searchable annotation resource that links published AD studies to AD-relevant sequence features and genome-wide annotations.
Results: As of May 2016, NIAGADS houses 35 datasets with ~50k subjects and >30 billion genotypes. With the completion of the Discovery Phase of ADSP, qualified investigators can retrieve sequencing data with ease and flexibility using the ADSP website and data portal (collaboration with dbGaP/SRA). The ADSP project generated sequencing data of >11,000 subjects; raw BAMs and quality controlled VCF files are available through the ADSP portal and dbGaP (phs000572). The ADSP is currently whole-genome sequencing (WGS) an additional 433 subjects to extend the discovery phase and is selecting ~3000 additional case/control subjects for WGS as a follow-up.
Conclusions: With a streamlined application and submission process, NIAGADS is a rich resource for AD researchers to utilize. Datasets, guidelines, and new features are available on our website at https://www.niagads.org and https://www.niagads.org/adsp/.

Disclosures: **L. Qu:** None. **A. Partch:** None. **P. Gangadharan:** None. **O. Valladares:** None. **M. Childress:** None. **R. Cweibel:** None. **J. Malamon:** None. **H. Lin:** None. **Y. Zhao:** None. **E. Greenfest-Allen:** None. **C. J. Stoeckert Jr.:** None. **A. Naj:** None. **G. Schellenberg:** None. **L. Wang:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

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Program#/Poster#: 308.14/H22

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

Support: NIA P30 AG19610

NINDS NS072026

Title: Gender differences in alzheimer's disease: brain atrophy, gender differences in alzheimer's disease: brain atrophy, histopathology burden and cognition

Authors: G. E. SERRANO, J. FILON, L. SUE, *T. G. BEACH;
Banner Sun Hlth. Res. Inst., Sun City, AZ

Abstract: Multiple studies have suggested that females are affected by Alzheimer's disease (AD) more severely and more frequently than males. Other studies have failed to confirm this and there has been no clear resolution of the issue. Difficulties include differences in study methods and male versus female life expectancy. Another element of uncertainty is that the majority of studies lack neuropathological confirmation of diagnosis. We compared clinical and pathological AD severity in more than 1000 deceased subjects with full neuropathological examinations. Age of dementia onset did not differ by gender but females were more likely to proceed to very severe disease, both clinically and pathologically, with significantly higher proportions of females having a Mini Mental State Examination score of 5 or less and Braak stage VI neurofibrillary degeneration. Median neuritic plaque densities were similar in AD females and males but females had significantly greater tangle density scores. In addition, we found that AD-control brain weight differences were significantly greater for females, even after adjustment for age, disease duration and comorbid conditions. These suggest that, when affected by AD, females progress more often to severe cognitive dysfunction, due to more severe neurofibrillary degeneration and greater loss of brain parenchyma.

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Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

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

Support: NIA Grant AG051496-01

Jane B. Cook Foundation JBC1983

Title: Identifying genetic modifiers of Alzheimer's disease-relevant phenotypes

Authors: *K. D. ONOS[1], K. J. KEEZER[1], C. J. ACKLIN[1], H. M. JACKSON[1], S. J. SUKOFF RIZZO[1], M. SASNER[1], G. W. CARTER[1,2], E. J. CHESLER[1,3], B. T. LAMB[4], G. R. HOWELL[1,2];
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Abstract: Alzheimer's disease (AD) pathology is characterized by accumulation of beta amyloid plaques, neurofibrillary tangles of tau, marked neuroinflammation and widespread neuronal loss. Unlike the human disease, mouse models of AD show minimal neurodegeneration. Therefore, The Jackson Laboratory, in collaboration with Indiana University, is working to generate, characterize, and make available improved resources for AD research. Traditional mouse models of AD involve the introduction of single or multiple AD-relevant genes onto a single mouse strain, C57BL/6J. One key difference between the AD patient population and current models concerns genetic diversity, thus we have established a panel of seven inbred strains that captures the greatest degree of genetic diversity available. Chosen strains represent the founders of the Collaborative Cross and Diversity Outbred (DO) (C57BL/6J, 129S1/SvlmJ, NZO/HiLtJ, NOD/ShiLtJ, WSB/EiJ, CAST/EiJ, PWK/PhJ). Transcriptional profiling of DO mice indicates naturally occurring variations in AD-relevant genes including *Trem2*, *Bin1*, *Abca7* and *Clu*. Each of these new strains carries the transgene developed by the Borchelt lab containing familial mutations in amyloid precursor protein (APP) and presenilin 1 (PS1). Male and female mice (APP/PS1 and wild type controls) were aged to 6 months and a variety of phenotypes were measured including metabolic profiles (e.g. weight, blood glucose and core body temperature), sleep patterns, motor activity, stress response and spatial memory. Initial analyses showed significant variation amongst the strains in response to the transgene, particularly in metabolic measures, sleep disturbance and in differential processing of APP, and plaque deposition. Both NZO.*APP/PS1* and PWK.*APP/PS1* (strains that are models of hyperinsulinemia) had significant weight loss and higher blood glucose, while WSB.*APP/PS1* (a strain resistant to diet-induced obesity) had significant weight gain, supporting a strong interaction between mechanisms involved in metabolic syndrome and AD. CAST.*APP/PS1* mice showed a significant decrease in overall plaque deposition and microglia activation state, while NOD.*APP/PS1* showed a significant increase in plaque load, evidence of tau hyperphosphorylation and neuronal loss. Transcriptional profiling is now being performed to identify genes and pathways that modulate susceptibility and resiliency to endophenotypes. This genetically diverse panel of AD mouse models represents a unique resource to improve translatability of preclinical trials and lays the foundation for further genetic studies to identify novel therapeutic targets for AD.

Disclosures: K.D. Onos: None. **K.J. Keezer:** None. **C.J. Acklin:** None. **H.M. Jackson:** None. **S.J. Sukoff Rizzo:** None. **M. Sasner:** None. **G.W. Carter:** None. **E.J. Chesler:** None. **B.T. Lamb:** None. **G.R. Howell:** None.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.16/H24

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

Title: Evaluation of cGMP concentrations in CSF as biomarker for PDE2A inhition in the dog brain.

Authors: *H. BORGHYS, P. BUIJNSTERS, D. DHUYVETTER, M. SOMERS, R. VREEKEN;
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Abstract: PDE2A is expressed both in the periphery and in the central nervous system, however, PDE2A is most abundant in the brain. More specifically, PDE2A is the most prevalent of all PDEs in the caudate putamen, the nucleus accumbens, all cortical regions and the hippocampus. This suggests that PDE2A plays a role in the regulation of intraneuronal cGMP and cAMP levels in brain areas involved in emotion, perception, concentration, learning, memory, and CNS disorders such as depression and anxiety. PDE2A inhibitors are being investigated for treatment of cognitive impairment in Alzheimer’s disease. In general, rodents are chosen as a preclinical species to look at activity of PDE2A inhibitors in the brain. However, since rodents are not the appropriate species to evaluate compounds which are Pgp substrates, an in vivo assay in a non-rodent species is sometimes needed. Additionally, an in vivo assay in a larger non-rodent species will have more translational value since biomarkers in CSF can be monitored longitudinally as in humans. We aim to use the dog as a non-rodent species to examine PDE2A inhibition in the brain. cGMP in CSF is chosen as a biomarker for functional activity following PDE2A inhibition. However, before evaluating compounds, the variability of baseline concentrations of cGMP in CSF needs to be assessed. In a first experiment, a sampling schedule mimicking a routine in-house PK/PD study for central active compounds in dogs was followed. CSF was sampled from the lateral ventricle in conscious animals instrumented with a needle guide in the skull. Six beagle dogs were sampled 4 times: in the morning (7-8 am, day -4), between 11 am and 12 pm and 3-4 pm (day 1) and again in the morning (day 2). In a second study, the group was increased to 12 animals and all animals were sampled in the morning, at noon and in the afternoon, with at least 2 days in between two consecutive samples. Thus, any effect of a previous sample on cGMP was minimized. Over this period no diurnal rhythm of the day and no sex differences were observed. The baseline cGMP concentrations varied in individual and between animals, however, they were all below 40 ng/mL albeit with exception of one measurement (49 ng/mL). The range of the CSF cGMP concentrations in the animals, which were sampled in the two schedules, was somewhat comparable for both profiles. Remarkably,

animals which are in general more active, show a trend to have higher baseline cGMP levels compared to less active animals. However, this needs to be confirmed in a larger group of animals. Based on these data, the group size required to pick up a two-fold increase from baseline cGMP concentration in CSF following compound administration was determined.

Disclosures: **H. Borghys:** A. Employment/Salary (full or part-time): Janssen R&D. **P. Buijnsters:** A. Employment/Salary (full or part-time): Janssen R&D. **D. Dhuyvetter:** A. Employment/Salary (full or part-time): Janssen R&D. **M. Somers:** A. Employment/Salary (full or part-time): Janssen R&D. **R. Vreeken:** A. Employment/Salary (full or part-time): Janssen R&D.

Poster

308. Neuropsychological, Biochemical and Imaging Biomarkers of Alzheimer's Disease and Other Neurodegenerative Diseases

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 308.17/H25

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NR014181

Title: Eeg markers of leukoaraiosis in older adults

Authors: ***N. B. LAM**, A. RAJAN, N. SCHWAB, R. LIU, M. DING;
Univ. of Florida, Gainesville, FL

Abstract: Leukoaraiosis (LA), detected by high intensity in MRI T2 images, is a white matter disorder thought to be related to small vessel disease. High levels of LA in older adults are associated with declined cognitive performance and increased risk of memory dysfunction. LA is known to be in higher concentrations along the cholinergic tracts. Considering that acetylcholine is an important neurotransmitter underlying the generation and modulation of EEG oscillatory activities, we hypothesize that impaired cholinergic transmission due to LA alters EEG alpha rhythms (8 to 12 Hz). To test this hypothesis, eight minutes of resting state EEG data, 4-minutes eyes-closed (EC) and 4-minutes eyes-open (EO), were recorded from eleven older adults with high LA and nine older adults with low LA. EC to EO alpha reactivity (i.e., alpha power in EC/alpha power in EO) and individual alpha frequency (IAF) during both EO and EC were calculated for each subject. We found that higher levels of LA were associated with lower alpha reactivity ($p = 0.0285$) as well as lower IAF (EC: $p = 0.0070$). These results demonstrate that features of EEG alpha rhythm may provide simple markers for the presence of LA. Future work will be directed at confirming these results with larger samples.

Disclosures: **N.B. Lam:** None. **A. Rajan:** None. **N. Schwab:** None. **R. Liu:** None. **M. Ding:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.01/H26

Topic: C.03. Parkinson's Disease

Support: PPRI/UBC Chair in Parkinson's Research (MJM)

Title: Frontal beta oscillations time-locked to corrective sub-movements in Parkinson's disease: modulation by L-dopa

Authors: ***A. HAJIHOSSEINI**[1], C. GONZALEZ[2], M. OISHI[2], M. J. MCKEOWN[1]; [1]Med., Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. of New Mexico, Albuquerque, NM

Abstract: Parkinson's disease (PD) is associated with abnormal oscillations in the basal ganglia (BG) and the cortex and electroencephalogram (EEG) studies have shown that L-dopa medication increases frontal beta power (13-30 Hz) in PD (George et al., 2013). In healthy subjects, frontal high-beta (20-30 Hz) oscillations are proposed to have a role in communication of task-related information (e.g., reward feedback) between the prefrontal cortex and the BG (HajiHosseini & Holroyd, 2015). This may relate to the notion that motor impairment in PD is due to a disrupted oscillatory link between motivational factors such as rewards and movement "vigour" i.e., speed and size of movement (Mazzoni, et al., 2007). We investigated whether EEG oscillations in PD during a manual-tracking task with performance feedback were affected by L-dopa.

EEG was recorded from14 subjects with PD (on and off L-dopa) and 9 age-matched control subjects in a joystick-tracking task with continuous visual feedback. The behavioural data were analyzed using a hybrid control theory framework (Oishi, et al., 2010) to extract the onsets of corrective sub-movements that were then used as temporal markers for the EEG. EEG data from all subjects were pre-processed by Independent Component Analysis followed by component clustering in EEGLAB. Bootstrapping revealed a significant frontal-central cluster where EEG was significant throughout the task.

We found that ~1s before a corrective sub-movement , beta power was higher in PD subjects on-L-dopa compared to the same group off-L-dopa. High-beta power was significantly greater in PD subjects on-L-dopa compared to healthy control subjects, but theta (4-7 Hz) power was lower in PD subjects off-L-dopa compared to healthy control subjects. In the time domain, the grand

average event-related potentials preceding the corrective sub-movements were significantly more positive in PD subjects off-L-dopa compared to on-L-dopa and compared to healthy controls. Our results suggest that L-dopa modifies frontal EEG oscillations preceding corrections guided by feedback in PD subjects. We speculate the increase in frontal beta power is a compensatory mechanism to correct for impaired movement vigour in PD. The decrease of frontal theta power in PD patients might represent impairment of frontal control mechanisms (perhaps due to medial prefrontal dopamine deficits), that remain largely unaffected by L-dopa medication. In sum, time-locked spectral and time-domain changes in EEG can provide useful information about the mechanisms underlying common treatments in PD and could serve as biomarkers to test alternative treatments for PD such as non-invasive brain stimulation.

Disclosures: A. Hajihosseini: None. **C. Gonzalez:** None. **M. Oishi:** None. **M.J. McKeown:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.02/I1

Topic: C.03. Parkinson’s Disease

Support: NSFC Grant:81371256

NSFC Grant:81171061

NSFC Grant:81361128012

Title: Movement related subthalamic ß oscillatory neurons in patients with Parkinson’s disease

Authors: *P. ZHUANG[1], R. CHEN[2], M. HALLETT[3], Q. CUI[1], Y. ZHANG[1], J. LI[1], Y. LI[1]; [1]Xuanwu Hosp, Capital Med. Uni, Beijing, China; [2]Krembil Res. Institute, Univ. of Toronto, Toronto, ON, Canada; [3]Human Motor Control Section, NINDS,NIH, Bethesda, MD

Abstract: Bacground: How the human basal ganglia processes information in generation of movement is still largely unknown. **Objective:** To explore voluntary movement related neuronal activity in the subthalamic nucleus in patients with Parkinson’s disease (PD).**Methods:** 17 PD patients (M:7; F:10) who underwent STN deep brain stimulation (DBS) for parkinsonian symptoms were studied.Their mean age was 58.5±5.4 years and mean disease duration was 6.9±3.6 years. Microelectrode recordings in the STN and EMG recording on the contralateral limbs to surgery were simultaneously performed.Patients performed at least 10 self-paced wrist extension movements at 10 sec intervals during the recordings. All patients were trained to

perform the task before surgery. Single-unit analysis with mean neuronal firing rate and coefficient of variation (CV) of inter-spike interval (ISI) were calculated.Power spectral analysis was used to evaluate neuronal oscillation patterns.Based on the movement onset, four periods were determined: baseline (2 s), pre-movement (3 s), movement execution and post-movement (2 s). ANOVA and Bonferroni test were performed to compare the change of mean firing rate and CV of neurons among four periods of movement. **Results:** 119 neurons were identified from 21 STN.Of these neurons,38.7% (n=46) were neurons with β oscillation at range of 13~30 Hz (mean 21.0 ± 5.1 Hz). Of these ß oscillatory neurons, 43.5% (n=20) were related wrist movement. 26.1% (n=12) were desynchronized with reduced firing rate, and 17.4% (n=8) were synchronized with increased firing rate. Bonferroni test showed that there were significant changes of mean firing rate between baseline and movement execution in neurons desynchronized (37.5±7.6 Hz vs 20.4±5.2 Hz, n=12) or synchronized (39.7±7.3 Hz vs 53.9±9.5 Hz, n=8) during self-paced wrist movement. Furthermore, these movement related neurons were localized in the dorsal half of the STN. **Conclusions:** These preliminary data support the view that the STN ß oscillatory neurons are modulated with the execution of self-paced wrist movement. The desynchronized or synchronized STN ß oscillatory activity might play a role in whether a movement is facilitated or suppressed. Moreover, the localizations of the movement related ß oscillatory neurons is within the dorsal portion of the STN, adding to the evidence that this is the motor part of the STN.

Disclosures: P. Zhuang: None. **R. Chen:** None. **M. Hallett:** None. **Q. Cui:** None. **Y. Zhang:** None. **J. Li:** None. **Y. Li:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.03/I2

Topic: C.03. Parkinson’s Disease

Title: Globus Pallidus internus low beta phase-amplitude coupling correlates with motor impairment in Parkinson’s disease

Authors: *M. MALEKMOHAMMADI[1], N. AUYONG[2], N. POURATIAN[2];
[1]Neurosurg., Univ. of California Los Angeles, Los Angeles, CA; [2]Neurosurg., UCLA, LOS ANGELES, CA

Abstract: Objective: The β (12-30 Hz) band is often investigated as a single entity in Parkinson’s disease (PD) pathophysiology. However, there is growing evidence that there exists functionally distinct “low” and “high” β sub-bands. Recent studies have suggested that low β

signal in the subthalamic nucleus (STN) is reflective of local activity and is responsive to therapeutic interventions. It remains to be determined if the the neuronal network within the Globus Pallidus internus (GPi), a main basal ganglia output nuclei also exhibits similar β characteristics. In this study, we aim to delineate the differential role of the low and high β sub-bands within the GPi, with respect to disease severity and contralateral hand movement in PD subjects undergoing Deep Brain Stimulation (DBS) implantation surgery. **Methods:** Intra-operative local field potentials (LFP) were recorded after placement of DBS leads (Medtronic Inc., model 3387) within motor part of GPi in 19 PD subjects bilaterally (38 GPi) (2.4 kHz sampling frequency, 500 Hz Low pass filtered). Rest trials without artifact, tremor, or voluntary movement, and trials with left hand movement were selected for analysis. Power spectral density analysis was done using Welch method (1 second windows, 90% overlap, 1 Hz frequency resolution) and Phase Amplitude Coupling (PAC) was calculated by modulation index (MI) (phase: 1-35 Hz with 2 Hz bandwidth; amplitude: 50-400 Hz with bandwidth of double phase encoding frequency). 100 surrogate data sets were generated to assess the statistical significance of MI values at $P< 0.05$ and P-values were corrected for multiple comparisons. Pearson's Correlation with clinical scores (lateralized UPDRS scores, and separate scores for tremor, rigidity and bradykinesia) was calculated ($P<0.05$) and one sample t-test was used to test significance level of movement related modulation in both power and PAC. **Results:** We found a bimodal distribution of spectral peaks at 15.63±2.44 and 24.5± 5.05 Hz for low and high β, respectively. Although both low and high β power decrease with contralateral hand movement; neither showed significant correlation to the lateralized UPDRS scores. In addition, low β-encoded PAC in motor GPi was significantly reduced with hand movement. The low β PAC ratio between movement and rest was significantly correlated to lateralized scores for bradykinesia and rigidity. **Conclusions:** With the emerging concept of the differential role of low and high β frequencies in PD pathophysiology, we present findings for shared β sub-bands properties between GPi and STN that suggests that β sub-bands oscillations are related to both movement and disease severity.

Disclosures: **M. Malekmohammadi:** None. **N. AuYong:** None. **N. Pouratian:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 309.04/I3

Topic: C.03. Parkinson's Disease

Support: NIH R37 NS040894

NIH R01 NS079312

Title: Neural and motor responses to temporally non-regular deep brain stimulation in Parkinson's disease

Authors: *B. D. SWAN[1], D. BROCKER[1], C. OZA[1], D. TURNER[2], W. GRILL[1];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Neurosurg., Duke Univ. Med. Ctr., Durham, NC

Abstract: Abnormal oscillatory and synchronized neural activity in the basal ganglia is increasingly implicated in the pathophysiology of Parkinson's disease (PD). Local field potentials (LFPs) recorded from the subthalamic nucleus (STN) of humans with PD exhibit increased oscillatory activity in the beta band (10-35 Hz) that correlates with decreased motor performance. Deep brain stimulation (DBS) of the STN is an effective therapy that alleviates motor symptoms in PD and suppresses beta band activity. We previously found that specific temporally non-regular patterns of DBS improved motor task performance and suppressed beta oscillatory activity in a computational model of the basal ganglia (Brocker et al., 2013). Here we conducted intraoperative measurements of STN LFPs and PD motor symptoms in human subjects during temporally non-regular DBS to investigate the effects on neural oscillations in the beta frequency band. Following informed consent, six human subjects with PD participated in our study during STN DBS implant surgery. We quantified neural and motor responses to high frequency temporally regular and non-regular DBS by recording STN LFPs during tremor or an alternating finger tapping task. Neural recordings showed high oscillatory activity in the beta frequency band in the absence of DBS, and behavioral measures reflected poor motor task performance. Temporally regular and non-regular patterns of DBS reduced beta band power and improved PD motor symptoms in some, but not all subjects. These data further demonstrate that temporal pattern influences the efficacy of DBS in PD. Additionally, neural recordings confirm that non-regular patterns of DBS suppress pathological oscillatory activity in the human STN. Further design of temporally patterned DBS may help elucidate the mechanisms of action of DBS in PD and improve the efficacy of the therapy in ameliorating motor symptoms.

Disclosures: B.D. Swan: None. **D. Brocker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity holder in Deep Brain Innovations, LLC. Inventor on licensed patents.. **C. Oza:** None. **D. Turner:** None. **W. Grill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity holder in Deep Brain Innovations, LLC. Inventor on licensed patents..

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.05/I4

Topic: C.03. Parkinson's Disease

Support: DARPA/ARO Contract # W911NF-14-2-0043

Title: High resolution electrocorticography in prefrontal cortex in Parkinson's disease patients

Authors: *C. DE HEMPTINNE[1], W. CHEN[2], A. MILLER[2], C. RACINE[2], P. STARR[2]; [1]Dept. of Neurolog. Surgery, Univ. of California San Francisco, San Francisco, CA; [2]UCSF, San Francisco, CA

Abstract: Background: Parkinson's disease (PD) is characterized by a constellation of motor and non-motor symptoms including depression, anxiety and apathy. Motor symptoms have been well studied and are associated with excessive neuronal synchronization in the beta band (13-30Hz) throughout the motor basal-ganglia-thalamo-cortical (BGTC) loop. Despite results from non-invasive and imaging studies suggesting a dysfunction of the cognitive and limbic BGTC loops, the pathophysiology underlying non-motor symptoms is still largely unknown. **Objective:** To investigate the neurophysiology of mood symptoms in PD using electrocorticography (ECoG), a technique that offers high spatial and temporal resolution. **Methods:** A 28-contact ECoG strip was temporarily placed over the prefrontal cortex of 22 PD patients undergoing deep brain stimulation (DBS) implantation surgery. A strip was placed over the dorsolateral prefrontal cortex (DLPFC) in 10 patients, spanning both the ventrolateral prefrontal cortex (VLPFC) and the orbitofrontal cortex (OFC) in 12 patients. Before surgery each patient's mood was evaluated using multiple standardized rating scales including the Edinburgh Depression Scale (EDS) and the Apathy Evaluation Scale (AES). The power spectral density (PSD) was computed for each ECoG contact and averaged across the entire beta band (13-30Hz), as well as sub-divided between the low (13-20Hz) and high (20-30Hz) beta bands. Given the importance of beta in the pathophysiology of PD, the contact associated with the maximum beta power was selected to investigate the correlation between beta power and mood symptoms. **Results:** We found a prominent beta peak in the power spectrum of the prefrontal ECoG potential in most patients (20/22). Topographically, beta power was maximal over the lateral portion of the OFC (8/12 patients) and anterior portion of the DLPFC (6/10 patients). We found that depression (measured with EDS) and apathy (measured by the AES) were both significantly correlated to low beta power in OFC, but not in DLFPC. **Conclusion:** These preliminary results suggest that neuronal synchronization in the beta band extends outside the motor system to the prefrontal cortex in PD, highlighting the importance of these oscillations in the diverse pathophysiology of PD. These findings could indicate a potential role of OFC beta power in non-motor symptoms of PD.

Disclosures: **C. de Hemptinne:** None. **W. chen:** None. **A. Miller:** None. **C. Racine:** None. **P. Starr:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.06/I5

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS069779

Title: Sensorimotor beta coherence with high-resolution electrocorticography in Parkinson's disease patients

Authors: ***W. CHEN**[1], C. DE HEMPTINNE[1], N. ROWLAND[2], P. A. STARR[1];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Introduction: In Parkinson's disease, the basal-ganglia-thalamo-cortical circuit is hypothesized to be in a pathological state of excessive beta band (13-30 Hz) neural synchronization. Studies using noninvasive techniques report greater global cortical beta coherence, a common measure of synchronization, when patients are clinically more symptomatic. This is reduced with dopaminergic medication or during basal ganglia deep brain stimulation (DBS). To date, electrophysiological studies lack adequate spatial and/or temporal resolution to assess cortical coherence within sensorimotor areas.
Objective: To investigate the role of cortico-cortical synchronization within and between premotor (PM), primary motor (M1), and primary somatosensory (S1) cortices in disease pathophysiology, we utilized high-resolution electrocorticography (ECoG) to compare oscillatory coherence in Parkinson's disease (PD) and essential tremor (ET) patients.
Methods: We collected intraoperative high-resolution ECoG (28-contact, 4 mm spacing center to center) data from 10 PD patients and 8 ET patients undergoing awake DBS surgery, with coverage over the superior frontal gyrus, precentral gyrus, and postcentral gyrus. Neural activity was recorded both at rest and during a cued movement reaching task, which cycled between intervals of movement and movement-hold. We analyzed magnitude-squared coherence across all electrode pair combinations, averaging across the beta band, during rest, movement, and movement-hold. Following anatomic localization of each electrode, we quantified between-gyrus and within-gyrus coherence.
Results: Both at rest and during movement-hold, beta band coherence between and within M1, S1, and PM cortices were not significantly different between PD and ET patients. During movement, however, there was decreased M1-PM and M1-S1 beta coherence in PD compared to

ET. Further analysis of beta band subdivisions revealed that low beta (13-20 Hz) coherence is significantly less in M1-PM and M1-S1 in PD.
Conclusions: Our preliminary results suggest that during voluntary movement, there is reduced beta coherence within and between M1, S1, and PM cortices in PD compared to ET. This may be a compensatory mechanism by which the cortex reduces the influence of PD-related hyper-synchronization.

Disclosures: W. Chen: None. **C. de Hemptinne:** None. **N. Rowland:** None. **P.A. Starr:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS054864

National Parkinson Foundation

NIH P01 NS-083514

McDonnell Foundation

Title: Resetting and saving of beta oscillatory changes during motor practice in healthy subjects and in Parkinson's Disease

Authors: J. LIN[1], P. PANDAY[1], A. B. NELSON[1], M. S. VENANZI[2], C. MOISELLO[1], A. DI ROCCO[3], *M. F. GHILARDI[1];
[1]CUNY, New York, NY; [2]Univ. of Milan, Milan, Italy; [3]The Fresco Inst. for PD and Movement Disorders, NYU Langone Med. Sch., New York, NY

Abstract: In a recent EEG study, we have found that the modulation depth of beta power during movement increases with practice over the sensorimotor and frontal areas of normal subjects but not over those of patients with PD (Moisello et al., Brain Behav. 2015 Sep 23;5(10):e00374). In line with studies suggesting that such beta power changes might reflect cortical plasticity phenomena and in particular use-dependent modifications, we concluded that reduction of beta enhancement in PD might represent saturation of cortical plasticity. However, a few questions remained open: Would a second task exposure on the following day increase such phenomenon in normal subject and restore beta modulation enhancement in PD? Do practice-induced increases of beta modulation occur within each block of consecutive movements? And, if so, do

these changes occur in patients with PD? We thus recorded high-density EEG in 11 patients with PD and 13 age-matched controls in two consecutive days during a forty-minute reaching task divided in fifteen blocks of 56 movements each. The results confirmed that on day 1, with practice, beta modulation depth over the contralateral sensorimotor area significantly increased across blocks in normal controls but not in PD, while performance indices improved in both groups without significant correlations between behavioral and EEG data. The following day, beta modulation values re-started at the same baseline values of day 1 and increased across trials within the same range of day 1 in both groups, without differences between the two days. Further analyses of data of both days revealed that the increases at the end of each block were partially carried onto the successive block in controls, while no significant carry over was observed in PD. We conclude that the lack of significant practice-related increase of mean modulation depth across each session we observed in PD is likely to deficient mechanisms of potentiation permitting between-block saving of beta power enhancement. Moreover, we speculate that the resetting of beta modulation values might reflect mechanisms of homeostatic regulation.

Disclosures: J. Lin: None. **P. Panday:** None. **A.B. Nelson:** None. **M.S. Venanzi:** None. **C. Moisello:** None. **A. Di Rocco:** None. **M.F. Ghilardi:** F. Consulting Fees (e.g., advisory boards); New York University.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.08/I7

Topic: C.03. Parkinson’s Disease

Support: Doris Duke Clinician Scientist Development Award

NIH Grant T32MH020068

Title: Rapid prediction of movement states from ongoing neural activity in Parkinson’s Disease

Authors: *M. AHN[1,2], S. LEE[1,2], J. A. GUERIN[1,2], D. J. SEGAR[3], T. V. SANKHLA[3], W. F. ASAAD[1,2,3,4,5];
[1]Neurosci., [2]Brown Inst. for Brain Sci., [3]Neurosurgery, Warren Alpert Med. Sch., Brown Univ., Providence, RI; [4]Neurosurg., [5]Norman Prince Neurosciences, Rhode Island Hosp., Providence, RI

Abstract: Parkinson’s disease (PD) is a debilitating movement disorder that affects approximately 1 million people in the US and is characterized by abnormal movements such as

resting tremor, bradykinesia, rigidity, and gait abnormalities that impair the quality of life of PD patients. Furthermore, motor deficits in PD are evaluated on a relatively long time scale by subjective measures such as the Unified Parkinson's Disease Rating Scale (UPDRS). In reality however, movement states may vary over the timescale of seconds or minutes. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) or the globus pallidus internus (GPi) is a commonly utilized therapy to treat PD motor symptoms, but remains relatively imprecise, because continuous electrical stimulation is delivered without responding to behavioral or neurophysiological states.To develop a closed-loop DBS system that can respond in real-time to functionally relevant movement fluctuations, we sought to quantify rapid changes of movement quality and determine the corresponding neural activity. States associated with or predictive of poor motor performance may be useful as biomarkers for controlling closed-loop DBS. We designed a continuous motor performance task to identify short timescale fluctuations in motor performance. PD patients used a joystick to move an on-screen cursor and were asked to follow a moving target. Some task sessions were collected alongside simultaneous local field potential (LFP) recordings from STN or GPi during DBS lead implant surgery, while others were collected pre-operatively in a clinical setting. We employed two metrics, quantifying instantaneous tremor magnitude (the absolute value of bandpass (4-12 Hz) filtered joystick data) and vector error (the difference between the velocities of the target and joystick). We observed that motor symptoms were differentiated well with those two metrics. We investigated the spectral power of LFP and the Phase-Amplitude Coupling (PAC) within STN/GPi to identify neural correlates of this behavioral variability. We found that the severity of abnormal movements was associated with increases in spectral power and with PAC features. We also applied machine learning that incorporates those features in order to predict movement states on a rapid timescale. We discuss the implications of our results in designing a closed-loop DBS system that may help to improve the efficiency of DBS treatments for PD patients.

Disclosures: **M. Ahn:** None. **S. Lee:** None. **J.A. Guerin:** None. **D.J. Segar:** None. **T.V. Sankhla:** None. **W.F. Asaad:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.09/I8

Topic: C.03. Parkinson’s Disease

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Title: Differential effect of bicycling and walking on subthalamic high-frequency oscillations in Parkinson's disease

Authors: *L. STORZER[1], M. BUTZ[1], J. HIRSCHMANN[1,2], O. ABBASI[1,3], M. GRATKOWSKI[4], D. SAUPE[4], J. VESPER[6], A. SCHNITZLER[1,7], S. S. DALAL[8,5];
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Abstract: Patients with advanced Parkinson's disease (PD) often suffer from severe walking difficulties, called freezing of gait, while they are still able to ride a bike easily [1, 2]. This phenomenon suggests functional differences in the brain networks subserving bicycling and walking. High-frequency oscillations (HFO; > 200 Hz) observed in the basal ganglia (BG) have been assumed to be prokinetic as they increase with voluntary movement and medication [4, 5]. This study aimed to contrast HFO dynamics during bicycling and walking in the BG of PD patients.
10 PD patients (mean age 55.8 +/- 6.3 y) clinically selected for bilateral deep brain stimulation (DBS) therapy were included. Using a portable EEG system, local field potentials from the subthalamic nucleus (STN) were recorded using externalized leads the day after implantation of DBS electrodes. Patients were recorded while OFF any dopaminergic medication during unconstrained walking and bicycling on a stationary bicycle and the respective baseline conditions, i.e., standing and sitting. Spectral peaks were predominantly found in the slow HFO range (200-300 Hz) in 13 STNs that were considered for further analysis. A two-way repeated measures ANOVA was computed for slow HFO power with the within-subject factor movement (rest/move) and posture (upright/sitting on bike).
HFO power was higher in the upright condition, irrespective of the movement state ($p < 0.05$). Furthermore, the ANOVA revealed a significant interaction between movement and posture ($p < 0.05$). Post-hoc paired t-tests showed that power was significantly higher during standing compared to sitting ($p < 0.05$), whereas power during walking and pedaling was similar ($p > 0.05$). This was especially due to a power increase from sitting to pedaling, as opposed to a slight power decrease from standing to walking. Our results are in line with previous studies associating the OFF state in PD with spectral peaks in the slow HFO range [3, 4]. Our analyses

revealed a differential effect of bicycling and walking on HFO power. HFO power increased from sitting to pedaling while it decreased from standing to walking. Therefore, one may speculate that bicycling boosts the basal ganglia's HFO output and thus facilitates movement. However, power was found to be overall higher during standing and walking. This may emphasize posture and balance influencing HFO power.
[1] Snijders et al. 2012 *J Neurol Neurosurg Psychiatry*.
[2] Snijders et al. 2011 *Mov Disord.*
[3] López-Azcárate et al. 2010 *J Neurosci.*
[4] Özkurt et al. 2011 *Exp Neurol*.

Disclosures: **L. Storzer:** None. **M. Butz:** None. **J. Hirschmann:** None. **O. Abbasi:** None. **M. Gratkowski:** None. **D. Saupe:** None. **J. Vesper:** F. Consulting Fees (e.g., advisory boards); Medtronic Inc, Boston Scientific, St. Jude Medical. **A. Schnitzler:** 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; Theva Pharma. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Medtronic Inc, Boston Scientific, St. Jude Medical, UCB, MEDA Pharma, TEVA Pharma, GlaxoSmithKline. F. Consulting Fees (e.g., advisory boards); Medtronic Inc, Boston Scientific, St. Jude Medical. **S.S. Dalal:** None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.10/I9

Topic: C.03. Parkinson's Disease

Title: Effects of STN deep brain stimulation on voice motor control in Parkinson's disease

Authors: *R. BEHROOZMAND[1], P. HERATH[2], K. JOHARI[2], R. KELLEY[3], E. KAPNOULA[3], K. BRYANT[3], N. NARAYANAN[3], J. GREENLEE[3];
[1]Communication Sci. and Disorders, [2]Univ. of South Carolina, Columbia, SC; [3]Univ. of Iowa, Iowa City, IA

Abstract: The present study investigated the effects of subthalamic nucleus (STN) deep brain stimulation (DBS) on the mechanisms of voice motor control in Parkinson's disease (PD). A total of ten PD individuals with bilateral STN-DBS implantation were tested in two separate blocks with DBS OFF vs. ON while they repeatedly maintained steady vocalizations of the vowel sound /a/ at their conversational pitch and loudness for 200 trials. The duration of each

vocalization was approximately 2-3 seconds and subjects took short breaks (~ 2 seconds) between successive vocalizations. During each vocalization trial, a brief (200 ms) pitch-shift stimulus was delivered to perturb voice auditory feedback. Pitch-shift stimuli were presented randomly at upward (+100 cents) and downward (-100 cents) directions and their onset time was randomized between 800-1200 ms relative to the onset of each vocalization. Vocal responses to auditory feedback perturbation were extracted for each PD individual during DBS OFF and ON conditions. In addition, the measures of pitch, loudness, harmonic-to-noise ratio (HNR), jitter and shimmer were calculated across all vocalization trials during DBS OFF and ON. Results of the analysis indicated that all subjects produced compensatory responses that changed the pitch frequency of their voice in the opposite direction to auditory feedback perturbation. We observed that DBS significantly modulated the magnitude of compensatory vocal responses only for downward pitch-shift stimuli and subjects produced upward vocal responses that were significantly larger in magnitude during DBS ON compared with DBS OFF condition. In addition, we found that the overall variability in voice pitch was significantly reduced for DBS ON compared with DBS OFF, as indexed by the measure of voice jitter. These findings indicate that STN-DBS has a positive impact on the mechanisms of voice motor control by helping individuals better control their voice pitch. However, the differential effects of DBS on vocal responses to upward and downward pitch perturbations in the auditory feedback suggest that the mechanisms that drive vocal folds muscle contraction (raising pitch) and relaxation (lowering pitch) are not equally facilitated during DBS. These findings provide new insights into the mechanisms that incorporate auditory feedback to regulate and control voice pitch in individuals with PD.

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Poster

309. Oscillatory Activity in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: Gifts to the Brain Restoration Center

National Center for Advancing Translational Sciences, through grant UL1TR000117

Title: Parkinson's disease outcomes of implanting deep brain stimulation leads to the globus pallidus internus while undergoing surgery under general anesthesia

Authors: N. S. TIMONEY[1], *J. E. QUINTERO[4,2], J. H. SMITH[1], F. N. MCCARRON[3], G. A. GERHARDT[4,2], C. G. VAN HORNE[4,2,1];
[1]Neurosurg., [2]Brain Restoration Ctr., [3]Surgery, Univ. of Kentucky, Lexington, KY; [4]Anat. & Neurobio., Univ. Kentucky, Lexington, KY

Abstract: One of the chief complaints of patients with Parkinson's disease (PD) who undergo deep brain stimulation (DBS) surgery is the awake portion of the surgery. As a result, individuals who would otherwise be good candidates for DBS may elect to not have the surgery and forgo the benefits of DBS therapy. Here we describe retrospectively and prospectively collected data on individuals with PD who have undergone standard DBS surgery while under general anesthesia at our center. Susceptibility weighted imaging (SWI) was used for pre-operative planning to identify the target locations in the globus pallidus internus (GPi). DBS leads were implanted under general anesthesia (typically ~ 1.2 - 1.8% sevoflurane). Single-pass microelectrode recordings (MER) and macrostimulation testing were used to intraoperatively identify the target and side effects. MDS-Unified Parkinson's Disease Rating Scale (UPDRS) testing was evaluated before and after surgery. Post-operatively, DBS electrodes were mapped and compared to planned targets. Over 60 individuals with PD have received DBS lead implants to the GPi while undergoing the surgery asleep at our center. In 14 individuals who underwent post-surgery UPDRS assessment 6 to 18 months after surgery: pre-surgery, mean UPDRS part III (motor) score OFF therapy was 59.3 ± 15.9 (Mean ± SD) and ON therapy was 36.8 ± 17.0. After surgery, ON stimulation/OFF medication mean score was 38.1 ± 15.6 (103% of pre-surgery ON therapy) and ON stimulation/ON medication score was 26.9 ± 13.2 (73% of pre-surgery ON therapy). Post-surgery OFF stimulation/OFF medication score for 10 individuals was 59.1 ± 14.9 (99.7% of pre-surgery OFF therapy). These results support intraoperative MER and macrostimulation testing as a method for electrode placement in asleep patients while showing that implanting DBS leads to the GPi while individuals are under general anesthesia provides an alternative means of offering DBS therapy.

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Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

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Topic: C.03. Parkinson's Disease

Title: Feasibility of 1.5T fMRI BOLD activation patterns for guiding deep brain stimulation targeting and parameter selection demonstrated in a large-animal model

Authors: T. A. JERDE[1], N. REINKING[2], M. KELLY[2], T. BILLSTROM[2], L. LENTZ[2], *R. S. RAIKE[2];
[1]New York Unveristy, New York, NY; [2]Medtronic Inc, Minneapolis, MN

Abstract: High-frequency subthalamic nucleus (STN) deep brain stimulation (DBS) is used to treat the movement disorders Parkinson's disease and dystonia. However, the therapeutic mechanisms of DBS are not well understood. Electroencephalographic (EEG) studies in patients implicate antidromic activation of the hyperdirect corticosubthalamic pathway in the therapeutic benefits of STN DBS. Similarly, functional magnetic resonance imaging (fMRI) in patients and large-animal models demonstrates that STN DBS can be associated with significant activation of blood-oxygen-level-dependent (BOLD) signals within motor cortical areas. However, due to a number of practical, technical and safety limitations, few of these studies have systematically explored stimulation parameters, which are known to drive therapeutic outcomes. Therefore, the purpose of this study was to assess the effects on motor cortical activation of different STN DBS parameters, including electrode location, and stimulation amplitude and frequency in an STN DBS sheep model. Animals were chronically implanted with clinical-grade DBS systems and imaging was performed using a 1.5T MRI system and human safety power guidelines for DBS. Activation maps revealed a dependence on DBS parameters, whereby an optimal electrode configuration, stimulation voltage and stimulation frequency range were identified that produced significant ipsilateral motor cortical BOLD activation. Conversely, non-optimal parameters were associated with more widespread or diffuse motor network activation patterns. Overall, the results support the feasibility of using 1.5T fMRI-BOLD activation patterns for guiding DBS targeting and parameter selection for movement disorders.

Disclosures: **T.A. Jerde:** F. Consulting Fees (e.g., advisory boards); Medtronic. **N. Reinking:** A. Employment/Salary (full or part-time): Medtronic. **M. Kelly:** A. Employment/Salary (full or part-time): Medtronic. **T. Billstrom:** A. Employment/Salary (full or part-time): Medtronic. **L. Lentz:** A. Employment/Salary (full or part-time): Medtronic. **R.S. Raike:** A. Employment/Salary (full or part-time): Medtronic.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 309.13/I12

Topic: E.03. Basal Ganglia

Support: The Swedish research council

Bertil Hallsten Foundation

Ahlén Foundation

Title: A novel approach for measuring light-evoked neurotransmitter release

Authors: *A. K. KONRADSSON-GEUKEN, T. VIERECKEL, G. SERRA, A. WALLÉN-MACKENZIE;
Organismal Biol., Uppsala Univ., Uppsala, Sweden

Abstract: A technical challenge so far has been to record fast *in vivo* neurotransmission such as, the release of glutamate. Electrochemical measurements with amperometry allow *in vivo* quantification of specific neurotransmitters with an outstanding temporal- and spatial resolution. Most studies today utilize electrical- or pharmacological stimulation to evoke neurotransmitter release. However, these approaches lack spatial selectivity. The aim of this study was to utilize optogenetic activation of restricted cell populations of transgenic animals to measure glutamate release with *in vivo* amperometry. Mice expressing Cre recombinase in Pitx2-positive cells in the subthalamic nucleus (STN) were injected with Cre-dependent adeno-associated viruses carrying Channelrhodopsin 2. Applying light pulses of 10-, 20- and 40 Hz, glutamate release was measured in these animals at a holding potential of +0.7, using glutamate Oxidase (GluOx)-coated multi-channel electrodes. Possible laser artifacts were identified with sentinel channels lacking GluOx and the use of a holding potential of +0.25 V, at which no glutamate activity is present. Enzyme activity and electrode efficacy were tested through local exogenous glutamate application. By combining two unique methods, optogenetics with amperometry in the anesthetized mouse, we have established a new protocol for the analysis of glutamate release from spatially restricted cell populations. By utilizing our transgenic animals and the combinations of methods described above, we will be able to further explain the importance of glutamate in the STN network which could be of clinical relevance as the STN is an area used for deep brain stimulation in patients suffering from Parkinson's disease.

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Poster

309. Oscillatory Activity in Parkinson's Disease

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Topic: E.03. Basal Ganglia

Support: T32 NS007480

Data collected in the lab of Dieter Jaeger

Title: Optogenetic stimulation parameter settings and candidate electrophysiological features for maximizing behavioral response

Authors: *T. H. SANDERS;
Biol., Emory Univ. Dept. of Biol., Atlanta, GA

Abstract: High frequency (100 - 200 Hz) electrical stimulation of the subthalamic nucleus is known to ameliorate bradykinesia in human patients. We have recently shown that high frequency optogenetic stimulation of motor-cortico-subthalamic neurons also ameliorates bradykinesia in the 6-OHDA unilaterally lesioned mouse model of Parkinson's disease. Here I report results from a study performed in a subset of these mice. In this study, mice (N = 4) were stimulated at frequencies ranging from 30-130 Hz with pulse widths that increased with decreasing frequency such that the amount of light delivered per unit time remained constant. The results suggest that certain optogenetic behavioral effects may depend on the amount of light delivered to transfected neurons, rather than the frequency of stimulation. I also show in this subset of mice that power, phase-amplitude coupling, and coherence features are significantly changed between normal, parkinsonian, and optogenetically treated states. Future studies will evaluate the suitability of these candidate features for feedback control of closed-loop stimulation.

Disclosures: T.H. Sanders: None.

Poster

309. Oscillatory Activity in Parkinson's Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: E.03. Basal Ganglia

Support: The Grainger Foundation

Title: Development and implementation of a large animal model for investigation of neurochemical activity during behavior

Authors: *J. TREVATHAN, A. D. BATTON, E. N. NICOLAI, K. H. LEE, J. L. LUJAN; Mayo Clin., Rochester, MN

Abstract: Introduction: Significant research efforts have studied the role of dopamine in motor control, motivation, and reinforcement learning, as well as implicated in pathologic changes in dopamine signaling in many neurologic and psychiatric disorders. Previous studies have used fast scan cyclic voltammetry (FSCV) to study neurotransmission by chronically measuring sub-second changes in neurotransmitter release in awake small animal models (Clark et al. 2010). However, these models are not suitable for clinical translation. Additionally, the stiffness of the recording electrode and wired connections to the neurochemical recording device make existing systems unsuitable for neurochemical sensing in freely-moving large animals. Here we describe the development and implementation of a freely-moving and chronically implanted large animal model for investigation of neurochemical activity. **Methods:** We used a custom built carbon fiber microelectrode (CMF) with a flexible lead system, a deep brain stimulation electrode, and a wireless backpack mounted device, WINCS Harmoni, for simultaneous neurochemical recording and stimulation in an awake swine. To manufacture the flexible CFM, we built a sensing element, T-300 carbon fiber, into the front-end of1.27 mm diameter Silastic^{TM} tubing. A ring reference electrode was mounted proximal to the tip of the electrode. Connections to the sensing element and reference electrode were made with stainless steel wire coiled around a tungsten stylet. Using image-guided stereotactic targeting, this flexible electrode was implanted into the swine striatum. Similarly, a stimulating electrode (NuMed, Hopkinton, NY) was implanted into the substantia nigra pars compacta/ventrotegmental area. WINCS Harmoni was placed inside a backpack and connected to the externalized electrodes. Neurochemical release and associated behavioral responses were recorded in the awake animal 48 hours after implantation and once weekly thereafter to characterize changes in the neurochemical response. **Results:** A flexible CFM was developed. This electrode allowed for chronic implantation and wireless monitoring of stimulation-evoked neurochemical responses when used in combination with WINCS Harmoni. **Conclusion:** The approach described herein allows for neurochemical monitoring of awake behaving animals, which will provide novel insight into the neurochemical correlates of behavior. As such, this technique represents the first step toward studies aimed at examining the role of sub-second fluctuations in neurochemical activity during behaviors associated with neurologic health, disease, and response to treatment.

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Poster

309. Oscillatory Activity in Parkinson's Disease

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Topic: C.09. Brain Injury and Trauma

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NRF 2015R1C1A2A01053318

Title: Optogenetics stimulation of entopeduncular input affects thalamic signaling and behavior in α-synuclein-induced hemiparkinson rat model

Authors: *H. MOON[1], Y. PARK[2], B. OH[2], C. CHUL BUM[3], Y. LEE[4];
[1]Med. Neuroscience, Col. of Med., Cheongju, Korea, Republic of; [2]Neurosurgery, Col. of Med., Chungbuk Natl. Univ. Hosp., Cheongju, Korea, Republic of; [3]Neurosurgery, St. Vincent's Hosp., Seoul, Korea, Republic of; [4]Radiology St. Mary's Hosp., Daejeon, Korea, Republic of

Abstract: Neuromodulation of the globus pallidus internus (GPi) alleviates Parkinson's disease symptoms, yet its mechanism is unclear. So we focus on entopeduncular nuclei(EP) in rats, which is homologous to the globus pallidus interna(GPi). We generated a hemiparkinson rat model by injecting an adeno-associated virus type-2 expressing α-synuclein (AAV2-α-syn) into the substantia nigra pars compacta (SNc) of the right brain to test whether optogenetic stimulation of the EP could alter parkinsonian behaviors or thalamic discharge. First, we confirmed parkinsonian behavior in our model using an amphetamine-induced rotation test. Then, we optogenetically activated or inhibited neurons, using the halorhodopsin (NpHR)/channelrhodopsin 2 (ChR2) system in the EP of the hemiparkinson rat model to determine downstream effects on parkinsonian behaviors and thalamic discharge *in vivo*. We assessed alterations in parkinsonian behaviors using the stepping and cylinder tests before, during, and after optogenetic stimulation. Importantly, we found that optogenetic inhibition of the EP improved parkinsonian motor behaviors. Moreover, we monitored thalamic neuronal activity following optogenetic neuromodulation *in vivo*, and we observed alterations in thalamic discharge. Taken together, our data demonstrate that optical neuromodulation in the EP can be used to successfully control contralateral forelimb movement and thalamic discharge to alleviate symptoms in an AAV2-α-syn-induced hemiparkinson rat model.

Disclosures: **H. Moon:** None. **Y. park:** None. **B. Oh:** None. **C. Chul Bum:** None. **Y. Lee:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Program#/Poster#: 310.01/J4

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS077954

William N. & Bernice E. Bumpus Foundation Innovation Award

DSF Charitable Foundation

Title: Evaluating mitochondrial biogenesis in a cell model of Parkinson disease via mitochondrial DNA replication in neuron cell bodies, axons, and dendrites

Authors: *V. S. VAN LAAR[1,2], L. H. SANDERS[1,2], B. ARNOLD[1,2], E. H. HOWLETT[1,2], J. T. GREENAMYRE[1,2], S. B. BERMAN[1,2];
[1]Dept. of Neurol., [2]Pittsburgh Inst. for Neurodegenerative Dis., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Evidence implicates dysregulation of mitochondrial homeostasis and quality control in neurodegenerative diseases such as Parkinson's disease (PD). We had previously found that exposure of primary neurons to chronic, sublethal doses of rotenone, a Complex I inhibitor linked to PD, was associated first with increased mitochondrial density in distal neurites, followed by later increases in mitochondrial density in cell bodies. The increased mitochondrial density in neurites prior to cell bodies was not accounted for by changes in mitochondrial transport or localized changes in mitochondrial degradation. This suggested the possibility that localized changes in mitochondrial biogenesis could be occurring in axons/dendrites, but methodology for direct studies targeting neuroanatomical localization of mitochondrial biogenesis was lacking. We have optimized methodology to directly image, localize, and quantify replicating mitochondrial DNA (mtDNA) in neurons using BrdU incorporation and immunocytochemistry. We are able to visualize newly synthesized mtDNA replication within minutes in neurons *in vitro*. The BrdU incorporation is inhibited, as expected, by the addition of 2',3'-dideoxycytidine (ddC), inhibitor of mitochondrial DNA polymerase gamma. Interestingly, as neurons 'age' in culture from 7 to 21 days, rates of mtDNA synthesis increase. This increase is also associated with increased expression of PGC1alpha, suggesting increased mitochondrial

biogenesis with senescence. To address the effects seen in our previous studies, we are also evaluating the effects of chronic, sublethal rotenone on localized mtDNA replication. These studies give us the ability to better elucidate mtDNA replication/mitochondrial biogenesis in neurons and under PD-relevant conditions.

Disclosures: V.S. Van Laar: None. **L.H. Sanders:** None. **B. Arnold:** None. **E.H. Howlett:** None. **J.T. Greenamyre:** None. **S.B. Berman:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Program#/Poster#: 310.02/J5

Topic: C.03. Parkinson's Disease

Support: Mitchel Center for Neurodegenerative Diseases

STARS Award

UT Startup Funds

Title: Investigating molecular mechanisms regulating mitochondrial quality control

Authors: *K. BUSH[1], A. M. BUCKLEY[2], K. R. BARBER[2], M. WOODSON[2], M. B. SHERMAN[2], Y. WAIRKAR[2];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]UTMB, Galveston, TX

Abstract: Parkinson's disease is a debilitating neurodegenerative disorder that affects an estimated 53 million people worldwide. Symptoms tend to arise later in life as characteristic tremors, and become severely incapacitating within 10-20 years, resulting in possible dementia, sleep disorders, depression, and or other emotional problems. As of now Parkinson's disease is untreatable though several proteins, genes, and their targets have been characterized as markers and possible triggers for the disease. The most well-established of these proteins are PINK1 and Parkin, both of which are part of a pathway that regulates mitochondrial fidelity in the form of quality control and fission. Mutations in the genes encoding these proteins result in loss of the ability to perform mitochondrial quality control. However, how the PINK1/PARKIN pathway regulate mitochondrial quality control is not well understood. The goal of the current project is to understand how PINK1/PARKIN regulates mitochondrial quality control. For this, we are utilizing the fly model to understand the molecular mechanism of mitochondrial quality control. One of the proteins that we found in a screen for defective synapse growth also affects mitochondrial morphology leading to the hypothesis that this protein may function in the

PINK1/PARKIN pathway. Current study is aimed toward identifying whether the protein of interest works in the PINK1/PARKIN pathway or regulates mitochondrial morphology independent of this pathway. The results of this analysis will be presented.

Disclosures: **K. Bush:** None. **A.M. Buckley:** None. **K.R. Barber:** None. **M. Woodson:** None. **M.B. Sherman:** None. **Y. Wairkar:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH/NINDS 1R01NS092667-01

Harvard Stem Cell Institute

The Consolidated Anti-Aging Foundation

Title: Altered lysosomal stress markers in idiopathic Parkinson's disease and following pharmacological-induced lysosomal dysfunction via glucocerebrosidase inhibition

Authors: E. B. MOLONEY, S. LEVY, J. A. KORECKA, O. ISACSON, *P. HALLETT; Neuroregeneration Res. Inst., Harvard Med. Sch. / McLean Hos., Belmont, MA

Abstract: *GBA1* encodes for the lysosomal hydrolase, glucocerebrosidase (GCase), and heterozygous mutations in *GBA1* dramatically increase the risk for developing Parkinson's disease (PD). Our data shows that sporadic PD (with no *GBA1* mutation) mirrors *GBA1* haploinsufficiency, given that GCase activity is reduced in several brain regions including the substantia nigra, and levels of the glycolipid substrate, glucosylsphingosine (GluSph), are elevated. Moreover, similar alterations to GCase activity and glycolipid levels occur in the brain of healthy subjects and mice in normal aging, and we hypothesize that disrupted glycolipid homeostasis and lysosomal function may precipitate degenerative processes in vulnerable neurons and lower the threshold for developing PD.
Our current studies aim to determine the effect of modulating GCase activity and glycolipid levels *in vivo* on relevant cell biological mechanisms, including markers of lysosomal integrity and stress. To model altered glycolipid homeostasis in mice, we use pharmacological inhibition of GCase with conduritol-β-epoxide, CBE, a selective, irreversible inhibitor of GCase. A 28-day systemic treatment with CBE in wildtype mice leads to PD-relevant neuropathological changes including significantly elevated brain levels of the glycolipids GluSph and glucosylceramide

(GluCer), aggregation of α-synuclein in the substantia nigra, and altered levels of proteins involved in the autophagy lysosomal pathway (Rocha EM et al, Antioxid. Redox Signal., 23(6) 550-564, 2015). As a readout of cellular responses to elevated glycolipids, we have performed analysis of levels of glycoprotein non-metastatic B (GPNMB), which is a transmembrane protein expressed in the brain in neurons and glia. GPNMB levels are elevated in the brain and CSF in lysosomal storage disorders that accumulate glycolipids, including neuronopathic Gaucher disease and Niemann-Pick Type C, and a SNP variant in the *GPNMB* gene has been associated with idiopathic PD risk. Our data shows that GPNMB levels are increased in the brain in CBE-treated mice, as well as in the substantia nigra in sporadic PD. Ongoing analyses will determine the relationship between GPNMB and lysosomal stress, and will evaluate additional markers of lysosomal function in models of altered glycolipid homesostasis.
These data show the remarkable overlap between lysosomal storage diseases and age-dependent idiopathic PD.
PH*, OI*: * Authors contributed equally to this work.

Disclosures: E.B. Moloney: None. **S. Levy:** None. **J.A. Korecka:** None. **O. Isacson:** None. **P. Hallett:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.04/J7

Topic: C.03. Parkinson’s Disease

Support: Academy of Finland (no. 2737991)

Jane and Aatos Erkko Foundation grant

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Title: Prolyl Oligopeptidase (PREP), a novel modulator of PI3K class III regulated autophagy pathway

Authors: *R. SVARCBAHS, J. JAKOLA, T. MYÖHÄNEN;
Univ. of Helsinki, Helsinki, Finland

Abstract: Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, Viikinkaari 5E (P.O.Box 56) 00014 University of Helsinki, Helsinki, FINLAND
Prolyl oligopeptidase (PREP) is a serine protease that hydrolyses small (less than 30 amino acids) proline containing neuropeptides. Recently, it has been shown that PREP enhances aggregation of alpha-synuclein, main component in Parkinson's disease pathology. Our previous results show that PREP overexpression reduced the autophagosome formation in cell culture while PREP inhibition by a small-molecule inhibitor, KYP-2047, led to increased macroautophagy (autophagy) via enhanced beclin1 levels in PI3K class III pathway and countered the effects of autophagy inhibitors. This led us to further investigate the effect of PREP catalytic inhibition and PREP deletion with CRISPR-cas9 system on autophagy markers in HEK-293 cells. Additionally, autophagy maker changes in PREP knock-out mouse brain tissue were measured.
PREP inhibition with 1 μM KYP-2047 in HEK-293 cells for 24 h led to a significant increase in total Bcl2 and phospho-(Ser70)-Bcl2 levels and was accompanied by increase in LC3BII and beclin1 levels. Moreover, PREP inhibition increased the levels of phospho-(Thr119)-Beclin1. PREP deletion by CRISPR-cas9 led to a significant difference in pBcl2/Bcl2 and pBeclin1/Beclin1 ratio, suggesting on positive beclin1 activity modulation.
In PREP knock-out animals there was a significant increase in total Bcl2, phoshpo-(Ser62)-Bcl-xl and LC3BII levels while decrease in Bcl-xl total level was observed, leading to increased pBcl-xl/Bcl-xl ratio.
Our results show that PREP inhibition in cell cultures can enhance the phosphorylation of Bcl2, leading to dissociation of Bcl2 from Beclin1 and Beclin1 phosphorylation that initiates autophagy in PI3K class III pathway. Although we saw increase in certain PI3K class III pathway proteins, in brain tissue of PREP knock-out mouse, Bcl-xL level was decreased. However, in contrast the pBcl-xl levels were increased, and pBcl-xl/Bcl was elevated similar to PREP inhibited and CRISPR-cas9 silenced cells. Our results suggest, that PREP inhibitors could be used as safe autophagy enhancers e.g. to degrade protein aggregates in neurodegenerative disorders.

Disclosures: **R. Svarcbahs:** None. **J. Jakola:** None. **T. Myöhänen:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

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Topic: C.03. Parkinson's Disease

Support: ANR-10-IAIHU-06

ANR-11-INBS-0011-NeurATRIS

Title: Blockade of SK channels with the scorpion venom peptide scyllatoxin protects midbrain dopamine neurons from degeneration.

Authors: *P. P. MICHEL[1], S. HAMADAT[1], D. SERVENT[2], G. MOURIER[2], E. C. HIRSCH[1]; [1]Brain and Spinal Cord Inst. (ICM), Paris, France; [2]CEA, iBiTecS, Service d'Ingénierie Moléculaire des Protéines, Gif-sur-Yvette, France

Abstract: Previous studies have suggested that inappropriate and sustained activation of Ca^{2+}-activated K^+ (SK) channels may participate actively to the death of dopamine (DA) neurons in Parkinson disease (PD). This concept is supported by studies showing that a blocker of SK channels, the bee venom peptide apamin (APA) is neuroprotective for DA neurons in cellular and animal models of the disease (Salthun-Lassalle et al, J Neurosci, 2004; Alvarez-Fischer et al, PloS One, 2013). Here, we wished to further explore this hypothesis. To this aim, we tested the neuroprotective potential of scyllatoxin (SCY), a SK channel blocker from scorpion venom whose peptide sequence is unrelated to that of APA.

Using a culture system of selective and progressive DA cell death, we showed that SCY was able to rescue a substantial fraction of DA neurons that degenerate spontaneously under these conditions. The protective effect was concentration-dependent and optimal between 0.1-1µM. Most interestingly, rescued DA neurons were functional as they had the capacity to accumulate tritiated-DA by active transport. The survival promoting effect of SCY was also highly specific to DA neurons. It was short-lived after treatment withdrawal and mimicked by the bee venom peptide APA.

Of interest, DA cell rescue mediated by SCY was compromised when Arg6 and Arg13 that are selectively required for SK channel binding, were replaced by corresponding Ala residues. Likewise, when the capacity of APA to bind SK channels was suppressed by substituting Arg with Ala residues at positions 13 and 14 of the sequence peptide, neuronal rescue was abolished. Combining SCY and APA did not further improve DA cell rescue, leading us to assume that the two peptides shared a common binding site on SK channels. Note that the neuroprotective effect of SCY and also that of APA, were entirely suppressed when voltage-gated Na^+ channels were blocked with tetrodotoxin. This suggested that SCY and APA improved the survival of DA neurons by maintaining the excitability of these neurons at a level that is also required for their survival, as suggested before (Michel et al, Faseb J, 2013). Overall, these data suggest that SK channels may represent an interesting target for neuroprotection in PD.

Disclosures: **P.P. Michel:** None. **S. Hamadat:** None. **D. Servent:** None. **G. Mourier:** None. **E.C. Hirsch:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

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Program#/Poster#: 310.06/J9

Topic: C.03. Parkinson's Disease

Support: DFG CRC1080

Title: Receptor-mediated endocytosis 8 is a new component of the protein homeostasis network in mammalian cells

Authors: *A. M. CLEMENT, A. BESEMER, J. MAUS, K. NALBACH, C. FREESE, C. VON HILCHEN, A. KERN, C. BEHL;
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Abstract: Cellular function and activity depend on an adaptive regulation of protein folding, stabilization and degradation that prevent protein aggregation. This is of particular interest in postmitotic cells such as neurons whose functional decline and degeneration leads to several late onset neurodegenerative diseases. Protein homeostasis, called proteostasis, is regulated by a fine-tuned system of chaperones that stabilize proteins and direct instable proteins towards the proteasome or autophagy degradation pathway. We performed an RNAi-based screen in *C. elegans* following an acute heat stress paradigm to identify new components of the proteostasis network and identified, among others, receptor-mediated endocytosis 8 (RME8). The human ortholog to RME8 is called DNAJ homolog subfamily C member 13 (DNAJC13) and is characterized by the presence of the J-domain that interacts with the chaperone HSP70. Recent evidence suggests that RME8 is an essential component of the sorting processes at the endosome and modulates endosomal trafficking. Mutations in RME8 have been linked to familial forms of Parkinson's disease with Lewy body pathology, although the pathogenic molecular mechanisms are so far unknown. We have now gathered evidence that RME8 is involved in the proteostasis network. The knockdown of RME8 in *C. elegans* as well as in human cell culture systems resulted in the accumulation of aggregation prone proteins like amyloid-beta 42 and α-synuclein or mutant SOD1. As protein aggregation is reminiscent of a defect in protein degradation pathways, we investigated the role of RME8 in autophagy. Based on siRNA and overexpression experiments we determined RME8 as a positive modulator of autophagy.
This project is supported by the CRC 1080 "Mechanisms of Neural Homeostasis" (Project A8). ASB and JM were fellows of the "Focus Program Translational Neuroscience" of the Johannes Gutenberg University of Mainz.

Disclosures: A.M. Clement: None. **A. Besemer:** None. **J. Maus:** None. **K. Nalbach:** None. **C. Freese:** None. **C. von Hilchen:** None. **A. Kern:** None. **C. Behl:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Program#/Poster#: 310.07/J10

Topic: C.03. Parkinson's Disease

Support: NHMRC Project: APP1082250

Title: The involvement of ASC in Parkinson's disease: beyond an adaptor protein.

Authors: *E. ALBORNOZ BALMACEDA[1,2], R. GORDON[2], A. B. ROBERTSON[1], K. SCHRODER[1], M. A. COOPER[1], T. M. WOODRUFF[2];
[1]Inst. for molecular Biosci. (IMB), Univ. of Queensland, brisbane, Australia; [2]Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia

Abstract: The Apoptosis-associated Speck-like protein with CARD domain (ASC), is a key component of multimeric inflammasome protein complexes that mediate innate immune and inflammatory responses. They function as intracellular sensors for infectious agents, as well as for host-derived dangers signals that are associated with neurological diseases such as Alzheimer's, stroke, meningitis and recently Parkinson's disease (PD). Inflammasome activation induces the oligomerisation of ASC and the assembly of ASC specks. ASC specks recruit and activate caspase-1, which drives the maturation of the cytokine interleukin 1β (IL-1β) and pyroptotic cell death. Recently, exciting evidence has emerged showing that ASC functions not only as an adaptor protein in the inflammasome complex, but also has extracellular and prionoid activities that propagate inflammation, revealing a new form of cell-to-cell communication. Although these studies have been in peripheral inflammatory conditions, they have profound implications for progressive neurodegenerative diseases in which cell-to-cell transmission of misfolded proteins in the CNS is emerging as a major pathological mechanism. In Parkinson's disease pathology, alpha-synuclein (Syn) aggregates have been implicated as potential candidates for inflammasome activation. The chronic neuroinflammatory response that accompanies dopaminergic degeneration has been shown to exacerbate pathology and drive disease progression. However, the role of ASC in propagating Syn pathology and spread in the CNS has not been investigated to date. Herein, we demonstrate that ASC is upregulated in PD patient brains and multiple pre-clinical models of PD. In primary microglia, we found that inflammasome activation by Syn aggregates causes ASC oligomerisation and extracellular secretion with delayed kinetics compared to canonical inflammasome activators. Crucially, we found that microglial activation with Syn aggregates caused the extracellular release of ASC specks, without causing detectable pyroptosis for up to 48 h after treatment. Collectively, our results suggest that extracellular ASC could be involved in the propagation of Syn pathology in PD, making it a potential therapeutic target to mitigate disease progression

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS074443

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Title: PKD1 activation positively regulates PGC-1α transcriptional activity and protects against dopaminergic neurodegeneration

Authors: **M. AY**[1], A. ASAITHAMBI[1], D. HARISCHANDRA[1], A. KANTHASAMY[1], H. JIN[1], V. ANANTHARAM[1], *A. G. KANTHASAMY[2];
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Abstract: Mitochondrial dysfunction has been associated with many neurological diseases including Parkinson's disease (PD). Impaired mitochondrial biogenesis also contributes to mitochondrial dysfunction in PD. Therefore, identifying the cell signaling mechanisms regulating mitochondrial biogenesis is critically important to the development of new neuroprotective strategies for alleviating mitochondrial dysfunction in PD. We have recently shown that protein kinase D1 (PKD1) activation plays a neuroprotective role and that positive modulation of PKD1 can offer neuroprotection against neuronal cell death in PD models. In this study, we sought to identify downstream effectors of the PKD1 pro-survival signaling and found that PKD1 activation positively regulates PGC-1α transcriptional activity. Overexpression of constitutively active PKD1 increased PGC-1α promoter activity and mRNA and protein expression in MN9D dopaminergic neuronal cells; however, PGC-1β and PRC mRNA expression were not affected by PKD1 overexpression. Moreover, treatment of MN9D cells with a rationally designed PKD1 activator peptide enhanced the mRNA and protein expression of PGC-1α and also the mRNA expression of mitochondrial biogenesis marker genes, including TFAM, CYTB, and Cox III. Interestingly, treatment of cells with a PKD1 inhibitor, kbNB-14270, strongly suppressed the mRNA expression of PGC-1α and TFAM. Additionally, PKD1

overexpression stimulated other downstream neuroprotective targets in dopaminergic neuronal cells. More importantly, pretreatment with the PKD1 activator peptide significantly protected against 6-OHDA-induced neurotoxicity in a human mesencephalic neuronal cell model. Collectively, our results suggest that positive modulation of PKD1 induces PGC-1α transcription and may offer neuroprotection to dopaminergic neurons (NIH grants NS074443, NS78247 and ES10586).

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Program#/Poster#: 310.09/J12

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

NIMH MH100600

NIMH MH104491

Title: LRRK2-G2019S alters developing synapse structure and function in dorsal striatum

Authors: *B. A. MATIKAINEN-ANKNEY[1], N. KEZUNOVIC[2], G. W. HUNTLEY[2], D. L. BENSON[2];
[2]Neurosci., [1]Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Leucine-rich repeat kinase II (LRRK2) is a large, multifunctional protein highly expressed by striatal spiny projection neurons (SPNs) during early postnatal development when corticostriatal circuits are established. Mutations in LRRK2 cause Parkinson's disease that is indistinguishable from idiopathic PD, the most common of which, G2019S, confers a gain-of-kinase activity to the protein. Thus we hypothesized that G2019S mutation would alter development of corticostriatal circuitry in a kinase-dependent manner. We examined SPN structure and function in acute slices of dorsal striatum from P21 wildtype (WT) mice or those expressing knock-in mutations of G2019S or D2017A (LRRK2 kinase-dead controls). In whole-

cell recordings from WT or mutant SPNs, we found that G2019S mice exhibited a four-fold increase in frequency of action-potential dependent sEPSCs in comparison with WT or kinase-dead mutants. There were no differences in sEPSCs between WT and D2017A MSNs, indicating a gain-of-abnormal function by the G2019S mutation. Such abnormally heightened activity in G2019S mice was evident in both direct- and indirect-pathway SPNs. Moreover, such aberrant activity was normalized by isolating striatum from neocortex or by inhibiting LRRK2 kinase activity with a small-molecule pharmacological inhibitor. These data suggest that aberrant activity is driven by corticostriatal input and is kinase-dependent. We found no differences across genotypes in density of synaptic molecular markers or SPN dendritic spines. However, G2019S SPNs exhibited significantly larger spine head diameters that were matched by a shift towards larger excitatory post-synaptic response amplitudes. Together, these data demonstrate that PD-associated LRRK2-G2019S mutation significantly alters normal structural and functional development of corticostriatal circuits. Such early alterations may predispose striatal circuits to PD-associated pathophysiology later in life.

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: 5R21NS088923-02

Title: Implication of 8-oxodG-mediated Transcriptional Mutagenesis in sporadic Parkinson’s disease

Authors: *S. BASU[1], S. GUHATHAKURTA[1], G. GOLDBLATT[1], S. TATULIAN[1], S. DAS[1], E. BOK[1], G. JE[1], A. C. CRISTOVAO[2], Y.-S. KIM[1];
[1]Univ. of Central Florida, Orlando, FL; [2]Dept. of Med. Sci., Univ. of Beira Interior, Covilhã, Portugal

Abstract: 8-oxodG(oxidized guanine) is a non-bulky DNA lesion that can lead to misincorporation of adenine instead of cytosine in mRNA during transcription (transcriptional mutagenesis, TM). Sporadic PD is classically characterized by aggregation of alpha-synuclein (α-SYN), which forms intraneuronal inclusions. Structural studies of α-SYN have shown the sensitivity of the molecule towards amino-acid changes which makes it more aggregation prone.

We hypothesized using *SNCA* (alpha-synuclein) gene as a model, that 8-oxodG-mediated TM event can generate novel variants which contribute to the aggregation of the wild-type protein as seen in Lewy bodies. We predicted the generation of 43 possible mutants, but focused on a few which had the highest potential towards aggregation (structural analysis by algorithm TANGO). We confirmed the presence of two of the predicted mutations (*Serine42Tyrosine* (S42Y) and *Alanine53Glutamate* (A53E)) in *SNCA* mRNA from the substantia nigra of human *post-mortem* PD brain using RNaseH2 PCR. Sequencing genomic DNA of the same PD sample and same region of α-SYN revealed no mutations at the DNA level. By using cell-based biochemical assays and recombinant protein assays we have seen that S42Y-α-SYN can accelerate the aggregation process involving the wild-type protein even when present in significantly lower amount. Importantly, we developed antibody to specifically detect the S42Y-α-SYN. Immunohistochemical analysis of serial *post-mortem* PD brain sections with H&E staining, anti-ubiquitin staining and anti-S42Y staining, showed Lewy bodies that stained positively with S42Y-α-SYN. To our knowledge, this is the first report about TM related mutations of α-SYN in Parkinson's disease and their role in the pathogenesis.

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS095053

Parkinson's Disease Foundation

Title: Alteration of cholinergic neuron activity in the l-dopa induced dyskinesia mouse model

Authors: ***S. CHOI**, T. C. MA, T. CHEUNG, Y. DING, D. SULZER, E. MOSHAROV, U. J. KANG;
Dept. of Neurol., Columbia Univ., New York, NY

Abstract: Striatal cholinergic interneurons (ChI) are implicated in motor control, associative plasticity, and reward-dependent learning. Previous studies have shown that ChIs become hyperactive in the L-DOPA induced dyskinesia (LID) mouse model (Ding 2011). Here we investigated alteration of electrophysiology and neuronal morphology of ChIs in the unilateral 6-

OHDA LID model. As shown previously (Ding 2011), spontaneous firing of ChIs decreased after 6-OHDA dopamine lesion (DA-lesion), but became hyperactive after LID induction by chronic L-DOPA treatment. However, there were no differences in basal intrinsic membrane properties such as resting membrane potential, voltage-current relationship, and rheobase between the sham, DA-lesion, and LID groups. Since HCN channels are among the factors that determine ChI spontaneous activity, we measured the ZD7288-senstive component of steady-state current in voltage clamp mode with step voltage injections (-100 to -20 mV at +10 mV increments). We found that HCN current in ChI was larger in the LID group compared to DA-lesion, which is consistent with increased ChI spontaneous firing in the LID group. To determine whether changes in input to ChI were altered by DA-lesion of LID induction, we measured spontaneous IPSCs (sIPSCs) and observed increased sIPSCs frequency in the lesion and LID groups, though the sIPSC amplitudes were unchanged. We hypothesized that changes in ChI input may indicate alterations in cell morphology. To assess this, we filled ChI with biocytin during electrophysiological recording and processed the slices for visualization with fluorescence secondary antibodies. 2D Scholl analysis showed no changes in the number of primary dendrites, but increased branching of dendrites in both the DA-lesion and LID groups, especially from 50-150 μm from the neuron soma. This was paralleled by increased total neurite length and branching for only the LID group. Analysis of the gene transcription changes relevant to these electrophysiological and morphological observations by ChAT-Ribotag RNAseq are ongoing. Together these observations indicate that the changes in ChI physiology are governed by both intrinsic (HCN channels and morphology) and extrinsic (GABAergic transmission) factors.

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Support: APP1044014

2012001396

LE130100078

Title: Munc18-1 controls a-synuclein self-replicating aggregation in early infantile epileptic encephalopathy

Authors: *Y. CHAI[1,2], E. SIERECKI[3,4], V. M. TOMATIS[2], R. S. GORMAL[2], N. ARIOTTI[3], N. GILES[3,4], D. XIA[2], R. PARTON[3], B. M. COLLINS[3], Y. GAMBIN[3,4], F. A. MEUNIER[2,1]; [1]The Univ. of Queensland, St Lucia, Australia; [2]Clem Jones Ctr. for Ageing Dementia Research, Queensland Brain Institute,The Univ. of Queensland, Brisbane, Australia; [3]Inst. for Mol. Bioscience, The Univ. of Queensland, Brisbane, Australia; [4]Single Molecule Sci. Centre, EMBL Australia, The Univ. of New South Wales, Sydney, Australia

Abstract: Munc18-1 is an essential element of the exocytic machinery controlling neurotransmitter release. Munc18-1 knockout mice show no developmental phenotype but die at birth from paralysis. Surprisingly, Munc18-1 heterozygous mutations are responsible for developmental defects, neurodegenerative and epileptic phenotypes including infantile epileptic encephalopathy (EIEE). suggestive of a gain of pathological function. Here, we used single molecule analysis, gene-edited cells and neurons to demonstrate that Munc18-1 EIEE-causing mutants promote the formation of large polymers that co-aggregate wild-type Munc18-1 *in vitro* and in neurosecretory cells. Surprisingly, Munc18-1 EIEE mutants also form Lewy body-like structures that contain α-synuclein (α-Syn). We reveal that not only Munc18-1 binds α-Syn but its mutants co-aggregate α-Syn. Likewise, removal of endogenous Munc18-1 increases the aggregative propensity of α-Syn^{WT} and that of Parkinson's disease-causing α-Syn^{A30P} mutant, an effect rescued by Munc18^{WT} expression indicative of chaperone activity. Co-expression of α-Syn^{A30P} mutant with Munc18-1 reduced the size of α-Syn^{A30P} aggregates. Munc18-1 mutations may therefore lead to a pathogenic gain of function through both the corruption of native Munc18-1 and a perturbed chaperone activity for α-Syn. Our results uncovers an unexpected function of Munc18-1 in controlling α-Syn propensity to aggregate.

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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Support: KAKENHI, Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science, No. 16K21283

Title: Characterization of alpha-synuclein-enriched periglomerular cells in the olfactory bulb

Authors: *K. TAGUCHI[1], Y. WATANABE[2], A. TSUJIMURA[2], M. TANAKA[1];
[1]Dept. of Anat. and Neurobiology, Grad. Sch. of Med. Sci., [2]Dept. of Basic Geriatrics, Kyoto Prefectural Univ. of Med., Kyoto, Japan

Abstract: alpha-Synuclein, the major constituent of Lewy bodies (LBs) and Lewy neurites (LNs), is normally expressed in presynapses and is involved in synaptic function. Abnormal intracellular aggregation of alpha-synuclein is observed as LBs and LNs in neurodegenerative disorders, such as Parkinson's disease or dementia with Lewy bodies. Accumulated evidence suggests that abundant expression of alpha-synuclein is one of the risk factors for pathological aggregation. Recently, we reported brain region-dependent differential expression of alpha-synuclein. Synaptic expression of alpha-synuclein is mostly accompanied by the expression of vesicular glutamate transporter-1, an excitatory presynaptic marker. In contrast, expression of alpha-synuclein in GABAergic inhibitory synapses is different among brain regions. Furthermore, some neurons show high expression of alpha-synuclein. Intense alpha-synuclein immunoreactivity within the cell bodies is observed in those cells. These results suggest that expression of alpha-synuclein is regulated in cell-type dependent manner. In the present study, we focus on the periglomerular cells (PGCs) with high expression of alpha-synuclein in the mouse olfactory bulb. These PGCs express NeuroD1, one of key transcriptional factors which are involved in neuronal differentiation in the olfactory neurogenesis. However, these cells are not stained by monoclonal antibody against NeuN, a mature neuronal marker. Interestingly, most of the PGCs with high expression of alpha-synuclein are Sox2-positive. Taken together, our results suggest that these PGCs sustain immature state and alpha-synuclein plays an important role in the regulation of the neuronal differentiation. Further studies of these PGCs will provide new insights for understanding physiological function of alpha-synuclein.

Disclosures: K. Taguchi: None. **Y. Watanabe:** None. **A. Tsujimura:** None. **M. Tanaka:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Hillman Foundation grant 109033

Title: Transmission of α-synucleinopathy from olfactory structures deep into the temporal lobe

Authors: *D. MASON[1], N. NOURAEI[1], J. HAN[2], D. PANT[2], K. MINER[2], K. LUK[4], J. STOLZ[3], R. LEAK[2];
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Abstract: Parkinson's disease is characterized by Lewy bodies filled with aggregated proteins such as α-synuclein. Lewy pathology emerges quite early in olfactory structures such as the olfactory bulb and anterior olfactory nucleus (OB/AON), which may contribute to smell deficits years before the onset of motor symptoms. We injected preformed α-synuclein fibrils into the OB/AON and observed dense inclusions containing pathologically phosphorylated α-synuclein (pSer129) 3 months later in deeper rhinencephalic structures (piriform and entorhinal cortices, amygdala, hippocampal CA1, and subiculum), all of which share anatomical connections with the OB/AON. Injections of the retrograde tracer FluoroGold into the OB/AON confirmed the existence of first-order afferents at these sites. In addition, some sites harbored FluoroGold$^+$ neurons but contained no inclusions, suggesting that some neuronal subtypes may be resistant to α-synucleinopathy. Animals sacrificed 1.5h following fibril infusions exhibited dense staining for total α-synuclein within the OB/AON relative to PBS controls, but did not yet harbor pSer129$^+$ inclusions. Multiple areas close to the injection site that do not send projections to the OB/AON remained free of inclusions, suggesting a lack of nonspecific uptake of fibrils from interstitial diffusion. Sonication of the fibrils for 1h in an inexpensive waterbath dramatically improved α-synucleinopathy transmission relative to 1 min-long probe sonication. Electron microscopy revealed that increasing sonication duration reduced fibril size. Young and aged mice exhibited largely similar patterns of α-synucleinopathy and fibril-infused mice exhibited subtle changes in olfactory function according to two independent smell tests. The amyloid stain Thioflavin labeled cellular structures at the infusion site and some, but not all inclusions contained ubiquitin, a hallmark of Lewy pathology. Three-dimensional confocal analyses revealed that pSer129$^+$ inclusions were wrapped around the nuclei of cells labeled with the specific neuronal marker NeuN. Preadsorption control experiments confirmed a loss of staining following incubation of the pSer129 antibodies with the pSer129 blocking peptide. Our results support the views that 1) α-synucleinopathy is transmitted through some, but not all neuroanatomical connections, 2) pathology is largely confined to first-order projection sites at 3 months, 3) transmission is perhaps most parsimoniously explained by retrograde transport, and 4) transmission in aged animals is largely similar to that in young controls 3 months post-infusion.

Disclosures: **D. Mason:** None. **N. Nouraei:** None. **J. Han:** None. **D. Pant:** None. **K. Miner:** None. **K. Luk:** None. **J. Stolz:** None. **R. Leak:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.15/J18

Topic: C.03. Parkinson's Disease

Title: Alterations of oscillatory activity in the striatal-cortical circuit following repeated sub-anesthetic ketamine administration in 6-OHDA-lesioned rats.

Authors: *T. YE[1,2], M. J. BARTLETT[1,3,4], M. B. SCHMIT[1,5], S. J. SHERMAN[1,3], T. FALK[1,3], S. L. COWEN[1,2,6];
[2]Psychology, [3]Neurol., [4]Pharmacol., [5]Neurosci., [6]Evelyn F. McKnight Brain Inst., [1]Univ. of Arizona, Tucson, AZ

Abstract: Sub-anesthetic administration of ketamine has been used to successfully treat a variety of disorders such as treatment-resistant depression, post-traumatic stress disorder, and chronic pain. Recent clinical case studies from our group indicate that low-dose ketamine infusion (0.15 – 0.3 mg/kg/hr for up to 72 hrs) acutely reduces motor impairments in Parkinson's disease (PD) patients and ameliorates L-DOPA-induced dyskinesias (LID) for up to one month. Abnormal patterns of hypersynchronous oscillatory activity have been reported in all of the disorders that have been treated successfully with ketamine, suggesting that ketamine may act to disrupt network-level oscillatory activity associated with the pathology. With regard to PD, hyper-synchronous beta-band oscillations (13 – 30 Hz) in the cortex and striatum may contribute to increased immobility, and beta is reduced with deep-brain stimulation (DBS) and levodopa treatment. Furthermore, gamma oscillations (30 – 70 Hz) in the motor cortex (M1) are associated with successful motor execution in healthy subjects. Administration of ketamine has been shown to increase high-frequency oscillations (HFOs) in rats and gamma-band activity in humans. As a result, it is proposed that sub-anesthetic ketamine-induced HFOs suppresses low-frequency activity associated with PD. We investigated this hypothesis by implanting electrode arrays in the striatum, M1, and hippocampus of healthy and unilateral 6-hydroxydopamine (6-OHDA) lesioned rats, a model of PD. To reflect clinical infusion protocols, we used repeated administration (*i.p.*) of sub-anesthetic ketamine (20 mg/kg every 2 hrs). Neural activity was continuously recorded from awake and freely behaving rats for an 11-hr period. Preliminary findings suggest that, like healthy controls (n=5), a single ketamine injection triggers HFO and gamma oscillations in the dorsolateral striatum (DLS) and M1. We observed that this ketamine regimen increased gamma-band activity in M1 (p=0.02, n=15 sessions in 3 rats) of 6-OHDA rats. This observation is consistent with the idea that ketamine enhances motor function by altering the oscillatory patterns of interneuron networks in M1. Furthermore, we observed that ketamine decoupled beta and gamma oscillations in M1 and DLS of both groups. Control animals had increased HFO-theta coupling in both regions, but 6-OHDA rats showed a relative

decrease in coupling in the DLS. Given the role of abnormal oscillatory patterns in PD pathology, this decoupling may contribute to the capacity of ketamine to mitigate motor impairments. Ongoing experiments aim to integrate more rats along with the examination of effects in treating LID.

Disclosures: T. Ye: None. **M.J. Bartlett:** None. **M.B. Schmit:** None. **S.J. Sherman:** None. **T. Falk:** None. **S.L. Cowen:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH R01 NS086100

NIH R01 NS085188

Title: Initiation and propagation of action potentials in the hyperdirect pathway during subthalamic deep brain stimulation

Authors: *R. W. ANDERSON, B. HOWELL, K. GUNALAN, C. C. MCINTYRE;
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Abstract: Deep brain stimulation (DBS) of the subthalamic region is an established clinical therapy for the treatment of late stage Parkinson's disease (PD). Direct modulation of the hyperdirect pathway, which consists of corticofugal axons originating from layer V pyramidal neurons that send a collateral to the subthalamic nucleus (STN), has emerged as an important component of the therapeutic mechanisms of action of DBS. However, given their complex axonal arbors, little is known about how action potentials (APs) are directly generated in hyperdirect neurons by subthalamic DBS electrodes, or how those DBS-induced APs propagate to distant brain regions. In addition, DBS stimuli are delivered at ~130 Hz and any DBS-induced APs must propagate antidromically through multiple branch points, suggesting that the fidelity of AP initiation and propagation may be limited. To address these questions we developed an anatomically and electrically realistic multi-compartment cable model of layer V pyramidal neuron with a detailed corticofugal axonal arbor that sent a collateral to the STN. We then coupled that hyperdirect neuron model to a finite element volume conductor model of the subthalamic DBS electric field. Our results show that DBS-induced APs are generated in hyperdirect neurons at or near their axon terminals located in the STN, and these APs propagate

faithfully throughout the entire axonal arbor of the hyperdirect neuron. We found that the distant terminals of the hyperdirect axonal arbor in cortex could reliably maintain a one-to-one ratio of firing to stimulus trains at rates in excess of 200 Hz. Interestingly, somatic AP invasion in cortex became intermittent under therapeutic DBS frequencies due to the impedance mismatch of the axon connection to the somatodendritic arbor and the slower membrane dynamics of the soma. However, we propose that somatic firing of hyperdirect neurons is actually irrelevant under DBS conditions, as the high frequency DBS-induced APs collision block and override any orthodromic signaling in these neurons. Therefore, the key finding of this study is that DBS-induced APs can reach all of the axon terminals of hyperdirect neurons with nearly 100% fidelity, irrespective of the activity in the soma. In turn, these DBS modulated cortical neurons likely have a strong impact on cortical microcircuit activity via tonic high frequency release of glutamate (and other neurotransmitters) at their axon terminals which are located throughout the cortical layers.

Disclosures: R.W. Anderson: None. **B. Howell:** None. **K. Gunalan:** None. **C.C. McIntyre:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

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Topic: C.03. Parkinson’s Disease

Support: JSPS

KAKENHI

Title: The relevant nuclei of Parkinson’s disease were elucidated by the quantitative activation-induced manganese-enhanced MRI in MPTP mouse model.

Authors: S. KIKUTA[1,2], Y. NAKAMURA[3], Y. YAMAMURA[3], Y. YANAGAWA[4], N. HOMMA[1,5], H. TAMURA[1], J. KASAHARA[3], *M. OSANAI[1,5];
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Abstract: Despite the biochemical characteristics of Parkinson’s disease (PD) have been well described, physiological properties of PD have been argued. Especially, brain regions related to the severity of the Parkinson’s disease have not been established. To reveal those regions, we

have recorded the history of the neuronal activities in entire brain regions, and have analyzed the correlation between the neuronal activities and the tyrosine hydroxylase (TH)-immunoreactivity, which is one of the biochemical markers of PD severity, in MPTP mice model of PD. For recording the history of the neuronal activities in entire brain regions, we used one MRI technique, quantitative activation-induced manganese-enhanced MRI, which is based on the use of Mn^{2+} as a surrogate marker of Ca^{2+} influx (Kikuta et al., 2015). This method has the potential to record the history of the neuronal activities, quantitatively, because of following reasons. Mn^{2+} inflow to the active neuron through voltage-dependent Ca^{2+} channels, and Mn^{2+} shortens the longitudinal relaxation time of H^+ (T_1), which was quantified by MRI. Thus, qAIM-MRI can visualize the neuronal activities in entire brain volume.
Within the basal ganglia, the region represented elevated activity was observed in caudate-putamen (CPu) of PD model mice compared with healthy controls. Several cortical and thalamic regions also activated in PD model mice. In the PD mice, the activities in dorsolateral CPu, sensorimotor area of cortex (Ctx), and parafascicular nucleus of thalamus (PF) were significantly correlated to the TH-immunoreactivity. These results suggested that the dorsolateral part of CPu involves in PD severity, and this hyperactivity in CPu may arise from increasing activity in Ctx and PF, whose neurons innervate CPu.
Our findings pave the way for significant progress in research on PD pathophysiology, and suggest that qAIM-MRI can be utilized not only for diagnosing PD, but also potentially for the study and diagnosis of various other neurological conditions.

Disclosures: **S. Kikuta:** A. Employment/Salary (full or part-time): JSPS. **Y. Nakamura:** None. **Y. Yamamura:** None. **Y. Yanagawa:** None. **N. Homma:** None. **H. Tamura:** None. **J. Kasahara:** None. **M. Osanai:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: Swedish Society for Medical Research

Bergvall

Crafoord

Kockska

Michael J. Fox

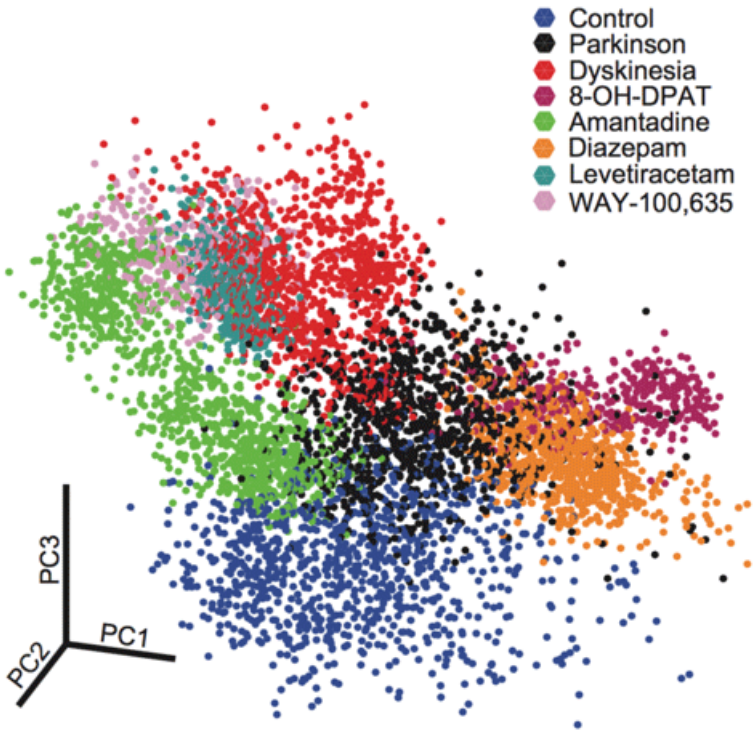
Olle Engkvist

Parkinson

Title: Systems-level neurophysiological state characteristics for drug evaluation in an animal model of levodopa-induced dyskinesia

Authors: *P. HALJE, M. TAMTE, U. RICHTER, I. BRYS, P. PETERSSON;
Lund Univ., Lund, Sweden

Abstract: Disorders affecting the central nervous system have proven hard to treat, and few novel therapies reach the clinics. A general problem is our poor understanding of how changes on the ligand-receptor level translate to changes on the systems level.
We here show a general framework for how comprehensive systems-level descriptions of electrophysiological brain states can be obtained and interpreted in relation to disease states and pharmacological interventions. Through large-scale recordings in distributed neural networks in multiple brain structures we characterize neurophysiological states based on local field potentials associated with parkinsonism and levodopa-induced dyskinesia in the 6-OHDA rodent model of Parkinson's disease (PD), together with pharmacological interventions aimed at reducing dyskinetic symptoms.
We found that all disease and treatment states (including PD, dyskinesia, 8-OH-DPAT, amantadine, diazepam, levetiracetam, Way-100635 and control) were clearly separable clusters (>99% classification performance) in the state space defined by the first 30 principal components of all measured features (power spectral densities from motor cortex, premotor cortex, dorsomedial striatum, dorsolateral striatum, substantia nigra pars reticulata, globus pallidus, subthalamic nucleus and thalamus). We also found that the geometrical distance between points in the state space was reflected in differences in motor symptoms. For example, the effectiveness of an antidyskinetic treatment (reduction of abnormal involuntary movement scores) could be related to the distance from the dyskinetic cluster. Similarly, antidyskinetic treatments with strong akinetic side effects were found close to the Parkinsonian cluster.
Our results add significant information to behavioral evaluations and elucidate the underlying effects of treatments in greater detail. The results open up for studies of neurophysiological mechanisms in neurological and psychiatric conditions that until now have been hard to investigate in animal models of disease.



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Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NSF DMS 1122279

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Title: A computational model for the progression of Parkinson’s disease in the basal ganglia

Authors: ***M. CAIOLA**, M. HOLMES;
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Abstract: The primary motor symptoms of Parkinson's disease (PD) are thought to be caused when dopamine producing neurons in the substantia nigra pars reticulata die, leading to changes in connection strengths and, ultimately, firing rates in the basal ganglia. This leads to net inhibition of the thalamus, restricting and slowing movement - symptoms that are characteristic with PD. As these motor symptoms advance, oscillations in the basal ganglia become pronounced at specific frequencies (such as beta). Since few human and primate studies characterize the basal ganglia changes during the transition to PD, computational models can help provide insight into how these oscillations emerge. To balance accuracy and simplicity, we use a population-based firing rate model developed by Dayan and Abbot. This model takes the average firing rate from a population of neurons and measures the effect on a connected population. Connections are either excitatory or inhibitory in nature and are assigned weights that measures the strength of the connection - the higher the value, the stronger the connection strength. Our model focuses on the cortico-basal ganglia-thalamo-cortical loop: the cortex, striatum, globus pallidus external (GPe) and internal (GPi), subthalamic nucleus (STN), substantia nigra pars compacta, and the thalamus. The GPe, GPi, and STN have been known to be the pacemakers of the basal ganglia and a possible origin of the antikinetic beta oscillations that plague PD. Allowing the PD dopaminergic change to affect just the connection weights between these populations, we are able to show a change in firing rate behavior that has been previously documented in animal models. More interestingly, we are able to derive physically realistic conditions necessary for the emergence of oscillatory behavior. The conditions are found to agree with recent experiments as well as the current understanding that the Indirect Pathway becomes more active, leading to high GPi activity and thalamic inhibition. In addition, we show that only a specific range of connection strengths lead to oscillations, while others lead to healthy steady firing rates. Further analyzing the disease progression, we parametrize the change in connection strength to effectively view the progression of PD. This allows us to visualize the dynamics of the disease and determine when and how the firing rate behavior changes. Surprisingly, we found that order of the weight changes plays a key role and it is not as linear as the previous models have assumed. By identifying these main changes in connection strength, we hope to provide new insight in the emergence of Parkinsonian symptoms and identify new targets for treatment.

Disclosures: **M. Caiola:** None. **M. Holmes:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH/NIBIB R01-EB016407

Title: Development of an *In vivo* model of basal ganglia pathway isolation for study of information transmission

Authors: *K. M. LAMBERT[1], J. A. WHITE[2], A. D. DORVAL[1];
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Abstract: The basal ganglia are subcortical nuclei implicated in Parkinson's disease, among other neurological disorders. Deep brain stimulation (DBS) of some basal ganglia regions — e.g., the subthalamic nucleus (STN) — can alleviate parkinsonian motor symptoms. However, despite its efficacy, the therapeutic mechanisms of STN-DBS remain unclear due in large part to an incomplete understanding of how neuronal processing within basal ganglia contributes to motor behavior. To improve our models of basal ganglia processing, we isolated the direct and indirect basal ganglia pathways, using selective pharmacological blockers against the opposing pathway, while applying STN-DBS at various frequencies (30, 200 Hz) and amplitudes (60-130μA). In otherwise naïve rats, we bilaterally injected the type-1 dopamine receptor (D1R) antagonist SCH23390 to acutely lesion the direct pathway, and/or the type-2 dopamine receptor (D2R) agonist quinpirole to acutely lesion the indirect pathway. We verified the behavioral success of each lesion — by quantifying changes in distance traversed and time spent near the chamber margins — in rodents in an open field task. The D1R antagonist SCH23390 decreased distance traversed, and increased margin time. Inversely, the D2R agonist quinpirole increased distance traversed and decreased margin time. A cocktail of both drugs reduced distance traversed, similar to the SCH23390 challenge, but also reduced margin time, similar to the quinpirole challenge. The selective nature of the drugs and the temporary nature of the behavioral results allow us to attempt multiple challenges in the same rat model interspersed with saline injection control challenges on sequential days. The acute lesion of a specific pathway should leave information through the opposite pathway to be studied in isolation. Recording from previously implanted microelectrode arrays during these behavioral challenges, we isolated single unit signals from two basal nuclei. In future analyses we will quantify changes in the neuronal activity and information content of these signals as a function of acute lesion condition and STN-DBS parameters.

Disclosures: **K.M. Lambert:** None. **J.A. White:** None. **A.D. Dorval:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH Grant R37 NS040894

Duke MSTP T32 GM007171

Title: Neural basis for bradykinesia/akinesia in Parkinson's disease: causality of beta frequency oscillations

Authors: *C. BEHREND[1,2], D. T. BROCKER[1], W. M. GRILL[1,3];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Duke Univ. Sch. of Med., Durham, NC; [3]Neurobio. & Surgery, Duke Univ. Med. Ctr., Durham, NC

Abstract: Substantial correlative evidence links the synchronized, oscillatory neural firing patterns that emerge in Parkinson's disease (PD) in the frequency range of 13-30Hz (termed "beta band") with the development of bradykinesia and akinesia. Yet, a causal link between these beta frequency oscillations (BFO) and symptoms of bradykinesia has not been demonstrated. We tested in healthy rats the hypothesis that the synchronized, BFO that emerge in PD are causal of symptoms of bradykinesia/akinesia. We designed novel stimulation patterns to mimic the temporal characteristics of the beta oscillatory bursting pattern seen in single units in PD rats and patients. We applied these beta frequency patterned stimulus trains (BPTs) along with continuous frequency (CF) controls over a range of amplitudes via stimulating electrodes implanted unilaterally into the subthalamic nucleus (STN) of healthy rats and assessed the effects on unit activity in the substantia nigra reticulata (SNr) and performance in motor tasks. We recorded 36 SNr units from four rats and quantified the degree of unit entrainment as a function of pattern and amplitude by calculation of the excitatory effective pulse fraction (eEPF) (Agnesi et al 2015). The eEPF is a ratio (0-1) that relates the number of unit firings evoked by a stimulus pulse at a consistent latency within the inter-stimulus interval (ISI) to the number of unit firings evoked by a 'virtual' stimulus pulse at the same latency during a baseline period (Agnesi et al 2015). Using RM-ANOVA we found effects of pattern ($F(6,211.5)=4.2$, $p=0.0005$) and amplitude ($F(1,38.3)=35.2$, $p<0.0001$) and a significant interaction of pattern and amplitude ($F(6,206.2)=2.4$, $p=0.029$) on whether unit entrainment occurred within the ISI. For BPTs at the highest amplitudes, the peak of entrainment occurred in a range of 3-7.2ms following a stimulus pulse. For CF controls at the highest amplitudes, two peaks of entrainment occurred in ranges of 2.6-4.4ms and 22.6-25.4ms following a stimulus pulse. The average eEPFs of units entrained within these phases were 0.060 ± 0.018, 0.046 ± 0.007, and 0.107 ± 0.042, respectively (mean ± SEM). Finally, we quantified the impact of stimulation on motor performance in the bar test, the forelimb-use asymmetry (FUA) test, and the adjusting steps (AS) test, which assess forelimb akinesia. In the bar test, no significant difference in length of time on bar was found among BPTs, CF controls, or no stimulation controls. In the FUA and AS tests, no significant difference was found between select BPTs and no stimulation controls. Our results suggest that BFO may not be necessary or sufficient for the generation of bradykinesia/akinesia in PD.

Disclosures: C. Behrend: None. **D.T. Brocker:** None. **W.M. Grill:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: FAPESP

CNPQ

CAPES

Title: Evaluation of cardiovascular risk factors in a rat model of parkinson's disease

Authors: ***A. A. HAIDAR**[1], L. F. OLIVEIRA[1], L. D. RODRIGUES[1], M. B. NEJM[1], P. P. GHAZALE[2], H. B. FERRAZ[1], C. SCORZA[1], E. CAVALHEIRO[1], L. R. BRITTO[3], F. SCORZA[1];
[1]Sao Paulo Federal Univ., Sao Paulo, Brazil; [2]Goiás Federal Univ., Goiânia, Brazil; [3]Sao Paulo Univ., Sao Paulo, Brazil

Abstract: This study aim to identify spontaneous arrhythmias, as well as evaluate heart rate in an experimental model of Parkinson disease, 6-hidroxidopamina (6-OHDA) model. Wistar rats (n=7) weighing 250-300g received two ECG electrodes, one implanted below the xiphisternum and the other between the junction of the two sternocleidomastoideus muscles. After that five (n=5) animals were injected with 6-OHDA (experimental animals) and two (n=2) of them (shams animals) received saline solution in the striatum. Animals' ECG started to be recorded immediately after surgery recovery. Recording was carried out for 4 days for 60 minutes 3 times a day. After the third day of ECG recording, experimental animals showed a significant raise in the heart rate (360 to 540 bpm, approximately) while shams animals showed a similar heart rate recorded before surgery. The induction and establishment of 6-OHDA model is able to induce ventricular tachyarrhythmias. The present study suggests a new avenue to better understand the cardiovascular dysfunctions observed in chronic stages of Parkinson disease.

Disclosures: **A.A. Haidar:** None. **L.F. Oliveira:** None. **L.D. Rodrigues:** None. **M.B. Nejm:** None. **P.P. Ghazale:** None. **H.B. Ferraz:** None. **C. Scorza:** None. **E. Cavalheiro:** None. **L.R. Britto:** None. **F. Scorza:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH Grant AG040261

Title: Experimental reduction of nigrostriatal dopamine similar to aging in substantia nigra, but not striatum, reduces open-field locomotor activity

Authors: *M. F. SALVATORE[1], T. MCINNIS[1], B. S. PRUETT[2], C. OWENS[3];
[1]Dept. of Pharmacol. & Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Ft Worth, TX; [2]Brown Univ., Providence, RI; [3]LSU Hlth. Sci. Ctr., Shreveport, LA

Abstract: Background: The risk of locomotor impairment, including bradykinesia, increases substantially during aging and arises from Parkinson's disease (PD) or aging-related Parkinsonism. It has been long believed that the neurobiological basis for the manifestation of bradykinesia in PD is >70% loss of dopamine (DA) in the striatum. However, this level of striatal DA loss has never been reported in aging studies. Coincident with bradykinesia onset in PD or in aging studies, 40-50% DA loss in the substantia nigra (SN) has been reported, but the possibility that this loss could contribute to bradykinesia has not been investigated. Here, we have experimentally created aging-related loss of DA in the striatum or the SN in a compartment-specific manner in young male (6 months) rats to determine if aging-related DA loss in the striatum or SN may reduce locomotor activity. **Hypothesis:** DA reduction in the SN alone is sufficient to reduce locomotor activity. **Approach:** To ensure that locomotor function would be devoid of aging-related confounds to locomotor performance, 6 month old Brown-Norway Fischer 344 F_1 rats were used. To selectively reduce DA tissue content in either striatum or SN in freely moving rats during the awake cycle, rats were first implanted with double guide cannula to target striatum (+1.0 AP, 2.5 ML, 5.0 DV) or the SN (-5.7 AP, 2.5 ML, 7.5 DV). Following recovery, rats were placed into an open-field for three hour sessions immediately following infusion of sterile saline or the tyrosine hydroxylase (TH) inhibitor, α-methyl-*p*-tyrosine (AMPT) in either striatum or SN. Each treatment was repeated 5 times to acquire locomotor activity and repeated measures 2 way ANOVA was used to determine each individual rats movement under AMPT infusion against movement under vehicle infusion . **Results:** Infusion of a quantity of 1.4 nmole AMPT into the SN, but not striatum, produced a significant decrease in open-field locomotor activity out to 2 hours past infusion. **Conclusions:** The inhibition of TH activity and associated reduction in DA tissue content (~40%) in the SN, but not striatum, reduces open-field locomotor activity in young rats. These results suggest that nigral DA neurotransmission contributes as a neurobiological mechanism of locomotor initiation and

activity. Aging-related reductions in nigral DA and TH function may therefore underlie aging-related bradykinesia.

Disclosures: **M.F. Salvatore:** None. **T. McInnis:** None. **B.S. Pruett:** None. **C. Owens:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

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BABEL/ERASMUS

Olle Engkvist

Parkinsonfonden

Parkinson Research Foundation

Title: Functional connectivity in the cortico-basal ganglia-thalamic circuit in rodent and primate models of Parkinson's disease

Authors: *P. PETERSSON, U. RICHTER, M. TAMTE, I. BRYS;
Lund Univ., Lund, Sweden

Abstract: The cerebral cortex, the basal ganglia and thalamus together constitute a closely connected neuronal network that is thought to have a key role in the control of behavior. In the classic model, changes in average firing rate in one part of the network lead to changes in downstream firing rates that are predicted by the strength of anatomical connections. It is likely, however, that periods of synchronized neuronal activity result in stronger interaction between cell groups and, hence, more pronounced alterations in downstream neuronal activity than uncorrelated rate changes of the same magnitude. If this is true, functional connectivity should change dynamically under most natural conditions, since synchronization and oscillations in the activity of neuronal populations are known to be strongly state-dependent. Moreover, disturbed functional connectivity could in itself have a pathological role in disease states associated with

aberrant oscillatory activity.
We have here analyzed neuronal activity obtained from large-scale multi-structure recordings from the cortico-basal ganglia-thalamic circuit in both rats and marmoset monkeys. Measures of functional connectivity are compared between recordings obtained in animals that have been exposed to chronic 6-OHDA medial forebrain bundle lesions and recordings from normal control conditions. Furthermore, many rats developed levodopa-induced dyskinesia following chronic levodopa-treatment. This made it possible to study the functional connectivity specifically during dyskinesia.
In our local field potential recordings we could confirm the previously reported relative increase in power in the beta-band in the parkinsonian state and synchronized oscillations in the theta and high gamma band (~ 80 Hz) in dyskinesia. Notably however, the main oscillation frequency in the beta-band differed both between individuals and species (range: 10-35Hz). We also found a relative increase of the functional connectivity related to oscillations in these states, as reflected by an increase in coherence in these frequency bands. Interestingly, more detailed analyses indicate that these increases are more pronounced in certain parts of the circuit: While coherence in the beta and high gamma band, are more pronounced in cortico-striatal circuits, the increase in coherence in the theta band in the dyskinetic state is more specific to deeper nuclei.
These results indicate that the functional connectivity between structures in interconnected circuits is highly state-dependent and contingent on the main oscillation frequency being present in that state, and may also differ for different parts of the circuits.

Disclosures: **P. Petersson:** None. **U. Richter:** None. **M. Tamte:** None. **I. Brys:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.25/K10

Topic: E.03. Basal Ganglia

Support: CONACYT - GRANT 222009

CONACYT - GRANT 660496

Title: White matter and functional connectivity changes in MPTP nonhuman primates (Cercopithecus aethiops) model using fMRI and DTI

Authors: ***G. RAMÍREZ GARCÍA**[1], C. CASTILLO-HERNÁNDEZ[3], J. FERNÁNDEZ RUÍZ[4], A. LÓPEZ ORNELAS[5], I. ESCOBEDO AVILA[2], A. CAMPOS ROMO[2];
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Abstract: The analysis of blood-oxygen-level dependent (BOLD) signals obtained with functional magnetic resonance imaging (fMRI) allows inferring neuronal activity patterns for cognitive and motor processes. However, it is also possible to analyse BOLD signals at rest, a kind of analysis that has been termed resting-state fMRI (RS-fMRI). It examines the synchrony of the temporal BOLD signal between anatomically separated brain regions (networks) without the subject performing any particular task. This functional connectivity analysis differs from the traditional white matter structural connectivity analysis which is mainly done with Diffusion Tensor Images (DTI). Both techniques have been used to evaluate changes in patients with neurodegenerative disorders like Parkinson's disease, in which the nigro-striatal-cortical dopaminergic pathway is affected causing motor and cognitive impairments. The aim of this work was to evaluate the functional connectivity in the resting state networks, as well as the structural connectivity, in green monkeys (*Cercopitecus aethiops: n=3 per group*) in basal conditions and after MPTP administration. RS-fMRI and DTI images were acquired in a GE Discovery MR750 (General Electric, Milwaukee, WI) 3T whole-body MR scanner. The animals were anesthetized with Tiletamine/Zolazepam 125/125 mg at dose of 2 mg / kg body weight (im). The DTI sequences consisted of *Single Shot Echo Planar Imaging sequences*, acquiring 65 volumes of 26 axial slices (2 mm slice thickness and no separation), one for each of the 60 independent directions of diffusion with b= 2000 s/mm^2 and five corresponding to b=0 s/mm^2. The functional connectivity networks were created using two methodologies, first using independent Component Analysis (ICA) and second the seed-based methodology, placing anchor seeds in different regions of the striatum. White matter changes were evaluated with the FSL Tract-Based Spatial Statistics tool. We found 6 networks at rest in the healthy subjects: somatomotor, frontal, default mode, primary visual medial cerebellar and visual networks. After the administration of MPTP, we found a decrease in the activation of striatal networks (caudate and putamen) and a decrease in white matter tracts of the internal and external capsules, *corpus callosum* and cerebellum. The results show the presence of six resting-state networks in green monkeys that can be analogous to functional networks previously characterized in humans. Decrease of striatal functional connectivity and white matter tracts are congruent with the loss of cortico-striatal communication due to the death of dopaminergic neurons in the striatum.

Disclosures: G. Ramírez García: None. **C. Castillo-Hernández:** None. **J. Fernández Ruíz:** None. **A. López Ornelas:** None. **I. Escobedo Avila:** None. **A. Campos Romo:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.26/K11

Topic: C.03. Parkinson’s Disease

Title: Limbic system and olfactory dysfunction in drug naive patients with parkinson's disease : a connectometry study

Authors: *N. HOSSEINI[1,2], B. POURMENNATI[1,2], F. RAHMANI[1], A. KAMALIAN[1], A. ANJOMSHOA[1], M. DOLATSHAHI[1], M. AARABI[3];
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Abstract: Introduction Diffusion Weighted Imaging(DWI) studies have shown correlation between microstructural White Matter(WM) changes in olfactory system, and decrement in smell ability in Parkinson’s Disease(PD). We examined the association of Olfactory Dysfunction(OD) in drug-naïve PD patients with structural brain connectivity, using connectometry. Methods We used the results of the odor identification test, and DWI data, for 87 PD patiens; divided into 4 groups: anosmic, severe microsmic, non-severe microsmic and normosmic patients.We mapped local connectoms and measured their associations with scores obtained from odor identification test, then we tracked the differences. Fig-1 Results Fig-2 Discussion Our results suggest that OD is correlated with changes in WM tracks, connecting Limbic System(LS) and the orbito-frontal areas containing the olfactory cortices, in drug naïve severe microsmics and anosmics. Our results reveal that the correlation between density changes of diffusion spins and odor identification scores is only present in severe microsmic and anosmic PD patients, but not in patients with mild OD. We may interpret that symptoms of OD precedes detectable DWI changes in WM. Calculating quantitative anisotropy for associated tracks may be more sensitive for detecting early WM changes.Integration of structural and functional findings e.g. fMRI may facilitate the comprehension of OD pathophysiology in PD.Conclusion We report a map of involved tracks from LS in OD among PD patients.

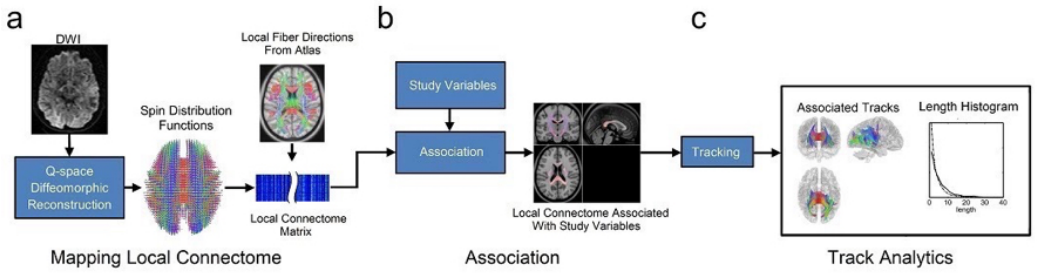

Fig-1

Adapted from: DSI-studio.labsolver.org

Method: We used odor identification test results-from the University of Pennsylvania Smell Identification Test (UPSIT), and Diffusion weighted imaging (DWI) data, acquired at a 3-T scanner, from Parkinson's Progression Markers Initiative(PPMI), for 87 Parkinson's Disease (PD) patiens. subsequently , we divided patients into 4 groups: 25 PD patients with anosmia, 23 with severe microsmia , 26 with non-severe microsmia and 9 normosmic patients. The DWI data were corrected for subject motion, eddy current distortions, and susceptible artifacts due to magnetic field inhomogeneity, using ExploreDTI toolbox. As you can see above, The diffusion data were reconstructed in the Montreal Neurological Institute (MNI) space using q-space diffeomorphic reconstruction to obtain the spin distribution functions. we matched the reconstructed diffusion data with an atlas that we created from our normosmic group. Diffusion MRI connectometry was conducted in 4 groups using a multiple regression model considering UPSIT and the local connectome matrices. A deterministic fiber tracking algorithm was conducted along the core pathway of fiber bundle to connect the selected local connectomes. To estimate the false discovery rate(FDR) , a total of 200 randomized permutations were applied to the group label to obtain the null distribution of the track length. FDR < 0.05 is deemed to be significant.

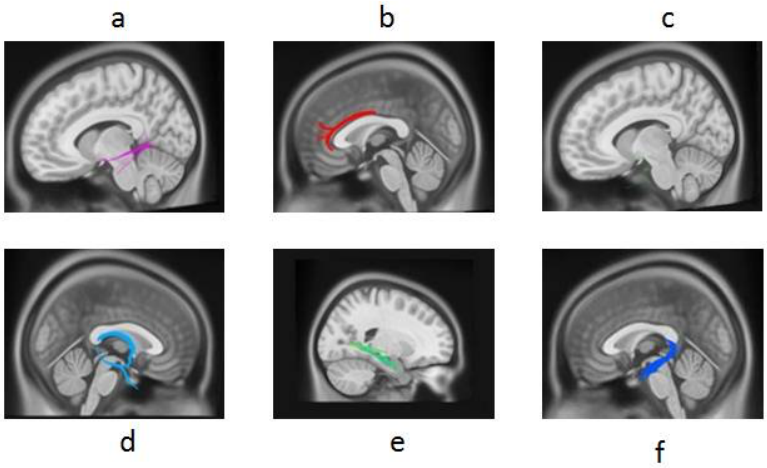

Fig.2. Results. The multiple regression analysis results illustrated positive association between UPSIT (University of Pennsylvania Smell Identification Test) score and left subgenual of cingulum(b), right inferior longitudinal fasciculus (ILF)(e), left inferior fronto-occipital fasciculus (IFOF)(a), fornix (d,f), and left uncinate fasciculus (UF)(c) in Parkinson's disease patients with anosmia [FDR=0.039]. In severe microsmia, UPSIT score positively correlate with left subgenual, right ILF, and left IFOF [FDR=0.048]. There was no association between structural brain connectivity and UPSIT score in patients without severe olfactory dysfunction. Above-mentioned tracks connect limbic system and the orbito-frontal areas.

Disclosures: N. Hosseini: None. **B. Pourmennati:** None. **F. Rahmani:** None. **A. Kamalian:** None. **A. Anjomshoa:** None. **M. Dolatshahi:** None. **M. Aarabi:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.27/K12

Topic: C.03. Parkinson's Disease

Support: National Parkinson's Foundation

Don Roberto Gonazlez Family Foundation

Title: Physical activity and resting state fMRI in parkinson's disease patients with mild cognitive impairment

Authors: B. JARRAHI[1], *G. PETZINGER[2], L. HAWTHORNE[2], M. GOMEZ[2], A. PETKUS[3], B. FISHER[4], V. FILOTEO[5], S. MCEWEN[1];
[1]Dept. of Psychiatry and Behavioral Sciences,, UCLA, Los Angeles, CA; [2]Dept Neurol, [3]Dept. of Psychology, [4]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA; [5]department of psychiatry, UCSD, San Diego, CA

Abstract: Physical Activity has been shown to promote brain connectivity within the aging brain, leading to improved cognitive-motor performance. In Parkinson's disease (PD), resting-state functional magnetic resonance imaging (rs-fMRI) has revealed abnormal functional connectivity in several intrinsic networks. It is unclear, however, how physical activity affects the spatial and temporal features of theses networks in PD patients with mild cognitive impairment (MCI). We applied multivariate analysis of covariance (MANCOVA) based on independent component analysis (ICA) of rs-fMRI to examine the influence of physical activity and dementia on three group ICA outcome measures: (a) the spatial map intensity associated with the intra-network connectivity; (b) the power spectra of network time course related to level of coherent activity within networks; and (c) resting-state functional network connectivity (FNC). Imaging data were acquired on eight PD individuals with MCI using a 3T Siemens Trio MRI scanner. MANCOVA was performed using the following covariates: (a) age, (b) composite score from Physical Performance Test (PPT), and (c) total score from the Mattis Dementia Rating Scale-2 (MDRS-2). Neural networks identified included cognitive/attention, cerebellar, motor, and default-mode (DMN) networks. Univariate tests yielded significant effects of PPT composite score on the spatial maps of IC 18 (medial prefrontal cortex), IC 6 (cerebellum), IC 7 (motor network), and IC 38 (DMN). Significant effects of DRS-2 score also found on the spatial maps of IC 18 (medial prefrontal cortex), IC 33 (dorsolateral prefrontal network) and IC 7 (motor network). Univariate test revealed significant effects of PPT composite score on the power spectra of IC 38 (DMN) (and MDRS-2 score on the power spectra of IC 22 (ventral attention network mainly in the right hemisphere). Our results provide preliminary evidence that physical activity in PD-MCI patients modulates the properties of neural networks, in particular the cognitive/attention, motor, and default-mode networks. Investigations of the effect of exercise in PD patients with MCI can provide a useful tool for understanding the role of exercise in PD for improving both motor and cognitive function.

Disclosures: B. Jarrahi: None. **G. Petzinger:** F. Consulting Fees (e.g., advisory boards); US World Meds. **L. Hawthorne:** None. **M. Gomez:** None. **A. Petkus:** None. **B. Fisher:** None. **V. Filoteo:** None. **S. McEwen:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.28/K13

Topic: C.03. Parkinson's Disease

Title: Does impaired reaction time cause rest tremor in Parkinson's Disease?

Authors: *V. V. SHAH[1], T. HOMAYOUNI[2], S. GOYAL[3], H. PALANTHANDALAM-MADAPUSI[1];
[1]SysIDEA Lab, Mechanical Engin., Indian Inst. of Technol. Gandhinagar, Gandhinagar, India; [2]Mechanical Engin., Univ. of California, Merced, Merced, CA; [3]SysIDEA Lab. IIT-GN, Mechanical Engin., Univ. of California Merced, Merced, CA

Abstract: Rest tremor is one of the most common motor symptoms of Parkinson's disease (PD). It occurs at a frequency of 4-6 Hertz in the extremities, such as hand, and characteristically disappears with action and during mental concentration or sleep. The patients suffering from PD are also known to have impairment in their reaction time (RT). For example, Wilson (1925) measured an average RT of 0.36 seconds in patients compared to 0.24 seconds in healthy individuals for a muscle response to single visual stimulus. Although the deterioration in motor control of human body can be intuitively attributed to the increased reaction time, how the increased reaction time is related to such biomechanical symptoms of PD as tremor is not yet understood. The prevailing dogma has been that the tremor originates from oscillations in the central neuronal activity. By contrast, a recent perspective based on feedback control theory (Palanthandalam-Madapusi et al., 2011) suggests that the increased reaction time, which contributes to transport delay in the sensorimotor loop involving extremities, triggers instability driven limit-cycle oscillations observed as rest tremor. This perspective attributes the tremor to the patient's intent to sense and stop it. In this work, we correlate several speculations of the recent perspective with clinical facts using arguments based on feedback control theory along with simple numerical and experimental examples. We contrast it with the prevailing dogma of central oscillator and analyze if any clinical facts can be used to comment further on the mechanism of rest tremors.

Disclosures: V.V. Shah: None. **T. Homayouni:** None. **S. Goyal:** None. **H. Palanthandalam-madapusi:** None.

Poster

310. Parkinson's Disease: Cell and Circuit Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 310.29/K14

Topic: C.03. Parkinson's Disease

Title: Multivariate pattern analysis of fMRI data reveals the discrete neural signature of target-specific deep brain stimulation in the pigs

Authors: *S. CHO, P. TESTINI, M. SETTELL, H. JO, H.-K. MIN, K. H. LEE;
Mayo Clin., Rochester, MN

Abstract: Subthalamic nucleus (STN) DBS is known to evoke non-motor effects such as psychiatric adverse effect (e.g., mania). However, it has been poorly understood the underlying neural mechanism on such non-motor neuromodulatory effect. Previous studies have shown that NAc/IC DBS evokes non-motor effect, which has been applied to treat psychiatric disorder (e.g., obsessive-compulsive disorder). Here we compared functional magnetic resonance images elicited by STN- and NAc/IC-DBS in order to figure out to what extent of similar and dissimilar neuronal activation patterns presents within a large-scale neural network.
We performed STN (n=7) and NAc/IC (n=7) DBS-fMRI experiments in a swine population. Multivariate pattern analysis was adapted to compare the correlational activation pattern of multiple brain regions induced between each DBS target. We trained the machine learning classifier (LDA) and tested whether the classifier can discriminate activation patterns evoked by each different DBS target. The analysis included whole-brain classification (25 ROIs), cortex-based classification (5 clusters), and principle feature set classification (5 ROIs).
The results showed that whole-brain classification (25 ROIs) yielded over 90% mean classification accuracy, and cluster-based classification (5 clusters; association, limbic, sensorimotor, and insular cortices, and thalamus) reached 75% mean accuracy for each cluster. We found that discrete neural signatures of STN and NAc/IC DBS were mainly represented in the three neuronal networks (association, thalamus, and sensorimotor system); however, similar activation patterns were also observed within limbic ROIs.
We conclude that STN- and NAc/IC-DBS evoked discrete neural patterns; nevertheless they also elicited similar neuromodulatory effect in non-motor circuitry. Furthermore, multivariate pattern analysis could possibly discriminate the different brain activation patterns in specific to different DBS-target. Our data suggests that both STN- and NAc/IC DBS shared common neuromodulatory mechanism for non-motor therapeutic and/or adverse effects. This study shows that fMRI with MVPA approach can be used to predict the presence of psychiatric therapeutic and adverse effects in Parkinson's patient who were implanted STN-DBS.

Disclosures: **S. Cho:** None. **P. Testini:** None. **M. Settell:** None. **H. Jo:** None. **H. Min:** None. **K.H. Lee:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.01/K15

Topic: C.04. Movement Disorders

Title: Region-specific transcriptomics of SCA1 mouse models highlight biological pathways underlying tissue vulnerability

Authors: ***T. DRIESSEN**, J. LIM;
Yale Univ., New Haven, CT

Abstract: Spinocerebellar ataxia type 1 (SCA1) is a progressive neurodegenerative disease caused by an expanded polyglutamine repeat in Ataxin-1 (*ATXN1*), and results in progressive ataxia, cognitive impairments, dysarthria, and subsequent respiratory failure. Degeneration of neurons, specifically in the cerebellum and brain stem is a hallmark of SCA1, though the underlying mechanisms dictating cell-type and tissue vulnerability remain largely unknown. In this study, we utilized high throughput transcriptomics to identify pathways unique to vulnerable tissues over the course of disease progression. We first utilized an $Atxn1^{154Q/2Q}$ knock-in mouse model that expresses polyglutamine expanded *Atxn1* throughout the CNS, and compared highly vulnerable tissues, such as the cerebellum and inferior olive, with relatively unaffected regions, such as cortex. Measuring transcriptional changes at the beginning and intermediate stages of tissue pathology allowed us to further elucidate how the transcriptional landscape in vulnerable tissues change over the course of the disease. In addition, transcriptional changes in a second ATXN1 [82Q] mouse expressing the human polyglutamine-expanded mutant ATXN1 in Purkinje cells was assessed at multiple ages and in different tissues to identify meaningful gene clusters common to the two mouse models. RNA-seq data analysis using a variety of bioinformatic tools identified multiple pathways associated with up- or down-regulated gene expression, including inflammatory response, synaptic transmission, and developmental pathways, all of which may be contributing factors in tissue vulnerability. A cluster of genes linked to other SCAs were also significantly altered in the ATXN1 [82Q] cerebellum, providing preliminary evidence that mutant ATXN1 may be an upstream regulator of a portion of SCA genes in affected tissue. In summary, we utilized large scale transcriptomics to identify gene expression changes that are unique to SCA1 affected tissues, paving the way for subsequent

studies to investigate which of these gene clusters predispose the cerebellum and other affected tissues to SCA1 pathology.

Disclosures: **T. Driessen:** None. **J. Lim:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.02/K16

Topic: C.04. Movement Disorders

Support: AMED Brain/MINDS

JSPS KAKENHI 26111701

JSPS KAKENHI 26890005

JSPS KAKENHI 15K18330

JSPS Grant BS251006

JSPS Grant BS261011

Title: Generation of a marmoset model of spinocerebellar ataxia type 3 via AAV9 vector-mediated gene transfer

Authors: ***A. KONNO**, Y. MATSUZAKI, H. HIRAI;
Dept. of Neurophysiol. & Neural Repair, Gunma Univ., Maebashi, Japan

Abstract: Spinocerebellar ataxia type 3 (SCA3) is a genetic disease caused by the expansion of CAG repeat in ataxin-3 (ATXN3) gene. Although there are many researches about possible therapies for SCA3 using a mouse model of SCA3, no therapy has proceeded to the clinic. One of the main reasons may be absence of non-human primate (NHP) model of SCA3, by which scientists can evaluate the effectiveness of possible therapies for future clinical use. In this study, we aimed to generate the marmoset model of SCA3 via adeno-associated virus serotype 9 (AAV9) vector-mediated gene transfer. The marmoset is a small NHP that has attracted remarkable attention as a potential experimental animal link between rodents and humans. Firstly, we used mice to assess the feasibility of the AAV9-mediated production of a SCA3 model, because the assessment of behavioral phenotypes for mice has been already well established. The ATXN3 gene having an expanded CAG repeat (ATXN3[Q89]) was used as the pathogenic gene for SCA3. Rotarod and beam-walking tests revealed progressive ataxia in mice

injected with AAV9 vectors expressing mutant ATXN3[Q89], whereas mice treated with AAV9 expressing ATXN3[Q15], which has the normal length of CAG repeat, were almost indistinguishable from PBS-injected control mice. Then, we proceeded to generate marmoset models by cerebellar injection of the same AAV9 vectors. The behavior of injected-marmosets was monitored and compared with pre-injection levels every two weeks using a tower-descending test and an upper-limb reaching test. In a tower-descending test, marmoset virally expressing ATXN3[Q89] initiated to show significantly worse performance at 4 weeks post-injection and continued to decline thereafter. In an upper-limb reaching test, the marmoset showed poorer performance similarly at 4 weeks after injection, restored to a level similar to the pre-injection period, and again became significantly worse at 14 weeks post-injection. In contrast, marmoset treated with AAV9 expressing ATXN3[Q15] remained almost unchanged in performances in both tests. About 1 year post-injection, immunohistochemical analysis of the marmosets was done. Notably, many large projection neurons in the deep cerebellar nuclei were lost, which was characteristic of SCA3 patients. Thus, we successfully generated SCA3 model of marmoset showing progressive ataxia with characteristic cerebellar pathology.

Disclosures: **A. Konno:** None. **Y. Matsuzaki:** None. **H. Hirai:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.03/K17

Topic: C.04. Movement Disorders

Title: Analysis of glucose metabolism during the pathogenesis of Spinocerebellar Ataxia Type 1

Authors: ***J. DIAZ**[1], A. PEREZ[1], M. GALLEGO[1], Y.-W. WAN[1], T. INOUE[1], A. CHAI[2], M. MALETIC-SAVATIC[1], H. ORR[3], M. GABER[1], Z. LIU[1], R. SAMACO[1], J. BOTAS[1]; [1]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [2]King's Col. London, London, United Kingdom; [3]Univ. of Minnesota, Minneapolis, MN

Abstract: Spinocerebellar ataxia type 1 (SCA1) is one of nine Polyglutamine Diseases caused by CAG expansion in the coding region of the corresponding gene. In SCA1, CAG expanded repeats cause an abnormally long glutamine tract in ATXN1 protein and trigger a gain of function pathogenic mechanism that leads to a progressive neurodegenerative disorder. The brain regions primarily affected are the cerebellum and the brainstem. Previous study reported that insulin sensitivity and insulin secretion are abnormal in SCA1 patients. We hypothesize that expression of mutant ATXN1 impairs glucose metabolism and use multiple approaches to test this hypothesis using mice and *Drosophila* disease models. Gene expression analyses in SCA1

mice and flies suggest glucose uptake deficiency in neuronal cells. Confirming these results, positron emission tomography imaging suggests lower glucose level in the cerebellum of SCA1 transgenic mice. Metabolomic analyses in SCA1 fly neurons reveal a decrease in glucose metabolism metabolites. We carried out a genetic screen in *Drosophila* of all genes encoding glycolytic enzymes for potential modifier genes of SCA1 pathogenesis. We found that knocking down glycolytic genes ameliorates neurodegeneration by reducing steady-state levels of mutant ATXN1 protein. Together these data suggest that glucose metabolism impairments contribute to SCA1 pathogenesis and reveal new therapeutic approaches to decrease ATXN1 neurotoxicity.

Disclosures: J. Diaz: None. **A. Perez:** None. **M. Gallego:** None. **Y. Wan:** None. **T. Inoue:** None. **A. Chai:** None. **M. Maletic-Savatic:** None. **H. Orr:** None. **M. Gaber:** None. **Z. Liu:** None. **R. Samaco:** None. **J. Botas:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.04/L1

Topic: C.04. Movement Disorders

Support: NIH Grant R01NS056224

NIH Grant R01NS074376

Title: Pathogenic polyglutamine expansion length correlates with polarity of the flanking sequences

Authors: M. KIM, I. BEZPROZVANNY;
UT southwestern Med. Ctr., Dallas, TX

Abstract: Polyglutamine (polyQ) repeat expansion within coding sequence of a soluble protein is responsible for eight autosomal-dominant genetic neurodegenerative disorders. These disorders affect cerebellum, striatum, basal ganglia and other brain regions. The pathogenic polyQ-expansion threshold in these proteins varies from 32Q to 54Q. Understanding the reasons for variability in pathogenic polyQ threshold may provide insights into pathogenic mechanisms responsible for development of these disorders.
Here we established a quantitative correlation between the polarity of the flanking sequences and pathogenic polyQ-expansion threshold in this protein family. We introduced an "edge polarity index" (^{E}PI) to quantify polarity effects of the flanking regions and established a strong correlation between ^{E}PI index and critical polyQ expansion length in this protein family. Based

on this analysis we subdivided polyQ-expanded proteins into 2 groups - with strong and weak dependence of polyQ threshold on ^{E}PI index. The main difference between members of the first and the second group is a polarity profile of these proteins outside of polyQ and flanking regions. PolyQ proteins are known substrates for proteasome and most likely mechanistic explanation for the observed correlation is that proteasome may have an impaired ability to process continuous non-polar regions of proteins, resulting in release of undigested products.
The proposed hypothesis provides a quantitative explanation for variability in pathogenic threshold among polyQ-expansion disorders, which we establish to correlate with polarity of flanking regions. Most likely mechanistic explanation of these results is that proteasome is not efficient in processing continuous non-polar regions of proteins. If supported experimentally, our hypothesis may have wide implications for further understanding the pathogensis of polyglutamine expansion disorders.
Keywords: polyglutamine disorders, primary sequence analysis, proteasome, Huntingtin, ataxin

Disclosures: **M. Kim:** None. **I. Bezprozvanny:** None.

Poster

311. Movement Disorders

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Program#/Poster#: 311.05/L2

Topic: C.04. Movement Disorders

Support: AFM-Telethon Grant #16161

Inserm

Paris Descartes University

Title: Upregulation of glial GLT1 glutamate transporter corrects Purkinje cell dysfunction and cerebellum-dependent motor incoordination in a mouse model of myotonic dystrophy

Authors: ***M. GOMES-PEREIRA**[1], D. M. DINCA[1], G. SICOT[1], S. O. BRAZ[1], A. LEROY[2], F. MEDJA[1], A. HUGUET[1], A. NICOLE[1], N. GUERIBA[1], C. CHHUON[3], C. PRIGOGINE[2], C. GUERRERA[3], G. CHERON[2], L. SERVAIS[4], G. GOURDON[1];
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Abstract: Myotonic dystrophy type 1 (DM1) is a multisystemic condition that affects many tissues as well as age groups. Compelling clinical evidence clearly demonstrates the impairment of the central nervous system (CNS), through cognitive/attention deficits, problems with

executive function, prevalent hypersomnia, behavioral changes and intellectual disability in the most severe cases. The neurological manifestations are highly debilitating and distressing for patients and their relatives, and there is no cure for this devastating condition. However, important gaps exist in our understanding of the disease mechanisms in the brain, which delay the design of rational therapies in the CNS. DM1 is caused by the abnormal expansion of a non-coding trinucleotide CTG repeat, which results in the toxic accumulation of expanded transcripts in the nucleus and changes in the activity of RNA-binding proteins, such as MBNL and CELF proteins. Nonetheless, we do not know the cell populations, the neuronal circuits or the molecular pathways primarily affected by the repeat expansion in the CNS. Using a transgenic mouse model of DM1, we found fine motor incoordination and abnormal Purkinje cell firing with fast oscillatory field potential, indicative of cerebellar dysfunction. This finding was unexpected since cerebellum is not typically associated with DM1 brain pathology. To investigate the mechanisms behind Purkinje cell dysfunction we looked for signs of RNA toxicity. Interestingly, RNA foci and misspliging were not detected in Purkinje cells, but were prevalent in the neighboring Bergmann glia. To find how defective astrocytes affect the physiology of Purkinje cells, we used a global proteomics approach and found significant downregulation of the glia-specific GLT1 glutamate transporter in DM1 mice and human patients. Further analysis revealed that MBNL1 inactivation through sequestration into CUG RNA foci was sufficient to downregulate GLT1. GLT1 abnormalities suggest glutamate missignaling and neuronal hyperexcitability in DM1. Pharmacological upregulation of GLT1 in DM1 mice by ß-lactam injection corrected Purkinje cell electrophysiology and motor deficits, indicating that cerebellar dysfunction is mediated by reduced GLT1 and suggesting that glutamate transport activation in glia may be beneficial in DM1 and other related disorders. Our results strongly suggest a role for cerebellum and glia dysfunction in DM1 brain disease and open avenues for future therapies.

Disclosures: **M. Gomes-Pereira:** None. **D.M. Dinca:** None. **G. Sicot:** None. **S.O. Braz:** None. **A. Leroy:** None. **F. Medja:** None. **A. Huguet:** None. **A. Nicole:** None. **N. Gueriba:** None. **C. Chhuon:** None. **C. Prigogine:** None. **C. Guerrera:** None. **G. Cheron:** None. **L. Servais:** None. **G. Gourdon:** None.

Poster

311. Movement Disorders

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Program#/Poster#: 311.06/L3

Topic: C.04. Movement Disorders

Support: NIH R01 NS076919-01

NIH R01 NS090335

Title: Deficient nuclear export of polyglutamine-expanded androgen receptor contributes to toxicity in a cell model of spinal and bulbar muscular atrophy

Authors: *F. ARNOLD, D. MERRY;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA) is an X-linked neurodegenerative disease caused by a polyglutamine (polyQ) expansion in the androgen receptor (AR). Upon ligand binding of testosterone or dihydrotestosterone (DHT) the AR undergoes a conformational change, inducing nuclear localization and transcription of target genes. In SBMA, both the presence of hormone and the nuclear localization of the AR are necessary for toxicity, with the formation of intranuclear inclusions of aggregated AR a hallmark of the disease state. Given the importance of nuclear localization in disease-mediated toxicity, we sought to determine if the nuclear export of polyQ-expanded AR is disrupted, and, if so, whether enhancing the nuclear export of polyQ-expanded AR is protective in cell models of SBMA. Heterokaryon analysis of cells inducibly expressing human AR reveals that polyQ-expanded AR is deficient in nuclear export compared to wildtype AR, even prior to the formation of intranuclear inclusions. Additionally, tagging the mutant AR with a nuclear export signal (NES) enhanced its nuclear export and reduced both inclusion formation and hormone-dependent toxicity. Moreover, the stabilization of polyQ-expanded AR that occurs upon hormone binding was substantially reduced when AR nuclear export was enhanced. All together, these experiments provide us with a new understanding of the role of nuclear export in SBMA pathogenesis and new insights into the effect of the polyQ-expansion on nuclear export.

Disclosures: F. Arnold: None. **D. Merry:** None.

Poster

311. Movement Disorders

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Program#/Poster#: 311.07/L4

Topic: C.04. Movement Disorders

Support: KAKENHI 26293207

Title: TFEB and Hikeshi influence the degradation of the disease-causative proteins in cellular models of neurodegenerative diseases

Authors: *H. ADACHI[1,2], Z. HUANG[1], K. OKADA[1], K. OHNARI[1], T. HASHIMOTO[1], T. TOYOTA[1], Y. IWANAKA[1], R. KATSUMATA[1], G. SOBUE[2];
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Abstract: A common characteristic of neurodegenerative diseases such as polyglutamine diseases is the abnormal accumulation of disease-causing proteins in the nervous system. The formation of nuclear and cytoplasmic inclusions is commonly observed. Under pathologic conditions, the accumulated level of such misfolded and toxic proteins may exceed the protective ability of the proteolytic machinery; the inability to either maintain misfolded proteins in a soluble form or degrade them results in their accumulation and the formation of inclusions. Autophagy refers to self-phagocytosis and is a process by which cells remove a large amount of proteins with long half-lives as well as damaged organelles. The degradation of proteins with long half-lives, organelles, and aggregated proteins is crucial for the maintenance of cell homeostasis. The transcription factor EB (TFEB) has been reported to regulate autophagy by upregulating genes that belong to the coordinated lysosomal expression and regulation (CLEAR) network, thereby controlling lysosomal biogenesis. Hikeshi is essential for the entry of the Hsc70/Hsp70 (Hsp70s) to the nucleus under stress condition. Hsp70s are rapidly and transiently relocated from the cytoplasm into the nucleus and nucleolus in response to heat shock. Inside the nucleus, Hsp70s dissociate from Hikeshi and bind native or non-native client proteins and function as a molecular chaperone, likely reversing and attenuating the multiple heat shock-induced nuclear phenotypes and therefore protecting the cells from heat shock damage. Homozygous missense mutations in the human Hikeshi gene cause congenital leukodystrophy associated with early onset spastic paraparesis, acquired microcephaly and optic atrophy. We examined the effects of the overexpression of TFEB and Hikeshi in cultured cell models of neurodegenerative diseases. Neuronal cells were transfected using Lipofectamine 2000 with plasmids encoding mutant androgen receptor, huntingtin, ataxin-1, ataxin-3, Hikeshi and TFEB. The overexpression of TFEB and Hikeshi decreased the expression of each causative protein in the neuronal cell models. On the other hand, reduction of TFEB and Hikeshi slows the turnover of mutant proteins. These findings demonstrated that TFEB and Hikeshi influence the degradation of the disease-causative proteins.

Disclosures: **H. Adachi:** None. **Z. Huang:** None. **K. Okada:** None. **K. Ohnari:** None. **T. Hashimoto:** None. **T. Toyota:** None. **Y. Iwanaka:** None. **R. Katsumata:** None. **G. Sobue:** None.

Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: Intramural Research Grant (23-9) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry

Japan Agency for Medical Research and Development (AMED)

Title: Generation of transgenic marmoset line with polyglutamine disease and behavioral phenotyping.

Authors: K. OWARI[1], N. NOGAMI[1], T. NAKATANI[1], M. KOIZUMI[1], H. ISHIBASHI[2], Y. NAGAI[3], I. TOMIOKA[4], *K. SEKI[1];
[1]Natl.inst.Neurosci., Tokyo, Japan; [2]Animal Res. Ctr., Tokyo Med. Univ., Tokyo, Japan; [3]Dept. of Neurotherapeutics, Osaka Univ. Grad. Sch. of Med., Osaka, Japan; [4]Inst. for Biomed. Science, Interdisciplinary Cluster for Cutting Edge Res., Shinshu Univ., Nagano, Japan

Abstract: Among non-human primates, common marmoset offers advantages for reproductive technology compared with the macaque species. Their characters such as shorter pregnancy, faster sexual maturation, and higher fecundity permit rapid establishment of a disease model line after the birth of the first transgenic animal. We have previously developed seven transgenic polyglutamine disease model marmosets carrying human SCA3 disease gene (*ATXN3*) with 120 CAG repeats (Tomioka et al 2016). Recently, we have succeeded to establish the disease model line by generating F1 animals by collecting sperm from a symptomatic male animal in the founder generation. Because of its lower motility, we have used a reproductive technique of intracytoplasmic sperm injection for generating Tg embryos and the embryos were transferred into surrogate mothers. Until now, 46 embryos were transferred into 19 surrogate mothers and 5 animals became pregnant. Among them, 4 offspring were delivered from 2 surrogate mothers and the transgene was detected from 2 infant ear tissues and a placenta from another recipient whose infant is too small for biopsy at current stage. These results show germline transmission of the transgene occurred. Since these F1 animals are too young to perform behavioral analysis, we have attempted to develop phenotyping strategies by using one F0 male animal who has seemed to exhibit disease onset by showing the decline of daily activities and muscle weakness. Behavioral profile was analyzed by capturing them by video images and the infra-red sensors equipped in their home cage. Forelimb grip strength was measured by bar grip test, and their locomotor function was evaluated by both foot print analysis and ladder climbing test. Results suggest that some of these evaluation are sensitive to movement disorder that is characteristic of the PolyQ disease, i.e. lower daily motility detected by the motion analysis as well as infra-red

measurement (Tg :0.621×10^7 a.u. vs WT :$1.49\pm0.26\times10^7$ a.u. as mean±SEM, $p< 0.05$), weaker force by voluntary muscle contraction detected by bar-grip test (Tg: 3.63 Kg/kg-BW vs WT :5.20±0.56 Kg/kg-BW as mean±SEM, $p<0.05$). In contrast, no difference was detected throughout measurement period so far (i.e. foot print analysis) suggesting that these motor functions may altered later in its life span and that this line could be a model for late-onset disease. It is likely that applying these test battery on the F1 marmoset line will successfully characterize their phenotype that is crucial for this model to be used in the translational research.

Disclosures: **K. Owari:** None. **N. Nogami:** None. **T. Nakatani:** None. **M. Koizumi:** None. **H. Ishibashi:** None. **Y. Nagai:** None. **I. Tomioka:** None. **K. Seki:** 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; Intramural Research Grant (23-9) for Neurological and Psychiatric Disorders from the National Center of Neurology and Psychiatry, Health Labour Sciences Research Grant for Research on Development of N.

Poster

311. Movement Disorders

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Program#/Poster#: 311.09/L6

Topic: C.04. Movement Disorders

Support: BBB- National Biological Honor Society

Minnesota State University- Mankato

Title: Expression of the novel polyglutamine protein FAM171B in the developing and adult mouse brain.

Authors: A. K. SUDASINGHE, D. S. SHARLIN, *G. M. GOELLNER;
Biol., Minnesota State Univ- Mankato, Mankato, MN

Abstract: Proteins containing polyglutamine (polyQ) tracts within their primary amino acid sequence are particularly interesting because expansion mutation within them has been shown to underlie a growing list of severe neurodegenerative disorders such as Huntington's Disease and several types of Spinocerebellar Ataxias. FAM171B is a novel polyQ protein, originally identified via large scale sequencing efforts, of which very little is known regarding its normal cellular function and expression pattern. In this study, we utilize both in situ hybridization and immunohistochemistry to assay whether FAM171B is expressed in the developing (postnatal

days 7, 21, 42) and adult mouse brain. Our experiments suggest that FAM171B is indeed expressed in brain with pronounced expression in the hippocampus, Purkinje cells of the cerebellum, and cerebral cortex. Considering these observations, FAM171B can be considered a candidate gene for an as yet molecularly uncharacterized neurodegenerative disease.

Disclosures: **A.K. Sudasinghe:** None. **D.S. Sharlin:** None. **G.M. Goellner:** None.

Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: Kennedy's Disease Association Research Grant

NIH Grant NS076919-01

NIH Grant NS090335

Title: Protein interaction networks in the pathogenicity of spinal and bulbar muscular atrophy

Authors: ***A. PLUCIENNIK**[1], T. BERGER[1], S. FINKBEINER[2], D. MERRY[1];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Gladstone Inst. of Neurolog. Dis. and the Univ. of California, San Francisco, CA

Abstract: Spinal and bulbar muscular atrophy is caused by a loss of brainstem and spinal cord motor neurons (and of the associated innervated muscles). The genetic basis of this disease is an expansion of a polyglutamine (polyQ)-encoding CAG repeat within the androgen receptor (AR) gene. Several hypotheses have been put forward to explain the cytotoxic consequences of polyQ expansion at the molecular level, including aberrant protein-protein interactions, altered post-translational modifications, and perturbations to global protein folding homeostasis. Here, we have employed a quantitative proteomics approach involving stable isotope labeling of amino acids in cell culture (SILAC) to identify alterations in the AR interactome due to polyQ expansion. Rat neuronal PC12 cells expressing polyQ-expanded AR or wild type AR were used in this study. Protein interaction partners of AR or polyQ-expanded AR were immunoprecipiated using an AR-specific antibody or the expanded polyQ- and conformation-specific antibody 3B5H10. Our approach has identified several promising protein candidates whose interactions with AR are altered by the polyQ expansion; one of these is Usp7 (ubiquitin specific protease 7). This protein preferentially interacts with soluble AR and does not colocalize with AR nuclear inclusions. Overexpression of human Usp7 in PC12 cells enhanced polyQ-expanded AR

aggregation and toxicity. Reduction of Usp7 levels by shRNA-mediated gene silencing in polyQ-expanded AR-expressing cells decreased the frequency of nuclear inclusions. The functional role of Usp7 in spinal motor neurons that express polyQ-expanded AR is currently being evaluated.

Disclosures: **A. Pluciennik:** None. **T. Berger:** None. **S. Finkbeiner:** None. **D. Merry:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.11/L8

Topic: C.04. Movement Disorders

Title: Behavioral characterization of Zip14 knockout mice; a potential model of manganism

Authors: ***C. G. JANUS**[1], S. JENKITKASEMWONG[2], H. KHAN[1], B. GIASSON[1], M. KNUTSON[2];
[1]CTRND and Dept. of Neurosci., [2]Dept. of Food Sci. and Human Nutr., Univ. of Florida, Gainesville, FL

Abstract: ZIP14 (SLC39A14) is a transmembrane metal-ion transport protein that can mediate the cellular uptake of a number of metals including zinc, iron, and manganese. Studies of *Zip14* knockout (*Zip14*$^{-/-}$) mice have demonstrated that ZIP14 participates among others in the hepatic uptake of zinc during inflammation. We have recently found that *Zip14*$^{-/-}$ mice display dramatic alterations in manganese metabolism. Most notably, *Zip14*$^{-/-}$ mice exhibit reduced concentrations of manganese in liver and massively elevated manganese concentrations in the brain and bone (10- and 65-fold higher than control). Concentrations of other metals (e.g., zinc, iron, copper) in the brain are unaffected, indicating that the defect is specific for manganese. We hypothesize that the absence of ZIP14 prevents the uptake of manganese in the plasma by the liver, causing subsequent increase of manganese in the blood and accumulation in peripheral tissues, bones, and the brain. In humans, manganese accumulation in the brain deposits in regions of the basal ganglia and can lead to a Parkinsonian-like disorder known as manganism. To define where manganese accumulates in the brain of *Zip14*$^{-/-}$ mice, we used laser-ablation inductively coupled plasma mass spectrometry, which provides a quantitative and detailed image of the distribution of manganese and other metals. We found that *Zip14*$^{-/-}$ mice accumulated manganese in a number of brain areas including the globus pallidus, a region that preferentially loads manganese in humans with manganism. We hypothesized that the accumulation of manganese in the basal ganglia of *Zip14*$^{-/-}$ mice is associated with neuropathological changes leading to alterations in motor behavior. The evaluation of *Zip14*$^{-/-}$, *Zip*$^{+/-}$, and wild type mice in a standard battery of tests of physical development and locomotor phenotype revealed lower body weight of *Zip14*$^{-/-}$

mice, and impairment in both spontaneous locomotor activity as well as motor coordination when the mice were challenged on accelerated rod. *Zip14*$^{-/-}$ mice also showed abnormal species specific spontaneous home cage behavior. The motor deficits of *Zip14*$^{-/-}$ mice were detected as early as 2.5 months and persisted at least until 10 months of age. Overall, our data suggest that *Zip14*$^{-/-}$ mice show impairments in multiple motor systems, whereas hemizygous *Zip14*$^{+/-}$ mice do not compared to WT mice.

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Poster

311. Movement Disorders

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Program#/Poster#: 311.12/L9

Topic: C.04. Movement Disorders

Title: Cas9 lipid nanoparticles as an efficient delivery tool for primary neural cultures

Authors: *E. RAMSAY[1], J. SINGH[1], G. THARMARAJAH[1], R. DESOUZA[1], E. OUELLET[1], A. THOMAS[1], S. GARG[1], T. LEAVER[1], A. WILD[1], A. WHITE[2], C. HANSEN[2], J. TAYLOR[1]; [1]Precision Nanosystems Inc., Vancouver, BC, Canada; [2]Physics and Astronomy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: In recent years, the demand for an efficient delivery tool capable of facilitating the use of RNA for modulating gene expression in the mammalian central nervous system has been a subject of many discussions. Furthermore, progress in the CRISPR-Cas9 gene editing arena has pushed the demand even further as more research programs are now poised in utilizing the technology for establishing new treatment strategies. Of the tools available, developments in the field of lipid nanoparticles (LNPs) has enabled significant gains for the reliable and efficient delivery of these precious payloads both in research and clinical settings. Here, we bridge that gap by describing the development of an LNP delivery system for Cas9, robustly manufactured with clinical-grade materials using microfluidic technology at suitable scales for screening applications. Cas9 LNPs of a defined diameter were engineered by modulating the lipid contents and parameters, such as mixing ratio and flow rate, associated with their manufacture via a proprietary microfluidic platform. The physicochemical characteristics of these novel nanoparticles was determined and correlated with their in vitro performance to guide rational design and optimization. Specifically, the particle size and encapsulation efficiency of Cas9 mRNA and Cas9 ribonucleoprotein (RNP) complexes into electron-dense lipid nanoparticles was determined using dynamic light scattering (DLS) and molecular assays. The performance of

these LNP delivery systems was initially characterized by manufacturing nanoparticles of about 80-100 nm harboring a reporter GFP mRNA. Initial studies demonstrated highly efficient particle uptake and minimal cellular toxicity in primary rat cortical astrocytes. Direct observations of the GFP fluorescence confirmed highly efficient gene expression 48h after a single-dose treatment. Looking at the effect of LNPs on individual cells, further studies facilitated by the use of a microfluidic device capable of high-throughput single-cell digital PCR provided insight into the response seen in a bulk population.These validation studies were paramount in providing suitable insights in establishing strategies for efficiently delivering Cas9 mRNA and/or Cas9 RNP LNP by administering them directly into E18 primary rat astrocyte cell cultures. The editing potential of those formulations was tested in cultures for HPRT target-gene editing.

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Poster

311. Movement Disorders

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Title: Micro-RNA351 alleviates denervation-induced skeletal muscle astrophy by targeting tumor necrosis factor receptor-associated factor 6

Authors: ***F. DING**, M. SHEN, J. QIU, Q. CHENG, Q. HE;
Nantong University, China, Jiangsu, China

Abstract: Denervation-induced skeletal muscle atrophy is one of the challenges of peripheral nerve regeneration. We have identified the differentially expressed proteins in the tibialis anterior (TA) muscles after sciatic nerve transection by the isobaric tags for relative and absolute quantification (iTRAQ) coupled with two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS), and showed that tumor necrosis factor receptor-associated factor 6 (TRAF6) was up-regulated in denervated TA muscle as the elapse of time. On the other hand, knockdown of TRAF6 could significantly inhibited glucocorticoid-induced C2C12 myotube atrophy *in vitro* and attenuated denevation-induced TA muscles atrophy after sciatic nerve injury *in vivo*. MicroRNAs (miRNAs, miRs) are involved in modulation of various physiopathological processes, but the impacts of miRNAs on muscle atrophy have not been fully investigated. We observed that the expression of miR-351 was differentially expressed in the tibialis anterior (TA) muscle at different time points after sciatic nerve transection, and the time-dependent expression profile of miR-351 was inversely correlated with that of TRAF6 at the mRNA and protein levels. The dual luciferase reporter assay indicated that miR-351 could dramatically down-regulate the expression of TRAF6 by directly targeting the 3'-UTR of TRAF6. Overexpression of miR-351 inhibited a significant decrease in the wet weight ratio or cross-sectional area of the TA muscle after sciatic nerve transection. Western blot analysis showed that the protein expression of TRAF6, muscle ring-finger protein 1 (MuRF1) and muscle atrophy F-box (MAFbx) in denervated TA muscle was respectively suppressed by overexpression of miR-351. In conclusion, miR-351 allivates denervation-induced atrophy of TA muscle after sciatic nerve transection at least partially through negative regulation of TRAF6 as well as MuRF1 and MAFbx, the two muscle-specific ubiquitin ligases, and two downstream signaling molecules of TRAF6.

Disclosures: **F. Ding:** None. **M. Shen:** None. **J. Qiu:** None. **Q. Cheng:** None. **Q. He:** None.

Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: Tourette Association of America Inc. - Research Grant Award 2014-2015

Title: Prominent phase-amplitude cross-frequency coupling between alpha and gamma oscillations underlies motor-tic encoding in cerebro-basal ganglia-cerebellar networks

Authors: *T. NINOMIYA[1], Y. NAGAI[2], T. SUHARA[2], T. MINAMIMOTO[2], M. TAKADA[1], M. MATSUMOTO[3], M. ISODA[4], K. W. MCCAIRN[1];

[1]Systems Neurosci. Section, Primate Res. Institute, Kyoto Univ., Inuyama, Japan; [2]Mol. Neuroimaging, Natl. Inst. of Radiological Sci., Chiba, Japan; [3]Fac. of Med., Univ. of Tsukuba, Tsukuba, Japan; [4]Sch. of Med., Kansai Med. Univ., Osaka, Japan

Abstract: Tourette syndrome (TS) is a complex hyperkinetic motor and neuropsychiatric disorder. While TS has traditionally been believed to arise from abnormalities within the cortico-basal ganglia-thalamo-cortical network (CBTC), recent studies suggest an involvement of the cerebellum (Cb). Utilizing our nonhuman primate model of motor tics, based on selective bicuculline-mediated disinhibition of functional territories of the striatum (dorsolateral putamen), we investigated how intrinsic and extrinsic networks of the CBTC encode tics in the spectral domain. Positron emission tomography (PET) imaging was initially undertaken to identify areas of activation associated with motor tics. Not only brain regions in the CBTC, but also Cb were found to be activated during the presence of motor tics. We then performed simultaneous multi-site electrophysiological recordings of local field potentials (LFPs) in the primary motor cortex (MI), the basal ganglia, and Cb, and compared them with signals derived from normal movement. The LFP data were subjected to phase-amplitude cross-frequency coupling analysis. Both normal and tic states were associated with increased coupling between the phase of alpha-band activity (7-14 Hz) of any recording sites and the amplitude of gamma-band activity (>50 Hz) of the striatum and MI. However, the strength of alpha-gamma coupling in the tic state was greatly accentuated relative to that in the normal state. The present investigation demonstrates that tic encoding is a global network phenomenon beyond the CBTC, associated with abnormally high and complex, long-range, spectral-domain interactions in the alpha and gamma bands.

Disclosures: **T. Ninomiya:** None. **Y. Nagai:** None. **T. Suhara:** None. **T. Minamimoto:** None. **M. Takada:** None. **M. Matsumoto:** None. **M. Isoda:** None. **K.W. McCairn:** None.

Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: NIH Intramural Program

Title: Combined malonic and methylmalonic aciduria (CMAMMA) presenting as myelopathy in eighth decade.

Authors: *D. NARENDRA[1,2], N. ATASSI[1], K. LINDGREN[1], F. EICHLER[1];
[1]MGH, Boston, MA; [2]NINDS Intramural Program, Bethesda, MD

Abstract: Mutations in the mitochondrial protein acetyl-CoA synthase 3 (ACSF3) were recently identified as a cause of combined malonic and methylmalonic aciduria (CMAMMA). To date 15 cases with confirmed homozygous or compound heterozygous mutations in ACSF3 have been reported. These have typically presented with vomiting, encephalopathy, and seizures in childhood or neuropsychiatric symptoms in adulthood. The full phenotypic spectrum of disease associated with ACSF3 mutations, however, is unknown. In particular, although myelopathy is a common presentation of elevated methylmalonic acid (MMA) and homocysteine levels in the setting of vitamin B12 deficiency and some forms of methylmalonic aciduria, it has not been associated with mutations in ACSF3 or other causes of elevated MMA with normal homocysteine levels. We identified homozygous ACSF3 missense mutations (c.1672C>T; p.Arg558Trp) in a 77 year-old woman who presented with subacute lower extremity weakness, numbness, and spasticity, consistent with myelopathy. She had no history of seizures or psychiatric disorder. Serum testing revealed highly elevated MMA (12.04; nlm <=0.40) in the setting of normal B12 (>2000; nlm >250). Subsequent urine organic acid analysis was notable for highly elevated methylmalonic acid and malonic acid, establishing the diagnosis of CMAMMA. Her homocyteine level was initially mildly elevated at 14.3 (nlm 0-14.2) and was within normal range at 13.4 on repeat testing. To our knowledge this is the first case of CMAMMA due to ACSF3 mutations presenting as a myelopathy in humans, and the first case to present after the age of 75. It expands the known phenotype of ACSF3 mutations and suggests that patients with myelopathy and high MMA and B12 levels should be screened for CMAMMA and ACSF3 mutations. More fundamentally, it suggests that elevated MMA (with near normal homocysteine levels) may be sufficient to cause myelopathy in humans.

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Poster

311. Movement Disorders

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National Natural Science Foundation, P.R.China.31471030

Title: Dexmedetomidine decreased the spinal motoneurons death in roots avulsion injury of the brachial plexus via AC-cAMP-PKA pathway

Authors: ***X. XU**[1], G. YU[1], X. CHEN[2], X. CHEN[2], J. LI[3], M. CAO[4], Y. TANG[5], L. LIU[5], R. AN[6], Z. QIU[6], L. ZHOU[1,7];
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Abstract: Dexmedetomidine(Dex) is a highly specific, potent and selective a2-adrenoceptor agonist. However, it remains unclear whether dex affects neurons in injured central nervous system. This study was performed in order to investigate if dex has effects on motoneurons in injured spinal cord and via which intracellular signaling pathway does it work. Forty female adult Sprague-Dawley rats underwent unilateral brachial plexus roots avulsion were randomly subdivided into the dex(20ug/kg, 0.1ml) and saline(0.1ml) treated groups by intraperitoneal injection following injury. Three days later, the treated rats were sacrificed and the cryostat sections of the spinal cord of the 7th and 8th cervical segments were stained by nertral red(NR), prepared for the immunohistochemistry reactions with CaMKII, p-CaMKII, PKA and nNOS antibodies. The number of survived motoneurons were counted and shown as the ratio of the ipsilateral to contralateral sides of C7 and C8 spinal cord. The results showed that the survival ratio of the motoneurons was significantly higher in the dex group when compared to that of the saline group. Meanwhile, the ventral horn motoneurons were p-CaMKII, PKA and nNOS positive in the ipsilateral but negative in the contralateral spinal cord. Since a2-adrenoceptor agonist can active glycine (gly) receptor via AC-cAMP-PKA pathway and glycine has a protective effect on cells, we think that dex may protect motoneurons in this way. The mechanism of the signaling pathway of the AC, cAMP, gly, p-CaMKII, PKA, and nNOS should be detected further to see whether the dex be used as a neuronal protective drug in the treatment of spinal cord injury.

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Poster

311. Movement Disorders

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International Essential Tremor Foundation

American Parkinson Disease Association (APDA)

Greater St. Louis Chapter of the APDA

Barnes Jewish Hospital Foundation

McDonnell Center for Higher Brain Function

Title: Altered resting-state functional connectivity in patients with essential tremor

Authors: *A. E. MORRIS[1], S. A. NORRIS[2], A. Z. SNYDER[3], J. M. KOLLER[2], J. W. MINK[1], J. S. PERLMUTTER[2];
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Abstract: Essential tremor (ET) is the most common movement disorder in adults. Yet, its pathophysiology is poorly understood. Deep brain stimulation (DBS) of the ventral intermediate nucleus of the thalamus (VIM) is an effective treatment for medically-refractory ET, but the therapeutic mechanism of action remains unknown. Neuroimaging studies have implicated cerebello-thalamo-cortical pathways in the pathogenesis of ET. However, most of these studies are motor task-based and therefore subject to chicken-egg interpretive ambiguities: do observed response abnormalities reflect abnormal performance, e.g., enhanced tremor, or do observed response abnormalities reflect primary pathology? Resting-state functional connectivity magnetic resonance imaging (rs-fcMRI) offers a means of interrogating brain function without the problem of performance confounds.
Subjects with ET were recruited from the Washington University Movement Disorders Center prior to receiving VIM DBS. Each subject had resting-state scans (2-5 x 6 min BOLD fMRI runs) with eyes closed and were observed for movement during scan acquisition. Tremor rating scale (TRS) severity was assessed pre-operatively. Age and sex-matched control data were previously obtained. fMRI data preprocessing minimized the effects of head motion during scanning. The present results represent 21 ET patients (out of an original sample of 33) and 41 control participants who passed rigorous data quality assurance criteria. Seed regions were defined within the cerebellum, striatum, sensorimotor cortex, anteroventral thalamus, and supplementary motor area (SMA), as well as in multiple cortical areas representing major resting state networks (RSNs). Composite within-RSN functional connectivity (FC) scores were evaluated and correlated with TRS scores.
In comparison to controls, ET patients showed weaker FC between anteroventral thalamus and primary motor cortex, SMA, and brainstem and between cerebellar right crus I and bilateral thalamus. Brainstem-thalamus FC correlated with pre-operative tremor severity. Composite

network strength in sensorimotor, default mode, dorsal attention, control, and salience networks did not differ between groups.
These results support the hypothesis that dysfunction in subcortical cerebello-thalamo-cortical circuits underlies the pathophysiology of ET. Sparing of cortical FC further supports the idea that ET is a disorder of subcortical origin. Finally, these findings support the concept that ET reflects dysfunction at the network level rather than focal dysfunction within a single structure.

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Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: R01 NS072446

Title: Lack of ABCD1 primes microglia for phagocytosis and axonal degeneration

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Abstract: Mutations in ABCD1 cause the neurodegenerative disease adrenoleukodystrophy that most commonly manifests as spinal cord axonopathy. Microglial dysfunction has long been implicated in pathogenesis. We examined spinal cord microglia in ABCD1-deficient mice displaying axonal degeneration and investigated the role of ABCD1 in microglia activity toward neuronal phagocytosis in cell culture. In a mouse model deficient in ABCD1, microglia activation characterized by CD68 and IBA1 expression was found proceeding neurodegeneration. We also demonstrated upregulation of several phagocytosis related markers within the spinal cord. Interestingly this occurs in the absence of overt inflammation. In addition, primary microglia isolated from Abcd1-/- mice didn't show distinct proinflammatory gene expression but revealed significant upregulation in phagocytosis related molecules including

Bai1, Gas6, mfgE8 and TREM2, indicating that microglia lacking ABCD1 are primed for phagocytosis, affecting neurons stressed by the metabolic milieu. Blocking phagocytosis or specific phagocytic receptors may alleviate axonal degeneration and be therapeutically beneficial.

Disclosures: Y. Gong: None. **N. Sasidharan:** None. **P. Musolino:** None. **J. El Khoury:** None. **F. Eichler:** None.

Poster

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Topic: C.04. Movement Disorders

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R01 HD076436

Goldsmith Foundation

Title: Extraction of motor spatial patterns in children with movement disorders via joint decomposition of brain and muscle activity

Authors: *A. BARACHANT[1,2], J. B. CARMEL[1,2,3], K. M. FRIEL[1,2,3], A. M. GORDON[4], D. GUPTA[1,2];
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Abstract: In children with neuromotor disorders such as in cerebral palsy (CP), electroencephalography (EEG) based motor spatial patterns can provide valuable information for functional motor connectivity assessment. However, conducting cue-based EEG motor paradigms in people with CP has 3 main challenges: First, they can have difficulty initiating, maintaining and stopping a movement, which means movement is not time-locked to cue stimuli, which is essential for extracting invariant spatial patterns. Second, their EEG motor patterns maybe weaker than background noise, which requires many more trials than healthy subjects, which can be challenging especially for children. Third, the brain injury that leads to CP causes reorganization of the cortical control signals, leading to atypical motor spatial patterns that are

not trivial to interpret. To overcome these challenges, we describe here a novel semi-supervised method that does not rely on cue based data epoching, but jointly decomposes the brain (EEG) and muscle (EMG) signals in the Riemannian space. It has the advantage of being objective, simpler and generalizable to signals of varying complexity, as compared to other recent methods such as SPoC and mSPoC that are quite complex and designed to handle a specific type of input signal. We demonstrate the method with the analysis of EEG and EMG data from 23 children with CP, to ascertain their reorganized functional motor connectivity. Subjects participated in an EMG-feedback based videogame experiment, where they controlled the lateral movement of a spaceship by pinching the index finger of either hand. In this way, the subject was engaged and focused and rewarded for being calm, while synchronized EEG and EMG was being acquired from hundreds of movement trials. The cue in this case was continuous rather than discrete. EEG and EMG covariance matrices were estimated and projected in their respective Riemannian tangent space and vectorized.Then a Canonical Partial Least Square was applied to find vector rotations in the tangent space to extract a latent variable that explains the maximum variation between EEG and EMG. Rotation coefficients were back-projected and diagonalized to produce a set of spatial patterns ranked by their importance. This method can enable the use of EEG based neurophysiological assessment of functional motor connectivity in people, especially children, with neuromotor disorders. A better understanding of brain physiology can help guide the development of more effective therapies. (Software implementation of this method is made available as an open-source python toolbox called *pyRiemann*)

Disclosures: A. Barachant: None. **J.B. Carmel:** None. **K.M. Friel:** None. **A.M. Gordon:** None. **D. Gupta:** None.

Poster

311. Movement Disorders

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Program#/Poster#: 311.20/M3

Topic: E.05. Brain-Machine Interface

Title: Closing the loop in essential tremor treatment: an adaptive approach to deep brain stimulation

Authors: *E. OPRI[1], J. SHUTE[2], R. MOLINA[3], K. FOOTE[4], M. OKUN[4], A. GUNDUZ[2]; [2]Biomed. Engin., [3]Electrical and Computer Engin., [4]Ctr. for Movement Disorders & Neurorestoration, [1]Univ. of Florida, Gainesville, FL

Abstract: Essential Tremor (ET) is one of the most common motor disorders, and it is characterized by the presence of a rhythmical, involuntary slow oscillatory movement of the

limbs (~5-10 Hz). Intention tremor occurs generally in the upper limbs, and it manifests during initiation and execution of target-aimed movements. Even though the pathophysiological basis of ET is still not well known, it is suggested that there is a synergic involvement of the cerebellum, ventral intermediate nucleus (Vim), premotor (PM) and primary motor (M1) cortex in the generation of tremor oscillations. Deep Brain Stimulation (DBS) is used to suppress this synergic involvement by targeting "tremor cells" in the Vim region. The aim of the study is to understand the effect of DBS on the Vim, PM and M1 regions, and to develop a closed loop stimulation delivery system that can activate based on specific neuromarkers in the Vim, PM and M1, such as presence of tremor and/or movement. In addition, coherence analysis between PM, M1 and Vim shed light on the relationship and network between the regions involved in the tremor generation. The closed loop system will be implemented in a Medronic Activa PC+S neurostimulator with Nexus E software to achieve a completely standalone solution, together with Nexus D to enable the interfacing with external sensing sources. Hence, the stimulation can be modulated by using neuromarkers together with other sources used as input features, such as accelerometers or gyroscopes, allowing to achieve better performance in detecting when stimulation is necessary. The fusion between internal neuromarkers and external sensor inputs can lead to the creation of "context aware" closed loop systems (e.g. knowing whether the patient is reaching out for a cup of coffee or driving a car) and increasing the quality of life of the patients. Our results suggest that with the adoption of an embedded closed loop DBS solution would be feasible to avoid side effects of stimulation, such as balance and speech impairment, while delivering an equally effective treatment and slowing battery depletion.

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Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

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Title: Functional correlates of the therapeutic and adverse effects evoked by thalamic stimulation for essential tremor

Authors: *H. JO[1], W. S. GIBSON[1], P. TESTINI[1], S. CHO[1], K. R. GORNY[2], J. P. FELMLEE[2], K. M. WELKER[2], B. T. KLASSEN[3], H.-K. MIN[1,2], K. H. LEE[1];
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Abstract: Deep brain stimulation (DBS) is an established neurosurgical therapy for movement disorders including essential tremor and Parkinson's disease. While typically highly effective, DBS can sometimes yield sub-optimal therapeutic benefit and can cause adverse effects. In this study, we tested the hypothesis that intraoperative functional magnetic resonance imaging could be used to detect DBS-evoked changes in functional and effective connectivity that would correlate with the therapeutic and adverse effects of stimulation. Ten patients receiving DBS of the ventralis intermedius thalamic nucleus for essential tremor underwent functional magnetic resonance imaging during DBS stimulation applied at a series of different electrode sites, followed by evaluation of DBS-evoked therapeutic and adverse effects. Correlations between the therapeutic effectiveness of DBS (three months post-operatively) and DBS-evoked changes in functional and effective connectivity were assessed using region of interest-based correlation analysis and dynamic causal modeling, respectively. Further, we investigated whether brain regions might exist in which activation resulting from DBS might correlate with the presence of paresthesias, the most common DBS-evoked adverse effect. Thalamic DBS resulted in activation within established nodes of the tremor circuit: sensorimotor cortex, thalamus, contralateral cerebellar cortex and deep cerebellar nuclei (FDR $q<0.05$). Stimulation-evoked activation in all these regions of interest, as well as activation within supplementary motor area, brainstem, and inferior frontal gyrus, exhibited significant correlations with the long-term therapeutic effectiveness of DBS ($p<0.05$), with the strongest correlation ($p<0.001$) observed within the contralateral cerebellum. Dynamic causal modeling revealed a correlation between therapeutic effectiveness and attenuated within-region inhibitory connectivity in cerebellum. Finally, specific sub-regions of sensorimotor cortex were identified in which DBS-evoked activation correlated with the presence of unwanted paresthesias. These results suggest that thalamic DBS in tremor likely exerts its effects through modulation of both olivocerebellar and thalamocortical circuits. In addition, our findings indicate that DBS-evoked functional activation maps obtained intraoperatively may contain predictive information pertaining to the therapeutic and adverse effects induced by DBS.

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Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Title: Evaluation of a wearable tremor modulation device for patients with essential tremor based on electrical peripheral nerve stimulation

Authors: *J. KIM[1], C. K. PARNELL[2], T. WICHMANN[3], S. P. DEWEERTH[1,2];
[1]Sch. of Electrical and Computer Engin., [2]Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Neurol., Emory Univ., Atlanta, GA

Abstract: Kinetic tremors in conditions such as essential tremor affect patient movements that require high degrees of dexterity and precision. Common methods of treatment are medications (primidone, beta-blockers) and thalamic deep brain stimulation. Peripheral nerve stimulation has also been tried in patients with treatment-resistant tremor (Prochazka et al. 1992; Hao et al. 2002), but this technique has not been extensively used in patients because of the bulkiness of the stimulation systems used in these experiments, and the perceived lack of efficacy of this treatment modality. To re-evaluate the usefulness of this technique, we developed a wireless wearable stimulation system that uses 3-D accelerometric measurements of arm tremor characteristics for closed-loop optimization of stimulation parameters. The motion sensor data are wirelessly sent to a PC or smartphone that then analyzes tremor movements. The stimulator (constant voltage mode) is powered by a 3.7 V rechargeable Li-ion battery, and can generate pulses up to ± 25 V in amplitude. All custom designed electronics (18 x 28 mm^2) are enclosed in a wrist-watch sized container.
Two subjects (19 and 20 years old) with kinetic tremor participated in this study. Round surface electrodes (1” diameter) were placed on two sites over the radial and ulnar nerves. We initially adjusted stimulation amplitudes so that they were noticeable, but not uncomfortable, using 200 μs wide biphasic stimuli. We then changed the amplitudes, frequency, and duty cycle of the stimuli, as well as the duration of the inter-pulse train interval while observing the amplitude of the kinetic tremor when subjects moved a small object from one cup to another with a spoon. The frequency, amplitude, and phase shift of the tremor were analyzed before and during the stimulation were compared. We found that the tremor amplitude was reduced by up to 63% when 5 stimuli (100 Hz) were applied with 500 ms inter-stimulation intervals. The placement of the electrodes and skin impedance differed between subjects, so that the stimulation parameters may have to be individualized for each patient. In future studies, we will develop an automated method of optimization of electrode positions and stimulation parameters, based on tremor characteristics.

Disclosures: **J. Kim:** None. **C.K. Parnell:** None. **T. Wichmann:** None. **S.P. DeWeerth:** None.

Poster

311. Movement Disorders

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Topic: C.04. Movement Disorders

Support: CurePSP Foundation

Title: Modeling Progressive Supranuclear Palsy by viral-mediated tau seeding in the cholinergic neurons of the pedunculopontine

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Abstract: Progressive Supranuclear Palsy (PSP) is the most common atypical Parkinsonism. Early stages of PSP closely match Parkinson's Disease (PD) to the point that many with PSP are initially diagnosed as PD and prescribed dopaminergic therapies, to which PSP patients respond poorly. The underlying etiology of PSP is pervasive and progressive tau protein aggregation resulting in neurodegeneration of multiple brain areas. Currently there is no preclinical model of PSP that combines 1) the isoform of tau that is prominent in PSP (1N4R), 2) tau aggregation in clinically relevant brain areas, and 3) clinically relevant behavioral deficits. However, our recent data in rats suggest that the targeted viral-mediated over-expression of tau in the cholinergic neurons of the pedunculopontine tegmentum (PPT) may be useful in understanding PSP.
The PPT projects to many brain regions implicated in PSP (e.g. SN, thalamus, globus pallidus) and undergoes considerable degeneration, with early and selective degeneration of the cholinergic subpopulation of neurons. The selective lesion of cholinergic PPT neurons produces deficits similar to that seen in PSP (e.g. deficits in motor performance, attention, and acoustic startle reflex). Therefore, to simultaneously ablate cholinergic neurons (to produce the behavioral effects) and introduce tau protein (to seed pathological tau) we stereotaxically injected an adeno-associated virus (AAV) encapsulated floxed double-inverse ORF wildtype 4-repeat tau vector into the PPT of a transgenic rat line expressing CRE recombinase in cholinergic neurons. At 12 weeks post-infection we see pathology consistent with early PSP: i) the loss of cholinergic neurons, ii) phosphorylated tau, and iii) spread of phosphorylated tau to the SN. Results to date suggest that targeting the over-expression of tau to specific neuronal populations in rats can produce progressive tauopathy not typically seen in other animal models.

Disclosures: **D.A. Maclaren:** None. **M.E. Griffin:** None. **S.D. Clark:** None.

Poster

311. Movement Disorders

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP NIH

BRINM

Title: Ligand for cell adhesion molecule PTPRD: Illudalic acid derivatives inhibit recombinant PTPRD phosphatase and are tolerated *In vivo*

Authors: ***G. R. UHL**[1], P. PAIK[2], M. MARTINEZ[4], A. SULIMA[5], K. RICE[3];
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Abstract: PTPRD is a receptor type protein tyrosine phosphatase with human variants that provide oligogenic contributions to risk for restless leg syndrome (RLS, WIlis-Ekbom disorder) and polygenic contributions to vulnerability to addiction. We have recently identified association between RLS-associated PTPRD SNPs and levels of PTPRD mRNA expression in postmortem human brain. Data from work by Hart and colleagues implicated PTPRD in individual differences in rewarding human responses to amphetamine. We have found that mice with altered levels of PTPRD expression display altered sleep onset as well as marked differences in preference for places previously paired with cocaine (CPP). Each of these features increases interest in drugs that could modify PTPRD activities. We have targeted ligands for PTPRD's phosphatase domains, and identified illudalic acid analogs as active at related phosphatases. Recombinant human PTPRD phosphatase domain fusion protein can be produced in E Coli strains in ways that allow it to be active in spectrophotometric phosphatase assays. Illudalic acid analogs, including a 6-butoxy analog, can be synthesized *via* an improved method. The 6 butoxy illudalic acid analog, but not three related compounds, provides micromolar potency inhibition of recombinant PTPRD phosphatase . This inhibition develops during preincubations up to 16 mins in ways that are consistent with pseudoirreversible kinetics and with models for interactions between related compounds and other phosphatases. Mice injected with 6 butoxy illudalic acid analog doses up to solubility limits display no gross alterations in behavior nor any signs of

systemic toxicity. 6 butoxy illudalic acid analogs provide potential lead compounds for inhibition of PTPRD phosphatase activity in ways that should contribute to understanding of the pathways whereby alteration of PTPRD activity influences addiction and RLS phenotypes and to development of drugs that may be useful treatments for these often-poorly-treated disorders.

Disclosures: G.R. Uhl: None. **P. Paik:** None. **M. Martinez:** None. **A. Sulima:** None. **K. Rice:** None.

Poster

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Topic: C.04. Movement Disorders

Support: R01 NS072446

Title: The role of ABCD1 in dorsal root ganglia in adrenomyeloneuropathy

Authors: *F. EICHLER, R. KOK, Y. GONG, N. SASIDHARAN;
Massachusetts Gen. Hosptial | Harvard Med. Sch., Boston, MA

Abstract: Mutations in ABCD1 cause the neurodegenerative disease adrenomyeloneuropathy (AMN) that manifests as spinal cord axonopathy. It has been reported that ABCD1 is highly expressed in DRG (sensory) neurons. Yet the role of ABCD1 in DRG has not been elucidated. Performing immunfluorescent experiments in wild type mice, we found expression not to be present in DRG neurons themselves but localized around them. Further containing experiments revealed ABCD1 expression in satellite cells as demonstrated by co-localization of ABCD1 and the satellite cell marker S-100. ABCD1 expression localized to peroxisomes that were abundant in satellite cells but sparse in neurons. A lack of costaining between ABCD1 and MBP excluded the possibility that Schwann cells are responsible for the strong expression of ABCD1 at the periphery of the neurons. The strong expression of ABCD1 observed in dorsal root ganglia satellite cells suggests an important role for ABCD1 in this cell type. To investigate this further, we inhibited the growth of satellite cells, using the mitotoxin 1β-arabinofuranosylcytosine (ARAC). After three days of growth we observed a strong reduction in neurite outgrowth and density. Finally we examined DRG explants from both wild type and ABCD1 knockout mice. After three weeks of culturing, a reduction in the neurite density of dorsal root ganglia lacking ABCD1 was observed (81% of WT explants vs. 56% of KO explants with dense outgrowth, paired two-tailed T-test $P<0.05$), accompanied by a lesser but significant (paired two-tailed T-test $P<0.05$) reduction in neurite length. In conclusion, peroxisomes are abundant in satellite cells

in mouse DRG but not their neurons. ABCD1 is a peroxisomal half-transporter and therefore found in DRG satellite cells and to a lesser extent in DRG neurons. Furthermore, satellite cells are important for neurite outgrowth in dorsal root ganglia explant cultures, and this appears to depend on a healthy and functional ABCD1. These insights provide a first anatomic and pathogenic link between the availability of the functional ABCD1 protein and the sensory neuropathy in AMN.

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Poster

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Topic: C.04. Movement Disorders

Support: NIH Grant R01

Title: Harmaline-induced tremor in large animals

Authors: *J. LEE[1], I. KIM[1], L. CHENG[2], S.-Y. CHANG[1];
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Abstract: Essential Tremor (ET) is the most common adult on-set movement disorder, which affects around 10 million Americans and millions more worldwide. However, underlying mechanisms of ET has not been fully understood. This lack of our understanding also limited to develop novel treatment method and/or strategy. It is important and necessary to develop an animal model of ET to investigate underlying mechanisms and pathophysiology of ET and screen novel drugs. B-carboline alkaloids (BACs), including norharman, harmane, harmine, harmaline, and ibogaine, are well known to induce action and postural tremor in mice, rats, rabbits, cats, and monkeys. However, a drug-induced tremor has not been tested in pig, even though the pig brain, which is gyrencephalic, resembles the human brain more in anatomy, growth and development than do the brains of commonly used small laboratory animals. Thus, we developed a pig tremor and analyzed the drug-induced tremor. Yucatan and domestic pigs were used to develop drug-induced tremor. Under general anesthesia, intravenous catheter and wireless three-dimensional accelerometers were placed on the ear and the limb, respectively. Once the animal was fully awake and its ambulatory behavior was stable, tremor started to be monitored. Video monitoring was performed simultaneously with a tremor monitoring. Harmaline (2.5 and 5mg/kg) was intravenously administered after 30 minutes of baseline recording. Recording session maintained for 3 hours. Harmaline was administsered once a day,

and this was repeated three times with 48 hours interval. Accelerometer data were obtained along three axis; x-,y-,z-, and fast Fourier transformation was used to yield power spectra. In pigs, harmaline could induce tremor with 10-15 Hz and the drug-induced tremor maintained for one and half hours. The amplitude of drug-induced tremor was significantly different with dose. In the 5mg/kg harmaline-injected animal, the amplitude was decreased according to repeated injection, while there was no significant difference in the 2.5mg/kg harmaline-injected animal. Also, the maintained duration of tremor showed a positive correlation with the dose. Our results suggest that harmaline-induced tremor model in pig can be useful as a preclinical animal model to identify the physiological and pathological mechanism for ET and test a novel treatment method.

Disclosures: J. Lee: None. **I. Kim:** None. **L. Cheng:** None. **S. Chang:** None.

Poster

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Topic: C.04. Movement Disorders

Title: Psychoactive compounds induce distinctive fine motor deficits and gait disturbance in mice

Authors: *T. HEIKKINEN, T. BRAGGE, T. TURKIA, A. NURMI, R. HODGSON; Charles River Discovery, Kuopio, Finland

Abstract: While many pharmacological treatments with multiple mechanisms of action have been shown to induce motor impairment in mice, more traditional measures for assessing these impairments tend to evaluate gross motor function and not fine movements. Moreover, the clinical utility of the traditional assays has been questioned, which increases the need to create novel assays that are more sensitive to subtle deficits in motor function. In the high precision kinematic assay, a mouse is placed on a runway and allowed to walk from one end to the other. The movement of the animal is recorded with a high speed camera assess multiple body parts from three angles allowing for a comprehensive analysis of the motor function. The analysis provides detailed information of e.g. changes in joint angles, trajectories and the acceleration rates of selected body points and their relation to each other, interlimb coordination etc. Previously, we have demonstrated preliminary data with some known psychoactive compounds that the kinematic analysis is more sensitive than traditional motor assays to the subtle motor deficits. In addition, by breaking the behavior into components, we have been able to provide a much richer characterization of the motor changes. The purpose of the present research was to

apply the kinematic analysis methodology to the assessment of pharmacologically induced impairments in motor function. In this study we assessed drugs with three different mechanisms of action, amphetamine, morphine, pregabalin in the kinematic analysis assay. Doses were selected to be below, at and above doses that have been shown previously to induce robust hyperactivity or anti-allodynia effects by using open field test or von Frey test, respectively. As an example, we observed expected increase in amphetamine induced hyperactivity in dose response manner and at highest doses, but kinematic analysis data revealed changes in the gait at lowest but not highest doses tested. Also, some parameters were only affected at lowest or highest amphetamine doses. Pregabaline, on the other hand did not induce clear locomotor changes in open field test but showed distinct changes in the kinematic analysis. Taken together, data in this presentation highlight the need to use of sensitive assays in vivo to examine therapeutics and their effects not only for their expected efficacy but also for their possible side effect profiles which may implicate unwanted motor impairments, coordination problems and/or sedation.

Disclosures: T. Heikkinen: None. **T. Bragge:** None. **T. Turkia:** None. **A. Nurmi:** None. **R. Hodgson:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.28/M11

Topic: C.04. Movement Disorders

Title: Identifying cannabis strains for treatment of epilepsy

Authors: *G. M. LEWITUS[1], P. BERMAN[2], K. FUTORAN[3], D. MEIRI[3];
[1]Fac. of biology, Technion, Haifa, Israel; [2]Technion, Hifa, Israel; [3]Technion, Haifa, Israel

Abstract: Epilepsy is the most common serious brain disorder worldwide with no age, racial, social class, national or geographic boundaries. Epilepsy's co-morbidities include cognitive decline, depressive disorders and schizophrenia which are worsened by poorly controlled seizures. There are many treatments available. However, 30% of the cases remain pharmaco-resistant, resulting in poorly controlled seizures. In the last years, the use of Cannabis as anticonvulsants was reintroduced to the clinic and is used as the last line of defense before brain surgeries in pharmaco-resistant children suffering from epilepsy. Cannabis oil extracts contain more than 100 different cannabinoids which have different pharmacology properties. The aims of this research are to identify the cannabinoids in different strains of cannabis grown in Israel and to evaluate their potential use as anticonvulsants. For this propose, we are utilizing mass-

spectrometry, coupled to liquid chromatography (LC-MS) to comprehensively profile the cannabinoid composition for a variety of cannabis strains that are clinically used in Israel. After profiling the cannabinoids, we are evaluating their anticonvulsant effects in the pentylenetetrazol (PTZ) test, which is a validated model to identify drug treatments that are effective in the treatment of epilepsy in humans.

Disclosures: G.M. Lewitus: None. **P. Berman:** None. **K. Futoran:** None. **D. Meiri:** None.

Poster

311. Movement Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 311.29/M12

Topic: C.04. Movement Disorders

Title: Neural connectivity and cortical activation in chronic tic disorders

Authors: *K. TUNG[1], M. MIYAKOSHI[3], S. MAKEIG[3], S. CHANG[1], J. PIACENTINI[1], S. LOO[2];
[2]Psychiatry, [1]UCLA Semel Inst., Los Angeles, CA; [3]SCCN UCSD, San Diego, CA

Abstract: Background: The etiology and pathophysiology of Chronic Tic Disorders (CTDs) remains poorly understood.One challenge in studying cortical activity in CTDs is involuntary movement that prevents the use of brain imaging due to motion artifacts. The necessity to remain still confounds true pathophysiological underpinnings with compensatory mechanisms. We propose the use of Mobile Brain-Body Imaging methods to study EEG during voluntary movements among children with and without CTDs. Both temporally resolved cortical activation as well as functional connectivity will be examined to test the hypothesis that although both groups will utilize a similar brain network in generating the voluntary movement, differing levels of activation and patterns of connectivity will be observed.
Methods: Participants included 44 children (27 with CTD, 17 healthy controls (HC)), between the ages of 8-12 years old. Diagnosis was made using the Anxiety Disorder Interview Schedule and tic severity was evaluated using Yale Global Tic Severity Scale. All EEG data were recorded using MoBI methods, which allows integration of multiple data sources within a single interface. EEG was recorded during the Voluntary Movement (VM) task in which all children were presented with a visual cue every 2 seconds and instructed to make an exaggerated blink. EEG data was processed and analyzed in EEGLAB.
Results: EEG data recorded during the voluntary movement condition were examined in order to characterize brain dynamics of effortful control over voluntary movement. Although CTD and HC subjects exhibited similar neural activation patterns, group differences emerged primarily in

the medial prefrontal, supplemental motor (SMA) and parietal areas. The HC group exhibited stronger alpha (8-12 Hz) event-related desynchronization before the VM cue whereas the CTD group shows higher gamma (40-50 Hz) activation immediately after the cue in medial frontal, parietal and left SMA.. Additionally, CTD children showed significantly stronger effective connectivity relative to HC ($p<0.01$) in connections to and from posterior regions and right insular cortex whereas HC children exhibited stronger connectivity in fronto-frontal and fronto-temporal connections ($p<0.01$).
Conclusion: CTD group exhibited altered activation and connectivity in frontal, parietal, and motor regions. Group differences in cortical dynamics and associated clinical characteristics such as tic severity and urge strength suggest the importance of multiple networks in generating voluntary movement and highlight the importance of examining both activation and connectivity for models of movement in CTD.

Disclosures: **K. Tung:** None. **M. Miyakoshi:** None. **S. Makeig:** None. **S. Chang:** None. **J. Piacentini:** None. **S. Loo:** None.

Poster

312. ALS

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 312.01/M13

Topic: C.05. Neuromuscular Diseases

Support: ALS Association

Les Turner Foundation

Title: Detailed genetic analysis of healthy and diseased CSMN reveals molecular determinants of vulnerability and progressive degeneration

Authors: *L. LABOISSONNIERE[1], B. GENC[2], J. TRIMARCHI[1], P. OZDINLER[2];
[1]Iowa State Univ., Ames, IA; [2]Northwestern Univ., Chicago, IL

Abstract: ALS is characterized by progressive degeneration of both spinal and corticospinal motor neurons (CSMN). However, the underlying causes for their selective vulnerability and progressive degeneration are not fully understood. The limitations for detailed cellular analysis are the complexity and the heterogeneity of the cerebral cortex, and the limited numbers of CSMN. To overcome these challenges, we generated and characterized UCHL1-eGFP mice, in which CSMN are genetically labeled with eGFP expression that is stable and long-lasting, allowing visualization and isolation of pure populations of CSMN from the motor cortex. By

crossbreeding this CSMN reporter line with mouse models that display CSMN vulnerability, such as the hSOD1^{G93A} mice, we generated hSOD1^{G93A}-UeGFP mice, in which the diseased upper motor neurons express eGFP and thus are purified by fluorescent activated cell sorting (FACS). We generated cDNA libraries from FACS-purified healthy and diseased CSMN at postnatal day (P)16, P30, P60, and P90, and performed microarray analysis to reveal global changes of gene expression at different stages of disease initiation and progression. Our preliminary results suggest the presence of signature events that occur at different stages, as well as the activation and/or inhibition of unique canonical pathways. These findings are important for suggesting novel drug targets for distinct stages of the disease. In addition, our findings will help determine potential early detection markers as well as biomarkers that would suggest the timing and extent of CSMN vulnerability and degeneration in ALS with upper motor neuron involvement.

Disclosures: L. Laboissonniere: None. **B. Genc:** None. **J. Trimarchi:** None. **P. Ozdinler:** None.

Poster

312. ALS

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 312.02/M14

Topic: C.05. Neuromuscular Diseases

Title: Altered ceramide generation in charcot marie tooth 2f

Authors: *N. SCHWARTZ[1], C. E. SENKAL[1], L. M. OBEID[2,1];
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Abstract: Charcot-Marie-Tooth (CMT) disease is the most commonly inherited neurological disorder, resulting in motor and sensory dysfunction that typically commences at middle age and progressively worsens throughout life. Nevertheless, the exact molecular mechanisms underlying CMT are unclear, limiting progress in developing potential therapeutics. As sphingolipids serve as bioactive signaling molecules in a plethora of pathways and have been implicated in neurodegenerative diseases including Alzheimer's, Parkinson's, and ALS, we sought to determine if changes in sphingolipid metabolism may mediate CMT phenotypes. One variant of CMT, CMT2F, is characterized by the presence of mutations in heat shock protein 27 (Hsp27), a member of the class of small heat shock proteins that serves many cellular functions. Using liquid chromatography/mass spectrometry, we have determined that peripheral nerve, but not brain nor spinal cord, tissue from mutant Hsp27 mice displays decreased ceramide levels compared to wild-type Hsp27 tissue, suggesting that these mutants suppress ceramide

generation. Mutant cell lines demonstrate decreased mitochondrial respiratory function, paralleling changes induced by blocking ceramide generation pharmacologically, suggesting decreased production of ceramide reduces mitochondrial respiratory function. Furthermore, autophagy is slightly upregulated in mutant cell lines. Taken together, these results suggest that CMT2F mutations in Hsp27 alter sphingolipid metabolism by dysregulating ceramide levels, producing cellular pathology that ultimately leads to neuronal degeneration in CMT2F. We seek to further characterize the role and regulation of sphingolipid signaling and biology in CMT2F. By understanding the effects of sphingolipids on the development of CMT2F, we will be able to identify targets that can be used in the development of novel treatments to prevent or reduce the severity of CMT2F or other neuropathies with similar mechanisms.

Disclosures: **N. Schwartz:** None. **C.E. Senkal:** None. **L.M. Obeid:** None.

Poster

312. ALS

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 312.03/M15

Topic: C.05. Neuromuscular Diseases

Support: Barrow Neurological Foundation

Title: ALS and artificial intelligence: IBM watson suggests additional RNA binding proteins linked to ALS

Authors: ***R. P. BOWSER**[1], E. ARGENTINIS[2], R. SATTLER[1], M. COLLINS[1], A. BOEHRINGER[1], I. LORENZINI[1], P. FERRANTE[1], A. LACOSTE[2], S. SPANGLER[3], N. BAKKAR[1];
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]IBM Watson Hlth., New York, NY; [3]IBM Res. - Almaden, San Jose, CA

Abstract: A number of RNA binding proteins (RNPs) are linked to amyotrophic lateral sclerosis (ALS), with known mutations in 11 RNPs that are causative in familial ALS and another 5 RNPs associated with ALS pathology but with no known genetic mutations. There are over 1,450 RNPs in the human genome, and therefore other RNPs may also be linked to ALS. We sought to identify additional RNPs associated with ALS using methodologies that analyze prior published information to suggest new RNPs with a connection to ALS. The cognitive capabilities of IBM Watson enable it to extract domain specific text features from published literature to identify new connections between entities of interest, such as genes, proteins, drugs, and diseases. This approach has been successfully applied to gain new insights into oncology, but has not been

applied to the neurosciences. We used IBM Watson to identify additional RNPs linked to ALS. IBM Watson analyzed published abstracts to learn the text patterns of a set of known RNPs related to ALS, and then applied that learning to a candidate set of proteins and ranked their similarity to the known RNP "training set". To test IBM Watson's predictive performance, we first restricted its knowledge to information prior to 2012 (known mutations in 8 RNPs linked to ALS). IBM Watson then rank ordered all other 1,445 RNPs with a probability to be linked to ALS. Since that time, mutations in three RNPs (Matrin 3, GLE1, and ARHGEF28) have been linked to familial ALS. Matrin 3 was the top candidate in this retrospective analysis, with both ARHGEF28 and GLE1 within the top 10% of all RNPs. Having shown that such analysis can successfully predict ALS-associated genes, we then applied a training set consisting of all known RNPs with mutations causative of ALS to predict other RNPs linked to ALS. Of the top 50-ranked genes, 5 have already been associated with ALS, even though no disease-causing mutations are known (RBM45, MTHFSD, SMN2, EWSR1 and hnRNPA3). Also included within the top 10 predicted genes were hnRNPU, hnRNPH2, SRSF2, SYNCRIP and CAPRIN1. To validate Watson's predictions, we examined the subcellular distribution of these RNPs in post-mortem tissue samples from ALS and control subjects. We identified reduced levels of SYNCRIP in ALS patients, but no changes in hnRNPH2. SYNCRIP functions in multiple steps of mRNA maturation and transport, and interacts with TDP-43, FUS, and SMN. CAPRIN1 binds G3BP and TDP-43 and accumulates in stress granules. SRSF2 is localized to antisense RNA foci in C9 patients. Overall, our approach using IBM Watson to mine scientific literature to find new ALS-linked RNPs is promising and may aid basic research efforts to understand mechanisms of disease.

Disclosures: **R.P. Bowser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iron Horse Diagnostics, Inc.. F. Consulting Fees (e.g., advisory boards); Above & Beyond, LLC.. **E. Argentinis:** None. **R. Sattler:** None. **M. Collins:** None. **A. Boehringer:** None. **I. Lorenzini:** None. **P. Ferrante:** None. **A. Lacoste:** None. **S. Spangler:** None. **N. Bakkar:** None.

Poster

312. ALS

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Program#/Poster#: 312.04/M16

Topic: C.05. Neuromuscular Diseases

Support: La Marató-TV3 Grant TV3201428-10

CIBERNED Grant 2015/01

Title: Abnormalities of the neuregulin and ErbB4 receptor pathway in Amyotrophic Lateral Sclerosis

Authors: *M. HERRANDO-GRABULOSA[1,2], B. GARCIA-LAREU[3,2], R. MANCUSO[4], A. MARTINEZ-MURIANA[1,2], G. MODOL-CABALLERO[1,2], A. BOSCH[3,2], X. NAVARRO[1,2]; [1]Cell Biology, Physiol. and Immunol., Univ. Autonoma De Barcelona, Bellaterra, Spain; [2]Ctr. de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Spain; [3]Dept. of Biochem. and Mol. Biol., Ctr. of Animal Biotech. and Gene Therapy, Univ. Autonoma de Barcelona, Bellaterra, Spain; [4]Ctr. for Biol. Sciences, Univ. of Southampton, Southampton, United Kingdom

Abstract: The death of motoneurons in Amyotrophic Lateral Sclerosis (ALS) is preceded by failure of neuromuscular junctions (NMJs) and axonal retraction, dependent upon deffective interaction of motor axons with terminal Schwann cells and skeletal muscle fibers. Neuregulin 1 (Nrg1) is a neurotrophic factor highly expressed in motoneurons and NMJs that supports axonal and neuromuscular development and maintenance. Interestingly, Nrg1 expression has been previously found reduced in both ALS patients and SOD1G93A mice. We have characterized the expression of Nrg1 isoforms and ErbB receptors in spinal cord and skeletal muscles of SOD1G93A mice and ALS patients. In SOD1G93A mice skeletal muscles we found a significant reduction of ErbB4 mRNA levels, correlating with the degree of muscle denervation. Skeletal muscles from sporadic and familial ALS patients also showed reduced levels of ErbB4 receptor at protein and mRNA levels. We confirmed that in the spinal cord of the ALS mice, Nrg1 type I mRNA levels increased along the progression of the disease, whereas Nrg1 type III mRNA levels were reduced. We also observed progressively reduced levels of ErbB4 receptor in the motoneurons of ALS mice with age. In conclusion, the abnormalities observed in the Nrg1-ErbB pathway suggest that it is an potential therapeutic target for improving motoneuron survival and/or NMJ maintenance in ALS.

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Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: NIH/NINDS R01 NS064224

Muscular Dystrophy Association (MDA4209)

Title: MAPK signaling mediates neurodegeneration in Spinal muscular atrophy

Authors: *S. AHMAD, N. GENABAI, X. JIANG, K. BHATIA, L. GANGWANI;
Texas Tech. Univ. Hlth. Sci. Ctr., El Paso, TX

Abstract: Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality. The deletion of the *survival motor neuron 1* (*SMN1*) gene causes low expression levels of full-length survival motor neuron (SMN) protein that results motor neuron loss in the spinal cord in SMA. The molecular mechanisms involved in degeneration of motor neurons in SMA are largely unknown. Recently, we have shown that JNK pathway mediates neurodegeneration in SMA. To further understand mechanism of motor neuron degeneration in SMA, we investigated the upstream MAP Kinase signaling that may regulate JNK activation. We examined G-protein mediated signaling, such as Rac1/Cdc42 (G-protein) and p21-activated kinase (PAK), a target of activated Rac1/Cdc42 kinases in WT and SMA mice as well as non-SMA and SMA type-1 patient spinal cord. We show that Rac1/Cdc42 and PAK/4/5/6 were activated in the spinal cords of SMA mice as well as in SMA patients. Protein tyrosine kinase 2 (PYK2), a brain specific isoform, and protein kinase C (PKC), which are known to activate Rac1 were also activated in SMA. Increased Ca^{2+} accumulation was found in neurons that may cause activation of PKC and PYK2 module. Together, these data suggest that the upstream signaling Rac1/Cdc42-PAK4/5/6 and PYK2/PKC kinase modules may activate JNK cascade that mediates neurodegeneration in SMA.

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Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: MRC Grant Number G0900688

Charity Funding: King's College Noreen Murray Studentship

Title: A novel Optineurin truncation mutation identified in an adult onset consanguineous Palestinian family with amyotrophic lateral sclerosis

Authors: *M. DE MAJO[1], M. GOTKINE[2], C. WONG[1], S. TOPP[1], R. MICHAELSON-COHEN[3], S. EPSZTEJN-LITMAN[4], R. EIGESS[4], A. NISHIMURA[1], B. SMITH[1], C. SHAW[1]; [1]Dept. of Basic and Clin. Neurosci., King's Col. London Inst. of Psychiatry, Psy, London, United Kingdom; [2]Hebrew Univeristy-Hadassah Med. Ctr., Jerusalem, Israel; [3]Obstetrics & Gynecology, Med. Genet. Inst. Shaare Zedek Med. Ctr., Jerusalem, Israel; [4]Stem Cell Res. Lab., Med. Genet. Institute, Shaare Zedek Med. Ctr., Jerusalem, Israel

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with an average survival of 3 years from diagnosis. A clear genetic component is apparent in approximately 10% of cases, and mutations in four major genes: *SOD1*, *TDP43*, *FUS* and *C9ORF72* account for approximately 50% of familial and 15% of sporadic cases. The genetic factors involved in the majority of ALS cases remain to be elucidated. Optineurin (*OPTN*) mutations have been described in open angle glaucoma and more recently, in ALS. Here we describe, for the first time, the genetic and functional characterisation of a novel recessive mutation in *OPTN* found in a consanguineous Palestinian family with an aggressive, adult form of ALS. Furthermore, this study is the first to characterise endogenous OPTN from primary fibroblast lines cultured from carriers of OPTN mutations.

OPTN is a 577 amino acid protein involved in a variety of cellular pathways, including autophagy. OPTN acts as autophagy receptor and it is involved in the clearance of protein aggregates through the autophagy-lysosome pathway. Within the same pathway OPTN is phosphorylated on Ser177 by tank binding kinase 1 (TBK1), a protein also linked to ALS.

A novel homozygous truncation mutation in OPTN (S174X) was identified in this family, via exome sequencing from DNA of affected family members; this results in removal of both the UBAN domain and the TBK1 phosphorylation site. Notably, the truncated protein retains the TBK1 binding region of OPTN. The unaffected mother was confirmed to be a heterozygous carrier, and sequencing of three unaffected siblings of the proband showed two of them to be heterozygous carriers and one to harbour a homozygous S174X change.Fibroblast primary lines were obtained from the unaffected mother and three siblings. Interestingly, Western blot analysis of fibroblast lysates, probing for endogenous OPTN, revealed OPTN expression for the wild type and heterozygous carriers but no expression of the truncated S174X OPTN allele. These observations support the hypothesis that early truncation results in an unstable protein. However, transcript analysis revealed that full length and truncated *OPTN* message was produced from both the heterozygous and homozygous carriers.

The absence of the mutated copy of OPTN suggests that the pathogenic mechanism could be associated with a loss of function, which could result in a knock on effect abolishing interaction with TBK1. Further research will aim to characterise the mechanism behind the pathogenicity of this truncation mutation in *OPTN* and how this affects its interaction with TBK1. The understanding of this mechanism could pave the way to gene replacement therapy approaches.

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Poster

312. ALS

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Program#/Poster#: 312.07/N1

Topic: C.05. Neuromuscular Diseases

Support: DFG (CEMMA GRK1789)

Helmholtz Virtual Institute "RNA dysmetabolism in ALS and FTD"

Title: Disease specific changes in sirtuin 3 levels in a mouse model of amyotrophic lateral sclerosis and Huntington's disease

Authors: E. BARTH[1], H. BAYER[1], J. HANSELMANN[1], P. WEYDT[1], K. LINDENBERG[1], *A. WITTING[2];
[1]Ulm Univ., Ulm, Germany; [2]Univ. Ulm, Ulm, Germany

Abstract: The loss of upper and lower motor neurons characterizes the progressive and fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS), also known as Lou-Gehrig's disease. Weight loss in patients and early changes in mitochondria indicate dysregulation of energy metabolism. These signs are also present in Huntington's disease (HD) patients. In mitochondria there are three members of the seven mammalian sirtuins, regulating energy metabolism. Of these mainly the NAD+ dependent sirtuin 3 (SIRT3) deacetylates proteins and is majorly involved in regulating ROS defense. Furthermore SIRT3 is a sirtuin implicated in human aging, a risk factor for neurodegenerative diseases. We therefore investigated the expression and function of mitochondrial sirtuins in ALS and HD mouse models. For this we quantified *Sirt3*, *Sirt4* and *Sirt5* during the course of disease in male ALS ($SOD1^{G93A}$) and HD (R6/2) mice on mRNA level. We found a specific reduction of *Sirt3* mRNA levels in the disease-affected areas cervical spinal cord and brain stem of end-stage ALS mice. Whereas in HD mice a tendency to increased *Sirt3* mRNA levels was found. Furthermore, the activity of SIRT3 was reduced in the spinal cord of the $SOD1^{G93A}$ mice, which was determined by analyzing the acetylation status of the SIRT3 target superoxide dismutase 2 (SOD2). The cell type specific analysis of cultured primary cells showed the highest levels of mitochondrial sirtuins (Sirt3, Sirt4, Sirt5) in neurons compared to microglia, astrocytes and oligodendrocytes. Surprisingly a cell-type comparison of knock-out cells for the peroxisome proliferative activated receptor gamma, coactivator 1 alpha (PGC-1α), a genetic disease modifier in ALS and HD, showed a shift of the highest *Sirt3* mRNA levels form the neurons to the astrocyte population. In summary our findings support opposite roles of SIRT3 in disease modifying processes related to PGC-1α during the course of ALS and HD.

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Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: Les Turner ALS Foundation

Title: Apical dendrite degeneration of Betz cells, a new cellular pathology in ALS

Authors: ***P. OZDINLER**[1,2], B. GENC[1], J. H. JARA[1], P. PAYTEL[3], R. ROOS[3], M. MESULAM[2], C. GEULA[2], E. BIGIO[2];
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Abstract: Upper motor neurons [a.k.a. corticospinal motor neurons (CSMN) in mice and Betz cells in humans] are important components of motor neuron circuitry because of their unique ability to initiate and modulate voluntary movement by receiving, integrating and conveying cerebral cortex input into spinal cord targets. Degeneration of Betz cells in ALS was documented as early as 1885. Even though this pathology was accepted as a defining characteristic distinguishing ALS from other motor neuron diseases, the timing and the extent of Betz cell degeneration has been actively debated. There is now evidence to suggest that upper motor neuron death is an early event and a significant contributor to disease pathology. Increased cortical hyperexcitability is observed prior to the manifestation of disease symptoms in ALS patients, as detected by transcranial magnetic stimulation. In addition, ALS mouse models display CSMN abnormalities manifested by spine loss and apical dendrite degeneration early in disease. Although large vacuoles are present in apical dendrites of CSMN that become diseased in mouse models of ALS, the health and integrity of apical dendrites of Betz cells in ALS patients have not been assessed. We investigated motor cortex from 11 normal control subjects with no neurologic disease, 5 fALS patients with mutations in *SOD1*, 10 sALS patients without a family history of ALS, and 6 AD patients. We find profound apical dendrite degeneration with large vacuoles in both familial and sporadic ALS patients. In contrast, Alzheimer's disease patients and normal controls retain cellular integrity in the motor cortex. These results suggest apical dendrite degeneration of Betz cells as a potential contributor to upper motor neuron pathology observed selectively in ALS.

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Poster

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MDA

Cure SMA/Families of SMA

Weissman Family Foundation

Title: SMN functions as a chaperone for the assembly of mRNP transport complexes.

Authors: *P. G. DONLIN-ASP[1], C. FALLINI[1], M. E. MERRITT[1,2], H. C. PHAN[3,4], G. J. BASSELL[1,2,5,3], W. ROSSOLL[1,2,5];
[1]Cell Biol., [2]Lab. of Translational Cell Biol., [3]Neurol., [4]Pediatrics, [5]Ctr. for Neurodegenerative Dis., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by a specific degeneration of motor neurons. SMA results from a reduction in the survival of motor neuron (SMN) protein, which is ubiquitously expressed with a well characterized role in promoting the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs).
We have previously shown specific defects in the axonal localization of poly A mRNA (including *β-actin* and *Gap43*) and mRNA-binding proteins (HuD, IMP1) in axons and growth cones of SMN-deficient motor neurons. We have also reported that overexpression of both HuD and IMP1 can restore axon outgrowth and *Gap43* mRNA and protein levels in growth cones of SMA motor neurons. Our findings led us to hypothesize that SMN plays an additional critical role in the assembly of messenger ribonucleoproteins (mRNPs), and that failure to assemble RNA transport complexes leads to the reported mRNA localization defects in SMA motor neurons.

To test our hypothesis, we have established a trimolecular fluorescence complementation (TriFC) assay as a sensor for the association of mRNAs with RBPs. TriFC performs consistently in cell lines, primary fibroblasts and motor neuron cultures. In motor neurons isolated from a severe SMA mouse model, as well as primary human fibroblasts from SMA patients, we readily detect a severe defect in the assembly of complexes containing IMP1 protein and *β-actin* mRNA, with no reduction in steady state expression. Furthermore, RNA immunoprecipitation experiments also show impairments in the association of IMP1 protein with *β-actin* mRNA. Adapting a novel biochemical method for size fractionation of RNP granules over a density gradient, we observe a consistent shift of IMP1-containing mRNPs toward smaller granules in SMA human fibroblasts. In addition, we have employed super resolution microscopy to compare the size of endogenous IMP1 granules. In SMA patient derived fibroblasts, IMP1 granules are consistently reduced in their volume relative to control lines, a phenotype consistent with both our TriFC and fractionation results. Finally, we can show a defect in the association of IMP1 mRNPs with both actin filaments and microtubules in the SMA patient fibroblasts, suggesting a mechanism to explain reduced mRNA localization reported in SMA motor neurons.
In summary, our results show that SMN plays a more general role in RNP assembly beyond the canonical role in snRNP assembly. Here, we demonstrate that SMN acts as a chaperone for the formation of transport-competent RNA granules, providing a mechanism for mRNA localization defects that may contribute to the motor neuron degeneration observed in SMA.

Disclosures: P.G. Donlin-Asp: None. **C. Fallini:** None. **M.E. Merritt:** None. **H.C. Phan:** None. **G.J. Bassell:** None. **W. Rossoll:** None.

Poster

312. ALS

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 312.10/N4

Topic: C.05. Neuromuscular Diseases

Support: JSPS KAKENHI #15K06763, #24500428

Title: Adenovirus-induced TDP-43 and FUS aggregates in cultured neuronal and glial cells demonstrated by time-lapse imaging

Authors: *K. WATABE[1], T. ISHII[2], H. MISAWA[2];
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Abstract: Formation of TDP-43- or FUS-positive cytoplasmic aggregates in neuronal and glial cells is one of the pathological hallmarks of amyotrophic lateral sclerosis (ALS). We have previously demonstrated that proteasome inhibition enhanced adenovirus-induced neuronal cytoplasmic aggregate formation of TDP-43 and FUS in vitro and in vivo, suggesting that impairment of protein degradation pathways accelerates formation of TDP-43 and FUS-positive aggregates in ALS. However, the relationship between the cytoplasmic aggregate formation and the cell death remains unclear. In this study, we performed time-lapse imaging analysis of neuronal and glial cells infected with adenoviruses encoding TDP-43 and FUS cDNAs under conditions of proteasome inhibition. Rat neural stem cell lines stably transfected with EGFP or Sirius under the control of tubulin beta III (TBB3p), GFAP or cyclic nucleotide phosphodiesterase (CNP) promoter were differentiated into neurons, astrocytes and oligodendrocytes, respectively, in the presence of retinoic acid. The differentiated neuronal and glial cells expressing EGFP or Sirius were then infected with adenoviruses encoding DsRed-tagged human wild type and C-terminal fragment (CTF) TDP-43 or mutant P525L FUS in the presence of proteasome inhibitor MG-132 or an adenovirus encoding shRNA for proteasome PSMC1. Time lapse imaging analysis revealed growing DsRed-positive cytoplasmic aggregates in the infected neuronal and glial cells followed by the cell collapse within 72 hours. Released cytoplasmic aggregates composed of WT and CTF TDP-43 remained insoluble in the culture media over 30 hours of the time course. These aggregates consisted of sarkosyl-insoluble granular materials and contained phosphorylated TDP-43. We are also attempting to develop time lapse imaging of cell to cell spreading of these cytoplasmic aggregates.

Disclosures: **K. Watabe:** None. **T. Ishii:** None. **H. Misawa:** None.

Poster

312. ALS

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Program#/Poster#: 312.11/N5

Topic: C.05. Neuromuscular Diseases

Support: NIEHS Grant R01 ES024064-02

NIEHS Grant T32 ES007255-27

Title: Methylmercury exposure alters fluo-4 fluorescence in spinal cord slices of mice expressing the human Cu^{2+}/Zn^{2+} superoxide dismutase 1 (hSOD1) gene mutation

Authors: *J. M. BAILEY, Y. YUAN, W. ATCHISON;
Pharmacol. and Toxicology, Michigan State Univ., East Lansing, MI

Abstract: Mice expressing the human Cu^{2+}/Zn^{2+} superoxide dismutase 1 (hSOD1) gene mutation ($hSOD1^{G93A}$) are used to model Amyotrophic Lateral Sclerosis (ALS), as these mice reliably exhibit an ALS-like phenotype as they age. This model has been used to describe the role of gene X environment interactions in the onset and progression of ALS. Previous work has shown that the environmental neurotoxicant methylmercury (MeHg) can hasten the onset of the ALS-like phenotype in these mice, on endpoints of motor function as well as intracellular calcium homeostasis in brain stem and cerebellum. However, ALS is associated with dysfunction and death of motor neurons and the spinal cord is a target of particular interest in understanding this disease's etiology. Here, we describe the effects on intracellular calcium regulation (tracked via fluo-4 epiflorescence) following acute 20 uM MeHg exposure in spinal cord slice taken from $SOD1^{G93A}$, $SOD1^{hWT}$ and wild type mice of both sexes and two age groups (PND 21-30 "adolescent" and 2-4 months old "young adult"). Both dorsal and ventral regions of the spinal cord were prepared. As a function of exposure time, acute MeHg exposure caused a greater increase in fluo-4 fluorescence in the dorsal spinal cord tissue of $SOD1^{G93A}$ mice compared to wild type animals. Spinal cord tissue from $SOD1^{G93A}$ mice was not only generally associated with greater relative fluorescence following acute MeHg exposure, but the time to peak fluorescence was also delayed among these animals compared to wild type controls. These data suggest that MeHg interacts with the $SOD1^{G93A}$ mutation to enhance neuronal dysfunction. This likely demonstrates evidence of a gene X environment interaction relevant to the etiology of ALS and points to a relevant mechanism of action (i.e. calcium dysregulation among neurons of the spinal cord).

Disclosures: J.M. Bailey: None. **Y. Yuan:** None. **W. Atchison:** None.

Poster

312. ALS

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Program#/Poster#: 312.12/N6

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant NS40433

IU Collaborative Research Grant 2214076

Title: Long-term systemic adipose-derived stem cell-conditioned medium therapy in a mouse model of amyotrophic lateral sclerosis

Authors: *C. L. WALKER[1], F. M. KENNEDY[1], C. M. E. FRY[1], A. K. IYER[1], Y. DU[2], K. MARCH[3,4], K. J. JONES[1,4];

[1]Anat. & Cell Biol., [2]Neurol., [3]Med., Indiana Univ. Sch. of Med., Indianapolis, IN; [4]Roudebush VA Med. Ctr., Indianapolis, IN

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating progressive disease involving the loss of motor neurons (MN), resulting in paralysis and death. The superoxide dismutase 1 (SOD1) transgenic mouse model of ALS exhibits is the gold standard animal model, exhibiting similar clinical pathological progression through pre-symptomatic, symptomatic, and end stages of disease. We, and others, have observed that disease onset occurs pre-symptomatically by 47 days of age $SOD1^{G93A}$ mutant mice. Previous research indicates initial hindlimb muscle denervation, with progressive neuromuscular junction (NMJ) loss over time. Our previous data indicate systemic daily therapy of adipose-derived stem cell conditioned medium (ASC-CM), beginning at symptom onset, delayed functional progression and extended survival in $SOD1^{G93A}$ mice, suggesting a central mechanism of action. In addition, we found that daily ASC-CM therapy beginning at 35 days of age, when NMJ innervation is similar to non-diseased mice, significantly attenuated NMJ loss by day 47 in $SOD1^{G93A}$ mice. We then hypothesized that long-term systemic ASC-CM therapy could provide functional and survival benefits, potentially through both peripheral and central mechanisms beyond that observed from post-symptom onset therapy. We began treating sixteen $SOD1^{G93A}$ and sixteen wild-type (WT) mice with either daily ASC-CM or vehicle control medium at 35 days of age until day 63, followed by a twice-weekly treatment schedule until animal end stage. At this time, we also began a battery of behavioral assessments testing gait, grip endurance, tail elevation, and rearing, among other behaviors, to identify any therapeutic effects on symptom onset and progression. The study is currently underway. Based on our previous pre-symptomatic and symptomatic treatment data, we hypothesize that the systemic ASC-CM therapeutic paradigm could prevent hindlimb muscle denervation and act centrally to ameliorate functional decline and impart survival benefits in the $SOD1^{G93A}$ ALS mouse model.

Disclosures: **C.L. Walker:** None. **F.M. Kennedy:** None. **C.M.E. Fry:** None. **A.K. Iyer:** None. **Y. Du:** None. **K. March:** None. **K.J. Jones:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: MoST Grant 102-2321-B-001 -068 -MY3

Title: The functional role of AMPK activation in amyotrophic lateral sclerosis.

Authors: *Y. CHERN, Y.-J. LIU, C.-C. LEE, H. PEI;
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Abstract: Many major neurodegenerative disorders (including Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS)) with distinct clinical features are protein-misfolding diseases. Regardless the remarkable efforts devoted to the development of therapeutic intervention, no effective treatment for these degenerative diseases is currently available. ALS is a late-onset and progressive motor neuron disease with the hallmark of TDP43 inclusions in the brain and spinal cord. Most of the ALS cases are sporadic (~90%), while only ~10% are caused by mutations of ALS-associated genes (e.g., TDP-43). The prevalence of ALS in Taiwan is 1.97 cases per 100,000. The clinical presentation includes muscle weakness and impaired voluntary movement resulted from degeneration of motor neurons. In addition to the formation of TDP43 inclusions, the mislocalization of TDP43 from nucleus to cytoplasm is considered an early event of ALS, because the mislocalized TDP-43 may form pathological oligomers and inclusions, and subsequently causes degeneration of motor neurons. We have previously demonstrated that abnormal localization of two RNA binding proteins (TDP-43 and human antigen R (HuR)) was associated with elevated AMP-activated protein kinase (AMPK) activity in the motor neurons of ALS patients. Molecular and pharmacological approaches demonstrated that activation of AMPK altered the location of TDP-43 and HuR in mouse motor neurons and in a motor neuron cell line (NSC34) via phosphorylation of importin-α1. Elevation of the cAMP/PKA-dependent pathway using different reagents effectively normalized the AMPK-evoked redistribution of TDP-43, HuR, and several other substrates of importin-α1. Collectively, our findings suggest that a therapeutic strategy focusing on the proper regulation of AMPK activity in motor neurons may produce beneficial effect for patients with ALS.

Disclosures: **Y. Chern:** None. **Y. Liu:** None. **C. Lee:** None. **H. Pei:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: ALSA grant 15-IIP-195

NIH/NINDS grant NS09387

Title: Bioenergetic characterization of human iPSC-derived ALS astrocytes

Authors: C. KONRAD[1], K. MCAVOY[2], D. TROTTI[2], A. ALMAD[3], N. MARAGAKIS[3], J. PHAM[4], J. ROTHSTEIN[4], R. SATTLER[5], G. MANFREDI[1], *H. KAWAMATA[1];
[1]Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [2]Dept of Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [3]Ansari ALS Ctr. for Cell Therapy and Regeneration Res., [4]Neurol., Johns Hopkins Univ., Baltimore, MD; [5]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease, which affects upper and lower motor neurons, causing progressive muscle paralysis and death. The majority of ALS cases are sporadic with unknown causes, but mutations in numerous genes have been identified in familial cases, which account for approximately 10% of all ALS. Familial ALS genes include Cu, Zn, superoxide dismutase (SOD1), C9orf72, Fused in osteosarcoma (FUS), and many others. It is widely accepted that in ALS non-cell autonomous contribution from astrocytes plays a significant role in the demise of motor neurons. Mitochondrial bioenergetics abnormalities have been widely reported in cultured neurons, in patient-derived fibroblasts, and in animal models of ALS. Mitochondrial defects have also been identified in ALS mouse primary astrocytes. Furthermore, we have described abnormal intracellular calcium signaling in spinal cord astrocytes of ALS mice. However, mitochondrial bioenergetic function and intracellular calcium signaling have not yet been investigated in human ALS astrocytes. Bioenergetic abnormalities can underlie the involvement of astrocytes in ALS, since they could cause energy depletion, but also alter cell secretion processes, calcium signaling, and metabolism of glutamate, lactate, and other substrates necessary for neuronal physiology. In this study, we examined cellular bioenergetics and intracellular calcium signaling in induced pluripotent derived cells (iPSC) derived astrocytes from patients affected by sporadic and familial ALS (mutant SOD1 and FUS, C9orf72), as well as healthy controls. Mitochondrial respiration and glycolytic lactate production were assessed by Seahorse flux analysis. Mitochondrial membrane potential was determined by tetra-methyl-rhodamine-ester (TMRM) accumulation. ATP content was measured by luminometry with and without the addition of specific inhibitors of mitochondrial oxidative phosphorylation (oligomycin) or glycolysis (2-deoxyglucose). ER calcium release and dynamics were assessed by live cell imaging with cytosolic fluorescent calcium reporters (Fluo4). Differences in several bioenergetic and calcium parameters were observed between ALS and control astrocytes, but also among different forms of ALS. These results suggest that primary alterations of bioenergetics exist in ALS astrocytes, which could underlie the involvement of astrocytes in ALS pathogenesis.

Disclosures: C. Konrad: None. **K. McAvoy:** None. **D. Trotti:** None. **A. Almad:** None. **N. Maragakis:** None. **J. Pham:** None. **J. Rothstein:** None. **R. Sattler:** None. **G. Manfredi:** None. **H. Kawamata:** None.

Poster

312. ALS

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Program#/Poster#: 312.15/N9

Topic: C.05. Neuromuscular Diseases

Support: 1R01NS089633-01A1

5R01DA034097-05

Title: Hud regulation of sod1 and fus mrnas in sporadic als

Authors: M. DELL'ORCO[1,2], A. S. GARDINER[1], C. CEREDA[2], *N. PERRONE-BIZZOZERO[1];
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Abstract: Altered RNA metabolism due to mutations in RNA-binding proteins (RBPs) or their dysregulation has been defined as a central pathogenic mechanism in motor neuron degeneration, such as Amyotrophic Lateral Sclerosis (ALS). Neuronal ELAV proteins, in particular HuD, have been previously associated with neurodegenerative diseases (NDs) (Amadio et al., JAD, 2009; 16:409-19), and *in vitro* and *in vivo* studies demonstrated the involvement of HuR in the regulation of SOD1 (Milani et al., Neurobiol Dis, 2013; 60:51-60), FUS and TARDBP (Lu et al., J Biol Chem, 2014 ; 289:31792-8042014) mRNAs. RBP accumulation in stress granules (SGs) and processing bodies (P-bodies) is a hallmark of ALS pathology. Using human neuroblastoma SH-SY5Y cells as an *in vitro* model of ALS pathophysiology, we found that HuD co-localization with SG and P-body markers is increased after H_2O_2 treatment. By immunoprecitation, we confirmed that HuD interacts with Argonaute and GW182 proteins. Bioinformatics analysis of human SOD1, FUS and TARDBP 3'UTRs demonstrated the presence of HuD consensus binding sequences in these mRNAs (Bolognani et al., Nucleic Acids Res., 2010; 38:117-30; Wang & Tanaka, Nat Struct Biol, 2001; 8:141-5). In our cellular model, the induction of a neuronal-like phenotype by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) differentiation triggers a significant increase in HuD mRNA levels and an oxidative stress-dependent overexpression of SOD1 and FUS mRNAs. Correspondingly, the overexpression of HuD in the *in vitro* cellular model leads to increased SOD1 and FUS mRNA levels. The increase in target mRNA levels is likely due to the binding of HuD to their 3'UTRs and consequent stabilization, as demonstrated by the significant reduction of SOD1 and FUS mRNAs after the overexpression of a dominant negative HuD mutant protein lacking the RNA Recognition Motif 3 (RRM3) required for target stabilization. By *in vitro* RNA immunoprecipitation (RIP) assays, we demonstrated that HuD binds and stabilizes SOD1 and FUS mRNAs leading to increased mRNA levels. By Immunohistochemistry (IHC) experiments in *post-mortem* tissues from sporadic ALS patients we found that HuD

protein levels were increased in the motor cortex compared to healthy controls. We also found increased HuD mRNA levels in the posterior frontal cortex and cerebellum from ALS patients, along with increases in SOD1 and FUS mRNAs. Uncovering HuD post-transcriptional regulation of SOD1 and FUS mRNAs and defining the potential involvement of additional intracellular pathways and trans-acting molecules will open novel perspectives for ALS research and the identification of new therapeutic targets.

Disclosures: M. Dell'Orco: None. **A.S. Gardiner:** None. **C. Cereda:** None. **N. Perrone-Bizzozero:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: IWT

FWO

ALS Liga Belgium

KU Leuven

VIB

Title: Arginine-rich DPRs perturb nucleocytoplasmic transport and RNA metabolism in the pathogenesis of C9orf72 ALS and FTLD

Authors: S. BOEYNAEMS, E. BOGAERT, P. VAN DAMME, W. ROBBERECHT, *L. M. VAN DEN BOSCH;
Lab. of Neurobiology, Vesalius Res. Center, VIB, Leuven, Belgium, Leuven, Belgium

Abstract: Neurodegenerative diseases are characterized by the presence of protein inclusions with a different protein content depending on the type of disease. Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are no exceptions to this common theme. In most ALS and FTLD cases, the predominant species of aggregated proteins are RNA-binding proteins (RBPs). Interestingly, these proteins are both depleted from their normal nuclear localization and aggregated in the cytoplasm. This key pathological feature suggests a potential dual mechanism with both a nuclear loss of function and cytoplasmic gain of function. The aim

of this study is to get a better insight into the mechanisms underlying these specific pathological events. We focused on hexanucleotide repeat expansions in *C9orf72* which are the most common genetic cause of the disease. Unconventional translation of these repeats yields five potentially toxic dipeptide repeat proteins (DPRs). We performed a targeted genetic screen in *Drosophila* model of DPR toxicity. We only observed toxicity of arginine-rich DPRs (GR and PR) and PR toxicity could be modified by silencing importins, exportins, Ran-GTP cycle regulators, nuclear pore components and arginine methylases. These data point at a key role for nucleocytoplasmic in PR toxicity. More recently, we found additional evidence as we observed a strong overlap in the genetic modifiers of our previous PR screen and a new screen in GR flies. In general, we identified several key transport factors which are potent modifiers of both PR and GR phenotypes. Bioinformatic analyses of the cargo sets of these transport factors strongly indicate that problems in nucleocytoplasmic transport could affect processes implicated in the disease, especially centering on RNA metabolism. Moreover, these cargo sets show a striking enrichment for proteins already implicated in ALS and FTLD, suggesting that defects in nuclear transport could indeed initiate pathogenic cascades. This implies that a defective nucleocytoplasmic transport could be a prime suspect as an initiating event in ALS and FTLD pathology and it could become a new therapeutic target. In recent work we are now investigating whether arginine-rich DPRs have additional roles in the initiation of RBP pathology.

Disclosures: S. Boeynaems: None. **E. Bogaert:** None. **P. Van Damme:** None. **W. Robberecht:** None. **L.M. Van Den Bosch:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: NIH NINDS #40433 (KJJ and VMS)

Title: Analysis and comparison of WT and mSOD1^{G93A} immune-mediated neuroprotection mechanisms in immunodeficient mice after facial nerve axotomy

Authors: *D. O. SETTER[1,2], E. M. RUNGE[1,2], N. A. MESNARD-HOAGLIN[3,4], M. M. HAULCOMB[1,2], R. J. BATKA[1,2], N. D. SCHARTZ[1], V. M. SANDERS[5], K. J. JONES[1,2]; [1]Anat. and Cell Biol., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Res. and Develop. Services, Richard L. Roudebush VAMC, Indianapolis, IN; [3]Neurosci. Program, Loyola Univ. Med. Ctr., Maywood, IL; [4]Res. and Develop. Services, Edward Hines, Jr. VA Hosp., Hines, IL; [5]Mol. Virology, Immunol. and Med. Genet., The Ohio State Univ. Col. of Med., Columbus, OH

Abstract: When a facial nerve axotomy (FNA) is performed on recombinase activating gene-2 knockout (RAG-$2^{-/-}$) mice lacking the adaptive arm of the immune system, there is significantly greater motoneuron (MN) death relative to wild type (WT) mice. Reconstituting RAG-$2^{-/-}$ mice with whole splenocytes restores MN survival to WT levels, and further studies indicate that CD4+ T cells are specifically responsible for immune-mediated neuroprotection. In the mouse model of ALS, (mSOD1^{G93A}), there is also significantly decreased MN survival after FNA. Relevant to these findings, recent studies have found evidence for immune dysregulation in both human ALS patients and the mouse ALS model. Reconstituting RAG-$2^{-/-}$ mice with mSOD1^{G93A} whole splenocytes fails to rescue MN survival, whereas reconstitution with isolated mSOD1^{G93A} CD4+ T cells does confer neuroprotection. These results indicate that an inhibitory factor in the mSOD1^{G93A} peripheral immune system prevents CD4+ T cell-mediated neuroprotection. To characterize this inhibitory mechanism, gene expression profiles of the axotomized facial motor nucleus were gathered from the following 6 experimental groups of mice: WT, RAG-$2^{-/-}$, RAG-$2^{-/-}$ + WT whole splenocytes, RAG-$2^{-/-}$ + WT CD4+ T cells, RAG-$2^{-/-}$ + mSOD1^{G93A} whole splenocytes, and RAG-$2^{-/-}$ + mSOD1^{G93A} CD4+ T cells. Genes associated with MN regeneration, glial activation, inflammatory cytokines, and cell death pathways were examined. While immunodeficiency in the RAG-$2^{-/-}$ group did not affect the MN regeneration response or cell death pathway gene expression, both glial activation and cytokine production in the neuropil of the facial motor nucleus that surrounds the MN cell body were significantly altered. Transfer of WT whole splenocytes or WT CD4+ T cells into RAG-$2^{-/-}$ mice restored glial activation and cytokine production to WT levels. These data suggest that the mechanism of immune-mediated neuroprotection involves CD4+ T cell-mediated regulation of the microenvironment surrounding injured MN, and not the MN directly. Future studies based upon these results are expected to provide direction in the development of therapeutic strategies targeting immune-derived inhibitory factors as a new treatment approach for MN disease.

Disclosures: D.O. Setter: None. **E.M. Runge:** None. **N.A. Mesnard-Hoaglin:** None. **M.M. Haulcomb:** None. **R.J. Batka:** None. **N.D. Schartz:** None. **V.M. Sanders:** None. **K.J. Jones:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: Target ALS

NIH R01 NS085207

NIH U24 NS078736

The ALS Association

Muscular Dystrophy Association

Robert Packard Center for ALS Research at Johns Hopkins

Title: Pathology and functional implications of the C9orf72 repeat expansion in ALS/FTD iPS astrocytes

Authors: *J. T. PHAM[1], J. C. GRIMA[1,2], L. HAYES[1], X. TANG[1], W. ZHOU[1], S. VIDENSKY[1], T. GENDRON[3], J. D. ROTHSTEIN[1,4];
[1]Neurol., [2]Neurosci., The Johns Hopkins Sch. of Med., Baltimore, MD; [3]Neurosci., Mayo Clin., Jacksonville, FL; [4]Brain Sci. Inst., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease resulting from progressive, irrevocable loss of upper and lower motor neurons as well as associated interneurons. Glial cells, especially astroglia, also play important roles in modulating disease progression by secreting neurotrophic factors as well as promoting excitotoxic and/or neuroinflammatory cellular environments. In 2010, a GGGGCC hexanucleotide repeat expansion (HRE) in the first intron of *chromosome 9 open reading frame 72* (*C9orf72*) was identified as the most common genetic cause of familial and sporadic ALS as well as also frontotemporal dementia (FTD), suggesting that the two disparate diseases may share common pathogenic mechanisms. While much of ALS and FTD research has focused on neurons, understanding of ALS and FTD pathogenesis is increasingly extending beyond neuronal death to encompass dysfunction in multiple cell types and differential cellular mechanisms, such as RNA metabolism and protein homeostasis in astroglia. We chose to investigate the astroglial contribution to c9ALS by differentiating patient fibroblast-derived induced pluripotent stem cells (iPSCs) into astroglia (iPSA). The HRE did not affect astroglial differentiation or maturation as evidenced by robust expression of astroglial markers such as GFAP, Aldh1L1, CD44, and S100β. In the presence of primary mouse cortical neurons, c9ALS iPSA also appropriately increased mRNA expression of excitatory amino acid transporter 2 (EAAT2), the principal glutamate transporter in the CNS found predominantly in astroglia, and demonstrated functional glutamate transport from the extracellular space. Total *C9orf72* transcripts were reduced in c9ALS iPSA as measured by qRT-PCR, and RNA foci containing the HRE were detected in c9ALS iPSA by RNA fluorescent in situ hybridization. The level of poly-glutamine-proline dipeptide repeats, a protein product of non-canonical translation of the HRE, was assessed in c9ALS iPSA cell lysates by immunoassay. New studies from our group and others have identified nucleocytoplasmic transport defects as fundamental to *C9orf72* pathophysiology. Ongoing studies are elucidating the composition of nuclear pore complexes and the nuclear proteome in c9ALS iPSA. Lastly, control human iPS motor neurons co-cultured with c9ALS iPSA exhibited decreased viability compared to co-culturing with control iPSA as measured by propidium iodide uptake. These

early studies suggest that *C9orf72* HRE can disrupt normal astroglial function and support of neurons, thereby contributing to c9ALS disease progression.

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Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: NIH NINDS 40443

Title: Elucidation of als mouse (sod1^{g93a}) locomotor transformation relative to a wt cohort: a lifetime, longitudinal, speed-controlled analysis.

Authors: ***R. J. BATKA**[1,2], M. M. HAULCOMB[1,2], S. L. DICKINSON[3], V. M. SANDERS[4], K. J. JONES[1,2];
[1]Anat. and Cell Biol., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Res. and Develop., Richard L Roudebush VAMC, Indianapolis, IN; [3]Epidemiology and Biostatistics, Indiana Univ. Sch. of Publ. Hlth., Bloomington, IN; [4]Virology, Immunology, and Med. Genet., The Ohio State Univ. Col. of Med., Columbus, OH

Abstract: Locomotion analysis is a prominent method for the characterization of ALS pathology, identification of symptom onset, and surveillance of disease progression. Previous work in our laboratory has established the interdependent relationship between speed and nearly all locomotion variables. Furthermore, we also illustrated how discrepancies in speed alone are sufficient to account for statistical differences in outcome measures. We present here a large-sample, high-resolution, comprehensive, longitudinal locomotion analysis using the CatWalk XT system. To our knowledge, this is the first study that incorporates the unique speed-variable relationships as they pertain to ALS mice. Sixteen wild-type (WT) mice (B6SJLF1/J) and 24 SOD1^{G93A} mice were allowed to run on the CatWalk apparatus on alternate days, beginning at postnatal day (PND) 38 as described previously. Data acquisition began at PND 45 through end-stage at PND 150. A minimum of 3 crossings up to a maximum of 20 crossings per animal per testing day was acquired. Each crossing from an individual animal produced means for 210 variables, 106 of which were used here. Two variables were significantly different throughout the time course between SOD1 and WT: Speed and Print Area of the Front Paws. Fifteen variables were never different between SOD1 and WT. Most variables were equivalent initially

and only diverged at or after conventional symptom onset (~PND 80-90). Five variables exhibited early (i.e., pre-symptomatic) differences, while four variables displayed unusual relationships. Electromyographic studies have established subclinical neuromuscular pathology as early as PND 40, with fast-twitch muscles being particularly susceptible. Progressive decrease in motor unit number estimation (MUNE) is the primary manifestation of ALS pathology. Muscle contraction force is unchanged until >50% MUNE loss, which coincides with detectable symptom onset. Compensatory mechanisms including collateral reinnervation, increased motoneuron excitability, and increased motor unit area account for the lack of overt symptoms. This remarkable compensatory ability was evidenced in this study by the maintenance of normal locomotion characteristics until symptom onset. We hypothesize that complex alterations in a few key variables help temporarily offset underlying pathology.

Disclosures: R.J. Batka: None. **M.M. Haulcomb:** None. **S.L. Dickinson:** None. **V.M. Sanders:** None. **K.J. Jones:** None.

Poster

312. ALS

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Topic: C.05. Neuromuscular Diseases

Support: R01NS-045195

Title: Early and androgen-dependent loss of neuromuscular transmission in two SBMA mouse models

Authors: *Y. XU[1], M. KATSUNO[2], H. ADACHI[3], G. SOBUE[2], M. BREEDLOVE[1], C. L. JORDAN[1];
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Abstract: Spinal bulbar muscular atrophy (SBMA) is an androgen-dependent neuromuscular disease caused by CAG/glutamine tract expansion in the androgen receptor (AR). We find multiple defects in neuromuscular transmission in chronically diseased males of three different SBMA mouse models (Xu et al., J. Neurosci. 36(18):5094, 2016), but whether such defects are androgen-dependent has not been established. Thus, we examined the function of neuromuscular junctions (NMJs) in the EDL muscle from diseased transgenic (tg) females treated with testosterone (T) in the myogenic (overexpression of wild-type AR receptor exclusively in muscle) and AR97Q (global expression of human AR gene with 97 glutamines) models. We also

examined synaptic function in young, pre-symptomatic AR97Q males (33-40 days) and castrated adult AR97Q males in which motor function is normal. Diseased AR97Q and myogenic females show significant impairments in synaptic function. Presynaptically, synapses are weak with significant deficits in quantal content (QC), similar to chronically diseased tg males of these same models. Postsynaptically, fibers in diseased females are depolarized (by 15-25 mV) at rest and have end plate potentials with prolonged decay times, comparable to diseased fibers in males, suggesting comparable changes in chloride channels and acetylcholine receptors to immature states. Interestingly, control asymptomatic females show similar but milder defects. Pre-symptomatic juvenile AR97Q males also show defects in the resting membrane potential (RMP) and QC of comparable magnitude to that of chronically diseased adult AR97Q males. Several other measures of neurotransmission were less severely affected, suggesting that the growing severity of deficits critically mediates the emergence of motor dysfunction. Castration largely rescues synaptic function in behaviorally-intact AR97Q males, with a phenotype similar to asymptomatic control-treated tg females. RMP and QC were each rescued by half whereas other deficits were completely reversed. These data indicate that AR toxicity occurs well before symptom onset and raises the question of whether subclinical toxic effects of AR are androgen-dependent. Together, these data show that deficits in neuromuscular transmission that correlate with motor dysfunction are androgen-dependent. We hypothesize that the depolarized state of muscle fibers in asymptomatic SBMA mice could be a primary trigger for late-stage motor dysfunction and early presynaptic defects. Postsynaptic ion channels and ion transports that control the resting voltage of muscle fibers are likely early molecular targets of AR toxicity.

Disclosures: **Y. Xu:** A. Employment/Salary (full or part-time): Michigan State University. **M. Katsuno:** None. **H. Adachi:** None. **G. Sobue:** None. **M. Breedlove:** None. **C.L. Jordan:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: C.05. Neuromuscular Diseases

Title: Novel principle component analysis (PCA) to assess gait in chronically exercised vs unexercised mice shows both exacerbation and amelioration of the underlying phenotype the MDX mouse model for Duchenne's muscular dystrophy (DMD)

Authors: ***P. J. SWEENEY**[1], T. BRAGGE[1], A. NURMI[1], T. HEIKKINEN[1], T. AHTONIEMI[1], J. PUOLIVÄLI[1], D. J. WELLS[2];
[1]Charles River Dicovery Services, Kuopio, Finland; [2]Royal Vet. Col., London, United Kingdom

Abstract: Duchenne muscular dystrophy (DMD) is a progressive disease that is caused by X linked mutation in the dystrophin gene. In DMD patients, the lack of dystrophin causes muscle damage including necrosis, fibrosis and fat infiltration which leads to a progressive decrease in mobility and eventual death. The MDX mouse, a mutant mouse model widely used for the preclinical study of DMD, displays the dystrophin deficiency from the early age of 3 weeks and the process of muscular degeneration and regeneration occurs the age of 8 to 10 weeks. During this "critical period", the process in these mice largely mimics that of DMD boys. The symptoms in these animals are largely subclinical and not evident in traditional functional tests, such as rotarod or open field. Even with chronic exercise regimes, it has been hard to discern changes in these mice without using more advanced technologies. Therefore, typical assays/analyses in this preclinical model are post mortem histopathology of muscles as well as biochemical analysis of serum creatine kinase and occasionally MRI studies.
In our study, we subjected a total 15 MDX animals to a 3 X 30 minutes per week 9 month intensive treadmill exercise regime in an attempt to exacerbate the underlying dystrophic phenotype and compared exercised animals to another group of 15 MDX mice without exercise. As controls, in both groups there were equal numbers of wild type (WT) exercised and unexercised mice. Treadmill speeds were gradually increased up to the level of 14M/min – animals were removed according to a predefined exhaustion criteria. In addition to treadmill exercise, all animals underwent fine motor kinematic gait analysis at the 3, 6, 9 and 12 month time points using kinematic analysis that involves a novel algorithm for Principle Component Analysis (PCA) and yields almost 100 distinct gait related parameters from each mouse.
Results showed that in general exercised MDX mice had to be removed more often, and sooner, from the treadmill - especially at later time points. Interestingly, although certain gait parameters were discernibly impaired according to kinematic analysis, it was also found that some aspects of gait in chronically exercised MDX mice showed marked improvement at various time points, almost approaching wild type mice performance. This suggests that kinematic analysis allows to identify certain gait parameters distinct for the MDX model, especially since deficits with traditional functional tests were found mild or absent. Kinematic analysis may provide a tool to examine efficacy of novel therapeutics alleviating animal models of neuromuscular diseases.

Disclosures: P.J. Sweeney: None. **T. Bragge:** None. **A. Nurmi:** None. **T. Heikkinen:** None. **T. Ahtoniemi:** None. **J. Puoliväli:** None. **D.J. Wells:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Title: High field (11.7) MRI reflects cyclic changes in muscle damage in chronically exercised vs unexercised MDX model of Duchenne's muscular dystrophy and can be confirmed by novel fine motor kinematic analysis *In vivo*

Authors: P. J. SWEENEY[1], A. SHATILLO[1], A. NURMI[1], A. HARTIKAINEN[1], *T. D. WOLINSKY[2], K. LEHTIMÄKI[1], T. AHTONIEMI[1], O. KONTKANEN[1], M. V. KOPANITSA[1], J. PUOLIVÄLI[1], D. J. WELLS[3];
[1]Charles River Discovery Services, Kuopio, Finland; [2]Discovery from Charles River, Wilmington, MA; [3]Royal Vet. Col., London, United Kingdom

Abstract: Duchenne muscular dystrophy (DMD) is a progressive disease that is caused by X linked mutation in the dystrophin gene. In human DMD patients, the lack of dystrophin progressive paralysis and death eventually results from cardiac or, more commonly, respiratory complications as the primary muscle involved in respiration - the diaphragm – is also severely damaged. The MDX mouse, a mutant mouse model widely used for the preclinical study of DMD, shows underlying DMD-like pathology primarily during the period of 3 – 10 weeks after birth. During this period muscle damage, the cycling of degeneration and regeneration that results in incomplete muscle fiber development and sustained injury, is over and little further damage extends beyond this phase. We have previously shown that with a chronic exercise regime this "critical period" may be extended and muscle damage, albeit less severe, can occur at later stages and can be evidenced by subjecting the animals to examination with high field MRI. However, recent evidence from chronically exercised MDX mice in our labs show that the continued damage is transient and, in fact, the exercise regime at some time points even ameliorates the effects of dystrophin deficiency at later ages. In this study we attempted to longitudinally correlate the deficiencies in gait with the exacerbation of muscle damage as evidenced by high field MRI.
In the study we subjected a total 15 MDX and 10 WT animals to a 3 X 30 minutes per week 9 month intensive treadmill exercise regime and compared these animals to another group of 15 MDX animals and 10 WT animals that did not undergo the treadmill training regime. In both groups there were equal numbers of wild type (WT) exercised and unexercised groups. All animals were subjected to baseline MRI and again at 3months, 6 months and 9 months. The unexercised age matched groups were added post hoc for comparison and subjected to kinematic gait analysis at the same time points as the exercised groups and Principle component Analysis (PCA) was performed on all animals at similar time-points.
Results showed that in general exercised MDX mice had a higher number of hyperintensity (indicating muscle damage) than both WT mice and unexercised MDX mice. However, at certain time points the exercise regime seemed to ameliorate the effects of exercise and this can be seen both in T2 MRI and the level of hyperintensity in certain areas of hind-leg musculature as well as in coincidental kinematic gait analysis. Therefore, the use of these these non-invasive techniques in tandem can be used to better gauge the therapeutic window and the preclinical efficacy of aimed at alleviating neuromusculoskeletal disease.

Disclosures: P.J. Sweeney: None. **A. Shatillo:** None. **A. Nurmi:** None. **A. Hartikainen:** None. **T.D. Wolinsky:** None. **K. Lehtimäki:** None. **T. Ahtoniemi:** None. **O. Kontkanen:** None. **M.V. Kopanitsa:** None. **J. Puoliväli:** None. **D.J. Wells:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant R01 NS064224

Title: Genetic rescue of spinal muscular atrophy by zinc finger protein ZPR1

Authors: X. N. JIANG, S. AHMAD, *L. D. GANGWANI;
Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., El Paso, TX

Abstract: Spinal muscular atrophy (SMA) is caused by mutation of the *survival motor neuron 1* (*SMN1*) gene that result in low levels of full-length SMN produced by *SMN2* gene in humans. The zinc finger protein ZPR1 interacts with SMN and is required for accumulation of SMN in sub-nuclear bodies, including gems and Cajal bodies. Interaction of ZPR1 with SMN is disrupted in cells derived from SMA patient. SMA patients express low levels of ZPR1. ZPR1 down-regulation in mice with SMA-like disease results in increased loss of motor neurons that increases disease severity and reduces the lifespan of SMA.

We have shown previously that the *in vitro* ZPR1 overexpression increases nuclear accumulation and levels of SMN in SMA patient cells. ZPR1 corrects axonal growth defects in spinal motor neurons from SMA mice. These findings suggest that ZPR1 is a modifier of SMA disease severity. To test whether ZPR1 is a protective modifier of SMA, we examined the effect of ZPR1 overexpression in mice with SMA. To examine the effect of overexpression of ZPR1 *in vivo* on SMA phenotype, we created transgenic mice overexpressing Flag-ZPR1 protein. ZPR1 transgenic mice [*Zpr1* (+/+); tg-*Flag-Zpr1* (+)] and [*Zpr1* (+/+); tg-*Flag-Zpr1* (+/+)] breed well and live normal lifespan without any obvious phenotype. We first tested *in vivo* functionality of the recombinant Flag-ZPR1 protein by crossing transgenic mice *Zpr1-tg* with *Zpr1* (-/+) knockout mice. Transgenic expression of Flag-ZPR1 protein was able to fully complement and rescue embryonic lethality of *Zpr1* (-/-) mice and transgenic mice [*Zpr1* (-/-); *Flag-Zpr1* (+)] displayed normal phenotype.

To test whether ZPR1 overexpression will help ameliorate severity of SMA disease, we examined the effect of ZPR1 overexpression in two SMA mouse models, severe SMA [*Smn* (-/-); *SMN2* (+/+)] and SMA delta 7 [*Smn* (-/-); *SMN2* (+/+); *SMN2D7* (+/+)] models. The phenotype of littermates, including growth and survival was examined. We report that the increase in ZPR1 expression improves overall growth and motor function, decreases severity of disease, and prolongs the lifespan of SMA mice in both severe and delta 7 SMA models. These data suggest that the increase in ZPR1 levels reduces severity of disease and improves SMA phenotype.

Therefore, ZPR1 may be a protective modifier of SMA. The ZPR1 represents a new therapeutic target to reduce the severity of SMA disease.

Disclosures: **X.N. Jiang:** None. **S. Ahmad:** None. **L.D. Gangwani:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH

Title: Acetylcholine receptor fragmentation is correlated with the extent of muscle fiber damage in a mouse model of muscular dystrophy

Authors: *R. MASSOPUST, W. J. THOMPSON;
Biol., Texas A&M Univ., College Station, TX

Abstract: The mouse neuromuscular junction (NMJ) is a tripartite synapse consisting of the motor axon terminal, muscle fiber, and surrounding terminal Schwann cells. The NMJ is a cholinergic synapse, which signals to an aggregate of acetylcholine receptors (AChRs) on the muscle fiber surface that resembles a “pretzel”. During the course of normal aging, AChR aggregates undergo a morphological change from pretzel-like shapes into fragmented and punctate structures. It has been proposed that these changes accrue slowly throughout the aging process. However, evidence from our lab suggests that this change is abrupt, on the time scale of days. In the *mdx* mouse model of Duchenne muscular dystrophy, AChR aggregates begin to change from continuous pretzels to fragmented structures at a much younger age than in aging wild-type (WT) mice. It is believed that these changes in *mdx* NMJs occur during a crisis period from 3 to 12 weeks of age. During this time, their muscle fibers undergo structural damage, necrosis and repair. Upon repair, *mdx* muscle fibers contain chains of central myonuclei aligned in the center of the muscle fiber. In normal, healthy muscles, myonuclei are found on the periphery of the muscle fiber, directly under the sarcolemma. Previous evidence suggests that central myonuclei in *mdx* animals remain central for at least 6 months. Central myonuclei are indicators of local damage and repair. In the present work, single muscle fibers were isolated from 2, 6, 12 and 24-week-old *mdx* and WT mice. Individual muscle fibers demonstrate two distinct morphological phenotypes. All muscle fibers with continuous AChR aggregates have central myonuclear chains running throughout less than 50% of the length of the muscle fiber. All muscle fibers with fragmented AChR aggregates have central myonuclear chains running

throughout more than 75% of the length of the muscle fiber. As central myonuclei are an indication of recent muscle fiber regeneration following damage, this suggests that a there is a damage-based threshold for AChR aggregate fragmentation.

Disclosures: R. Massopust: None. **W.J. Thompson:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant

Heep Fellowship Texas A&M

Title: Postsynaptic participation in synapse elimination at the developing neuromuscular junction.

Authors: *I. W. SMITH[1], W. J. THOMPSON[2];
[1]Biology/Neuroscience, Inst. For Neurosci., College Station, TX; [2]Biol. / Neurosci., Texas A&M Univ., College Station, TX

Abstract: During development of mature neuromuscular synapses, the innervation of skeletal muscles is extensively remodeled: each muscle fiber transitions from receiving innervation by multiple axons at birth to a singly innervated state in the adult. Termed synapse elimination, this process of loss of redundant motor inputs has typically been characterized as an activity-dependent competition amongst the individual inputs for sole occupation of each muscle fiber endplate. Evidence from our lab indicates an “axo-centric” perspective is an oversimplified explanation for how neuromuscular synapses ultimately become singly innervated. Using light and electron microscopy to investigate mouse neuromuscular junctions (NMJs) during early postnatal development we have found evidence that terminal Schwann cells (tSCs) are capable of altering the competitive balance between inputs. Previously, we have shown how tSCs promote the elimination of inputs, acting as a to drive competition. Additionally, in a series of ongoing experiments we have observed marked differences in the ultrastructure of postsynaptic areas covered by nerve as opposed to tSCs. Our observations indicate muscle fibers undergo extensive remodeling and/or growth, evidenced by pocket-like invaginations in the muscle fiber membrane that appear to be a mechanism for postsynaptic membrane trafficking. Interestingly, pockets appear to contain acetylcholine receptors and are not observed beyond the endplate region at

birth. Moreover, within the endplate region pockets are almost exclusively located beneath areas of nerve terminal contact, suggesting a mode of reinforcement through focal expansion of synaptic contact areas. Interestingly, pockets are not distributed evenly amongst the presynaptic inputs, suggesting selective expansion beneath individual inputs. Collectively our observations and analysis indicate a role for the muscle fiber during the early stages of synaptic competition. We propose that the muscle fibers assess the relative firing patterns and levels of activity of individual inputs and respond through differential distribution of newly synthesized and recycled receptors.

Disclosures: I.W. Smith: None. **W.J. Thompson:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

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Program#/Poster#: 313.06/O2

Topic: E.10. Motor Neurons and Muscle

Title: Interaction of age and myostatin in neuromuscular function.

Authors: D. TAVOIAN[1], W. D. ARNOLD[2], *S. DE LACALLE[3];
[1]Ohio Univ., Athens, OH; [2]Neurosci., Ohio State Univ., Columbus, OH; [3]Biomed. Sci., Heritage Col. of Osteo. Med., Athens, OH

Abstract: In our increasingly aging society, finding ways to slow and reverse loss of muscle mass (sarcopenia) and strength (dynapenia) has become an urgent task. Sarcopenia begins in mid-life and accelerates significantly by age 70. The accompanying decrease in strength leads to serious health problems from falls, poor posture, and limited muscular endurance. The causes of sarcopenia are multifactorial but nerve degeneration and a decrease in muscle fiber size play a prominent role. In the last 20 years, the muscle hypertrophy that follows constitutive deletion of the myostatin gene (MSTN) has sparked a wealth of research to investigate its role in muscle mass and strength maintenance in aging. We have tested the effects of deleting MSTN in adulthood and throughout aging in a conditional knockout mouse model. We used 24 adult female mice genetically modified to contain an inducible Cre recombinase transgene and a critical segment of MSTN (exon 3) flanked by loxP sites. At age 12 months, half of the mice were treated with doxycycline in the chow, which rendered MSTN non-functional by excising the DNA flanked by the LoxP sequences. Motor behavior, electromyographic analysis and measurements of muscle strength were collected at baseline (age 11 mo.) and after one year of treatment.
Age alone had an impact in multiple measures of motor function. Compared to baseline, 24

months-old mice showed a slight (4.5%) increase in lean mass and total weight (12.5%), impairment in rotorod performance (33% decrease), a reduction in the number of motor units (by 30%) and an increase in motor unit size (45%). When doxycycline was administered in the diet (MSTN KO), the effects of aging were ameliorated, as indicated by a more pronounced (9%) increase in lean mass, an attenuation in the impairment in rotorod performance (20%), and the slight improvement in motor unit number (a smaller reduction of 28%) and size (an increase of 26%), as well as some improvement in front grip strength (25% increase). A direct comparison of MSTN KO with littermate controls at 24 months of age also suggested a slight beneficial effect of deleting MSTN (albeit not as pronounced as has been shown in constitutive KO male mice) with better performance in motor behavior tests (rotorod) and in front limb strength tests. Our results agree with our previous studies (Williams, PLoS ONE 10(8): e0134854, 2015) reporting a sexual dimorphism in the role of MSTN, and suggest that further evaluation of the potential advantageous effects of inhibiting or counteracting the actions of MSTN in muscle must address sex differences. Ongoing work by our group is also seeking to characterize the neurological changes that we find associated with MSTN deletion.

Disclosures: D. Tavoian: None. **W.D. Arnold:** None. **S. de Lacalle:** None.

Poster

313. Neuromuscular Disease

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Topic: E.10. Motor Neurons and Muscle

Support: AHA 15POST25720070

NIH Grant

Title: Mechanically patterned extracellular matrix improves neuromuscular junction formation *In vitro*

Authors: *C. L. WEAVER, A. VU, L. FIJANY, G. YEO, A. ENGLER;
Bioengineering, Univ. of California San Diego, La Jolla, CA

Abstract: Motorneuron diseases (MND) are progressive, debilitating disorders resulting from the loss of muscle innervation from MN degeneration. *In vitro* models of the neuromuscular junction (NMJ), the connection between MNs and muscle fibers, have been developed to isolate and examine cellular dysfunctions associated with MN diseases; however these co-culture models with myotubes and MNs have randomly oriented myotubes with immature synapses that

contract asynchronously. Mechanically patterned (MP) extracellular matrix with alternating soft and stiff stripes improve current *in vitro* NMJ models by inducing mouse myoblast durotaxis to stripes where they aligned and differentiated to form patterned myotubes. MP substrates supported increased differentiation, fusion, significantly larger acetylcholine receptor (AchR) clusters, and increased expression and localization of MuSK and Lrp4, two cell surface receptors required for NMJ formation. Primary mouse MNs cultured with patterned myotubes formed functional synapses that more faithfully recapitulate mature *in vivo* NMJs by supporting extensive neurite outgrowth with processes that co-localized with myotube AchRs. Most importantly, robust spontaneous contractions were observed, indicating that functional synapses were developing. Co-cultures containing myotubes and human embryonic stem cell-derived motorneurons expressing channelrhodopsin were optically stimulated to demonstrate NMJ functional connectivity. The mechanically patterned NMJ platform described here represents a significant improvement over current *in vitro* NMJ systems by creating a model that more accurately recapitulates *in vivo* physiology. Future work adapting the model to include MND patient-derived cells will elucide disease pathology and advance drug discovery, potentially leading to new treatments for this class of debilitating disorders.

Disclosures: C.L. Weaver: None. **A. Vu:** None. **L. Fijany:** None. **G. Yeo:** None. **A. Engler:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: R15 NS074367-01A1

Title: Assessment of TrkB receptor expression and function at the neuromuscular junction and sciatic nerve retrograde transport complexes in mice missing muscle-synthesized BDNF

Authors: L. A. VANOSDOL[1], R. L. DANGREMOND[1], A. M. VANDERPLOW[1], B. WILMOT[1], A. L. JUDKINS[1], *E. N. OTTEM[2];
[2]Biol., [1]Northern Michigan Univ., Marquette, MI

Abstract: Neuromuscular diseases (NMDs) are characterized by degeneration and atrophy of motoneurons and muscles they innervate. To determine the importance of muscle-synthesized BDNF to the health of the motor unit, we utilized a transgenic mouse model lacking the BDNF gene only in muscle. Assessment of pathological phenotype of mice revealed adult-onset,

progressive motoneuron pathology and muscle degeneration. We observed somal atrophy of gastrocnemius (gastroc)-associated motoneurons in 120 day-old mice lacking muscle-synthesized BDNF. Gastroc myofibers and motoneurons in transgenic animals are particularly susceptible to progressive pathology when compared to other muscle groups. At the NMJ, we observed significant fragmentation at both the presynapse and postsynapse of knockout animals. Evidence indicated transgenic mice have disrupted retrograde transport processes as phosphorylated-neurofilament-H (p-NF-H) was significantly accumulated in the presynapse NMJ of gastroc-associated NMJs. We found after sciatic nerve ligation there was a significant decrease in accumulating p-NF-H at the distal ligation site. We assessed the distribution of tyrosine receptor kinase B (TrkB), the BDNF receptor, at the NMJ of transgenic and control mice. To assess pathology of the NMJ we labeled presynaptic vesicular acetylcholine transporters, postsynaptic acetylcholine receptors, and the non-phosphorylated (inactive; TrkB) and phosphorylated (active; p-TrkB) isoforms of the TrkB receptor. Preliminary data from confocal microscopy and image analysis suggests mice missing muscle-synthesized BDNF (homozygous knockouts) have much reduced pre- and postsynaptic TrkB expression when compared to control mice or mice with reduced muscle-synthesized BDNF (heterozygous knockouts). Data suggest control and heterozygous animals have similar TrkB receptor expression at both the pre- and postsynapse of the NMJ. Presynaptic BDNF-TrkB signaling has been implicated in initiating retrograde transport, continuing studies will address whether there is a difference in expression of p-TrkB in the pre- and postsynapse of the gastroc NMJ in control and transgenic animals. We will address whether there are differences in p-TrkB inclusion in retrograde transport complexes in control and transgenic mice using a sciatic nerve ligation protocol. Because both homozygous and heterozygous mice with missing or reduced muscle-synthesized BDNF display retrograde transport deficits, but seem to display different levels of pre- and postsynaptic NMJ TrkB expression, determining the levels of p-TrkB expression in the NMJ and retrograde transport complexes is important.

Disclosures: **L.A. VanOsdol:** None. **R.L. Dangremond:** None. **A.M. Vanderplow:** None. **B. Wilmot:** None. **A.L. Judkins:** None. **E.N. Ottem:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH NS04519 (CLJ)

Title: Increased BDNF expression in muscle slows disease in a mouse model of spinal bulbar muscular atrophy

Authors: *K. HALIEVSKI[1], M. KATSUNO[2], H. ADACHI[3], G. SOBUE[2], S. M. BREEDLOVE[1], C. L. JORDAN[1];
[1]Neurosci., Michigan State Univ., East Lansing, MI; [2]Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; [3]Univ. of Occup. and Envrn. Hlth., Fukuoka, Japan

Abstract: Muscle-supplied neurotrophic factors are crucial for the normal development and maintenance of the neuromuscular system and have also been implicated in neurodegenerative disease. Notably, such factors become depleted in diseased muscles, such as in spinal bulbar muscular atrophy (SBMA), an androgen-dependent, slowly progressive neuromuscular disease that occurs in men with an expanded CAG (glutamine/Q) tract in the *androgen receptor* (*AR*) gene. Muscles in SBMA mouse models are deficient in brain-derived neurotrophic factor (BDNF) mRNA and this loss correlates with muscle weakness. To determine whether remedying this loss can ameliorate disease symptoms, we experimentally increased muscle BDNF expression in an SBMA mouse model that globally overexpresses a human AR with 97 CAG repeats. We used a floxed stop model that in presence of a muscle-specific Cre (controlled by the human skeletal actin promoter, HSA-Cre) expresses a human BDNF transgene only in skeletal muscles. Such "BDNF-muscle-overexpressors" were then crossed to the SBMA model (HSA-Cre/BDNF^{stop}lox/97Q combination referred to as "Triple"), resulting in overexpression of human BDNF only in muscles of diseased SBMA mice. Controls were 97Q mice with either the BDNF^{stop}lox or the HSA-Cre transgene, or neither, but not both ("Non-triples"), expected to express normal levels of muscle BDNF. We examined motor function (latency to hang up to 120sec) and bodyweight, and monitored disease progression from the day of onset (defined as two consecutive days of reduced hang time). Criterion for endstage was when animal hang time dropped to below 30sec. We found that increased muscle BDNF slows disease progression, doubling the time to endstage, with Triples reaching endstage ~20days after symptom onset compared to ~11 days when transgenic BDNF is lacking in diseased muscle. Age at symptom onset and maximum weight did not differ between groups. Ongoing studies ask what cellular (neurotransmission at the neuromuscular junction) and/or molecular (gene expression) underpinnings may be responsible for this improved phenotype. Understanding mechanisms muscle BDNF acts upon to slow disease progression may suggest therapeutic targets for improving motor function of men affected by SBMA.

Disclosures: **K. Halievski:** None. **M. Katsuno:** None. **H. Adachi:** None. **G. Sobue:** None. **S.M. Breedlove:** None. **C.L. Jordan:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH

Title: Vital imaging of aging neuromuscular junctions

Authors: *R. HASTINGS, W. J. THOMPSON;
Biol., Texas A&M Univ., College Station, TX

Abstract: Neuromuscular junctions (NMJs) are relatively stable structures that appear to change little during the majority of an animal's life. Changes do occur, however, during "old age". These changes are rapid and increase in frequency with age. The phenotype of these changes is remarkably similar to the changes that occur at NMJs present on muscle fibers that have been damaged and underwent degeneration/regeneration. Therefore, we hypothesize that the changes seen at NMJs in aging are the result of muscle fiber damage. To observe these changes, we have used repeated vital imaging to investigate NMJs in the mouse sternomastoid (STM) muscle. Mice used for vital imaging express transgenic cyan fluorescent protein (CFP) in axons and transgenic green fluorescent protein (GFP) in Schwann Cells. This allows the visualization of the axonal and glial components of each NMJ of living mice. In order to visualize the acetylcholine receptors (AChR), a non-saturating dose of α-bungarotoxin (BTX) conjugated to Alexa Fluor 555 was applied to the muscle. The mice were anesthetized using a ketamine/xylazine cocktail. A depilatory cream was used to remove the hair, and a midline incision was made along the neck of the animal to expose the STM. The mouse was secured on a metal platform in a supine position and intubated in order to control its respiration with a ventilator. A small metal stage was positioned underneath the STM and the mouse was placed under an epifluorescence microscope to be imaged. Over the course of the repeated vital imaging sessions, the majority of NMJs changed very little. There were instances, however, of drastic changes to individual NMJs that happened rapidly, i.e. between two imaging sessions. Furthermore, these changes at the NMJ were accompanied by central myonuclei underneath the endplate and smaller cross-sectional area than those whose NMJs appeared normal. Central nuclei and decreased myofiber cross-sectional area are hallmarks of muscle fibers that have undergone damage. These phenomena correlated with the drastic changes at the NMJ, and therefore we conclude that these changes are the result of the muscle fiber undergoing degeneration/regeneration.

Disclosures: R. Hastings: None. **W.J. Thompson:** None.

Poster

313. Neuromuscular Disease

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Program#/Poster#: 313.11/O7

Topic: C.05. Neuromuscular Diseases

Support: NIH

Title: Neuromuscular acetylcholine receptor dynamics in dystrophic mice.

Authors: *S. HADDIX[1], W. J. THOMPSON[2];
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Abstract: Duchenne muscular dystrophy (DMD) is a fatal disease that has no cure. While it is characterized as a myopathy, there are also abnormalities of the neuromuscular junction (NMJ) in animal models of DMD, which include fragmentation and expansion of the acetylcholine receptor (AChR) endplate, increased axon branching, and an increased number of terminal Schwann cells. The remodeling is likely initiated by muscle fiber necrosis and regeneration at the junction, as in muscle damage models. However, it is difficult to prove such an event causes the rearrangement in dystrophy. To investigate the events underlying the restructuring of dystrophic NMJs, we utilize a technique to visualize the dynamics of AChRs in *mdx* mice, a murine model of DMD. A low concentration of bungarotoxin (BTX) conjugated to Alexa Fluor® 555 is applied to the sternomastoid muscle of an *mdx* mouse to label junctions *in vivo*. This first dose of BTX labels AChR at the synapse, identifying the endplate morphology at the time of application. Following a 10-day recovery period, which also allows for natural myofiber degeneration and regeneration, the animal is sacrificed and labeled with BTX conjugated to a different fluorophore (Alexa Fluor® 647). We hypothesize that if a necrotic event occurs at the endplate region during the 10-day period the first color BTX will be lost or remodeled. The second color BTX will stain robustly, as receptors must be inserted into the sarcolemma for transmission to resume or as a result of the ongoing AChR turnover. In control animals the first and second BTX applications label the same population of receptors and show no difference in morphology. These we interpret to be fibers that have not undergone a necrotic event at the junction during the 10-day interval. However, in a significant number of the *mdx* synapses the first color BTX has mostly disappeared and the second color of BTX labels a fragmented endplate. This indicates that receptor turnover is higher in dystrophic muscles and corresponds to synaptic remodeling. Often the muscle fibers of these remodeled junctions contain central nuclei, a marker of myofiber regeneration. We suggest that the cause of increased receptor dynamics is necrosis and subsequent regeneration of the muscle fiber at the endplate region. The proportion of these “first color weak, second color strong” junctions is elevated and constant in *mdx* mice from 5 to 9 weeks of age. These time points were chosen as they represent the commonly cited “crisis”

period of myofiber damage in this model. Our results indicate that muscle fiber degeneration and regeneration is occurring at a consistent rate during this period and contributes to synaptic remodeling in dystrophy.

Disclosures: S. Haddix: None. **W.J. Thompson:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.12/O8

Topic: C.05. Neuromuscular Diseases

Support: Kennedy's Disease Association Research Award

Title: Androgen receptor p160 co-activators in spinal and bulbar muscular atrophy

Authors: *J. ZENCHAK[1], J. JOHANSEN[2];
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Abstract: Spinal bulbar muscular atrophy (SBMA) is a neurodegenerative disorder that exhibits myopathic and neuropathic characteristics, including muscular weakness and atrophy, and lower motor neuron loss in the brainstem and spinal cord. The molecular basis of the disease is a polyglutamine (CAG) expansion in the *androgen receptor* (AR) gene, resulting in a misfolded and dysfunctional protein.
The AR protein is a major transcription factor, responsible for driving gene transcription that regulates development and maintenance of male reproductive functions and phenotype, and other sexually dimorphic processes. Coregulator proteins aid in this process by binding to AR and altering the topology of chromatin to allow or prevent transcription. Coregulators bind to the amino-terminal of AR, the site of mutation in SBMA, and their recruitment can be affected by the length of the polyQ tract in the AR. The p160 family of coactivators, which includes steroid receptor coactivators 1, 2, and 3, is well-characterized and known to interact with and enhance the activity of AR. Identifying changes in coregulator proteins associated with disease progression may offer a novel therapeutic approach to SBMA.
The AR113Q mouse is one popular model used to study SBMA. The AR113Q knock-in (KI) mouse has 113 CAG repeats inserted in the AR gene, and shows decreased motor function and survival. In AR113Q-KI SBMA mouse skeletal muscle, we examined the p160 co-activator family: steroid receptor co-activators-1, -2, and -3 (SRC-1-3). We used western blotting and immunohistochemistry to detect differences in SRC protein levels in skeletal muscle from SBMA mice. Preliminary data suggests an increase in protein expression of all of the SRC

coactivators, though this difference was only significant for SRC-2. Understanding how the AR interacts with co-regulatory proteins in diseased muscles may provide a tissue-specific therapy for SBMA.

Disclosures: J. Zenchak: None. **J. Johansen:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

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Program#/Poster#: 313.13/O9

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant

AHA Grant

Title: Deletion of pre-B-cell colony-enhancing factor in neurons results in motor dysfunction and paralysis of adult mice

Authors: *X. WANG[1], Q. ZHANG[1], R. BAO[1], N. ZHANG[2], S. DING[1];
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Abstract: Pre-B-cell colony-enhancing factor (PBEF), also known as Nicotinamide phosphoribosyl transferase (Nampt), is the rate-limiting enzyme in the salvage pathway of mammalian Nicotinamide adenine dinucleotide (NAD^+) biosynthesis through conversion of nicotinamide (NAM) to nicotinamide mononucleotide (NMN). NAD^+ is an important co-factor as well as a cosubstrate for many biochemical reactions. Our previous study showed that global deletion of PBEF using heterozygous knockout mice exacerbates neuronal death and brain damage after ischemic stroke. Here we found that inducible and neuronal specific deletion of NAMPT results in motor dysfunction and paralysis of adult mice. We generated homozygous inducible and neuron-specific PBEF conditional knockout (cKO) mice, i.e., Thy1-$Pbef^{-/-}$ cKO mice, by crossing $Pbef^{f/f}$ mice with Thy1-Cre-ERT2 mice. The deletion of neuronal PBEF in mice can be achieved by tamoxifen administration. We show that after tamoxifen administration, PBEF expression levels in Thy1-$Pbef^{-/-}$ cKO mice significantly decreased as compared to the control $Pbef^{f/f}$ mice; consistent with the role of PBEF as a rate-limiting enzyme in the NAD^+ salvage pathway, Thy1-$Pbef^{-/-}$ cKO mice exhibited significant lower levels of NAD^+ and NADPH. Moreover, conditional deletion of PBEF in neurons following tamoxifen administration resulted in progressive decrease of body weight, paralysis and died within three weeks.

Meanwhile, the cKO mice exhibited impairments in general motor ability, muscle strength, locomotor activity, motor coordination as shown by different behavioral tests. Administration of NMN, an immediate enzymatic product of PBEF in the NAD^+ biosynthesis salvage pathway, significantly delayed the onset of motor deficits, body weight reduction, paralysis and death induced by PBEF deletion in neurons in the knockout mice. Furthermore, Thy1-Pbef$^{-/-}$ mice exhibited abnormality of neuronal muscular junction. In summary, our results suggest that PBEF-mediated NAD^+ biosynthesis is essential for motor function and survival and PBEF-NAD^+ cascade might be a potential therapeutic target in motor neuron degenerative disorders.

Disclosures: X. Wang: None. **Q. Zhang:** None. **R. Bao:** None. **N. Zhang:** None. **S. Ding:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.14/O10

Topic: E.10. Motor Neurons and Muscle

Support: NIH

Title: Activation of Schwann cells in a rat model of "Critical Illness Myopathy"

Authors: *Y. LEE[1], L. LARSSON[2], W. J. THOMPSON[1];
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Abstract: Patients with “Critical Illness Myopathy” (CIM) are characterized by general paralysis of all limb and trunk muscles but have intact cognitive and sensory function. CIM is observed in intensive care unit patients who have been mechanically ventilated and immobilized for long durations and is considered a consequence of modern intensive care. The weakness is manifest in changes in the contractile proteins in muscles, but we have been investigating whether there are changes in the innervation of the muscles. The Larsson lab has developed a rat model in which normal, adult rats are anesthetized, given paralytics, and respired by mechanical ventilation. We have examined neuromuscular junctions (NMJs) in the soleus muscles of such rats paralyzed for 4 and 8 days by fluorescently staining the muscles for postsynaptic acetylcholine receptors, presynaptic nerve terminals, and Schwann cells (SCs). We find the nerve terminals begin to sprout short processes that follow SC processes extended from the synapses. The most profound changes and the initial changes we see involve extension of numerous processes from the terminal Schwann cells. Observations of NMJs in the rat CIM model are, therefore, consistent

with previous studies that show SC involvement in synaptic remodeling in cases of partial denervation or toxin-induced paralysis.

Disclosures: **Y. Lee:** None. **L. Larsson:** None. **W.J. Thompson:** None.

Poster

313. Neuromuscular Disease

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Program#/Poster#: 313.15/O11

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant PO1 NS058901

Title: Reduced corpus callosum and somatosensory pathway function in a CCUG mouse model of myotonic dystrophy type 2: an autofluorescence optical imaging and electrophysiological study

Authors: ***G. CHEN**[1], R. E. CARTER[1], J. D. CLEARY[3], J. M. MARGOLIS[2], Y.-L. KANG[2], C. M. CHAMBERLAIN[3], L. P. W. RANUM[3], T. J. EBNER[1];
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Abstract: Myotonic dystrophy (DM) is a progressive, multisystem disorder with widespread effects on skeletal muscle, heart and the central nervous system including cognitive deficits. In DM1/DM2, CUG/CCUG expansion transcripts sequester muscleblind-like proteins leading to alternative splicing abnormalities that contribute to the symptoms in multiple systems. Anatomical and diffusion tensor imaging documents widespread changes in the cerebral cortical white matter in DM patients including in the corpus callosum. In this study, we use autofluorescence imaging and electrophysiology *in vivo* to assess cerebral cortical circuit and corpus callosum function in a CCUG transgenic mouse model of DM2.
The CCUG mice express tet-responsive (TRE) (CCUG)300 transcripts under the control of the tet-transactivator (tTA) driven by the *Camk2a* promoter. These mice express CCUG expansion transcripts in the forebrain and show molecular CNS features of DM2, including RNA foci and repeat-associated non-ATG (RAN) protein expression. In anesthetized mice, the cerebral cortex was exposed bilaterally and intracortical microstimulation (ICMS) delivered with a tungsten microelectrode. Field potentials evoked by paired-pulse ICMS were recorded from the contralateral hemisphere to assess presynaptic circuit function. Paired-pulse facilitation (PPF) was completely lacking in the double-positive transgenic mice (tTA[+];TRE[+]) compared to singly transgenic control mice (tTA[+] or TRE[+]) and wild type littermates. Furthermore,

paired-pulse depression at short time intervals was accentuated in double-positive mice. Additionally, the corpus callosum conduction velocity as measured from the time of stimulation to the arrival of fiber volley was significantly slower in the double-positive mice, with a 11-18% reduction compared to control lines. Using flavoprotein imaging, we also evaluated the responses evoked in the somatosensory cortex by air puff stimulation of the contralateral C3 whisker. Compared to the control lines, the responses in the double-positive transgenic mice were reduced from 18-30%. Similar results were obtained from a second line of CCUG mice, but additional experiments are needed to determine significance.
Together the findings of compromised PPF and reduced conduction velocity demonstrate impaired corpus callosum function in the CCUG mice. Importantly, many studies of both DM1 and DM2 suggest abnormalities in the white matter tracts including the corpus callosum. The decrease in the responses to air puff stimulation evoked in the primary somatosensory cortex suggests that other major neuronal pathways are also affected in this DM2 mouse model.

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Poster

313. Neuromuscular Disease

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Program#/Poster#: 313.16/O12

Topic: C.05. Neuromuscular Diseases

Support: CIHR

ALS Canada doctoral award

Title: Zebrafish models to validate mutations in CAPN1 causing hereditary spastic paraplegia

Authors: *A. LISSOUBA[1,2], M. LIAO[1], P. DRAPEAU[1,3];
[1]CRCHUM, Montreal, QC, Canada; [2]Pathologie et biologie cellulaire, [3]Neurosciences, Univ. de Montreal, Montreal, QC, Canada

Abstract: Hereditary spastic paraplegia (HSP) is a genetically and clinically heterogeneous disease characterized by spasticity and weakness of the lower limbs with or without additional neurological symptoms, due to the degeneration of upper motor neurons. Although more than 70 genes and genetic loci have been implicated in HSP, many families remain genetically undiagnosed, suggesting that other genetic causes of HSP are still to be identified.Our collaborators performed whole-exome sequencing to analyze a total of nine affected individuals

in three families with autosomal-recessive HSP. Rare homozygous and compound-heterozygous nonsense, missense, frameshift, and splice-site mutations in *CAPN1* were identified in all affected individuals, and sequencing in additional family members confirmed the segregation of these mutations with the disease (spastic paraplegia 76 [SPG76], Ziv et al., AJHG 98:1038-46, 2016). *CAPN1* encodes calpain 1, a protease that is widely present in the CNS. Calpain 1 is involved in synaptic plasticity, synaptic restructuring, and axon maturation and maintenance. We validated calpain 1 deficiency using zebrafish. Antisense morpholino knockdown of *calpain 1a*, a *CAPN1* paralog in *Danio rerio*, resulted in several developmental defects visible at 2 days postfertilization (dpf), and a moderate to severe phenotype was exhibited by 78% of injected embryos at 5 dpf, indicating that these defects are long lasting. The *capn1a* Mo was also injected in the Islet1::GFP transgenic fish expressing GFP in the motor neurons, including the upper branchiomotor neurons. We observed a disorganization of these motor neurons in comparison to those of the control, as well as migration defects of the nV trigeminal nuclei and of the VII facial branchiomotor neuronal cell bodies. Furthermore, the vagal motor neurons had an aberrant positioning and spacing, probably because of a defect in cell motility. Additionally, reduced acetylated-tubulin staining could be observed at the level of the optic tectum and cerebellum and strikingly, clusters of acetylated tubulin could be observed in some cells in the dorsal-most part of the brain. In order to further study calpain 1a defiency in the zebrafish, we generated CRISPR/Cas9 knockout lines of both paralogs of *CAPN1*, *calpain 1a* and *calpain 1b*, and crossed these lines together to obtain a complete calpain 1 knockout in the zebrafish. The identification of mutations in *CAPN1* in HSP and the study of the resulting defiency in the zebrafish expand our understanding of the disease causes and potential mechanisms, opening the door for new therapeutic avenues by high-throughput screening.

Disclosures: **A. Lissouba:** None. **M. Liao:** None. **P. Drapeau:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: SEED/TIDE/007/2013/G

Title: Improved motor performance in spastic cerebral palsy children after repetitive transcranial magnetic stimulation

Authors: ***D. BHATIA**[1], B. L. RAJAK[1], M. GUPTA[1], S. PAUL[1], A. MUKHERJEE[2], T. K. SINHA[1];

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Abstract: Spastic cerebral palsy (SCP) is a non-progressive neuromuscular disorder caused due to brain injury affecting motor ability in children during birth or at an early age. An important treatment approach being employed for SCP is standard therapy (ST) comprising of stretching, positioning and weight bearing exercises. However, recently brain stimulation techniques such as repetitive Transcranial magnetic stimulation (r-TMS) have been explored for the benefit of these patients. This study evaluated the motor performance of spastic CP children after r-TMS therapy using universally accepted scales - gross motor function measure (GMFM) and quality of upper extremity skill test (QUEST) along with EEG analysis. 30 subjects were recruited for this study, out of which 20 were spastic CP children and 10 were normal healthy children. The age, height and weight were matched in selected SCP subjects and then divided into interventional group (IG) and reference group (RG). Subjects in IG were administered 5Hz and 10Hz r-TMS therapy of 15 minutes duration followed by ST of 30 minutes daily for 20 days. The other 10 subjects in RG were provided only ST for 30 minutes daily for 20 days. Statistical analysis of pre versus post scores of both assessment scales (GMFM and QUEST) between different groups (IG and RG) showed significant improvement ($p<0.05$). The GMFM mean change was 2.2% in IG as compared to 0.4% in RG. Similarly, QUEST mean change was 2.3% in IG as compared to 0.6% in RG. Additionally, further analysis of pre and post r-TMS effect was performed using power spectrum density (PSD) analysis of electroencephalogram (EEG) signals of SCP subjects and compared with EEG of normal subjects. EEG recording was performed on all subjects using four electrodes placed on pathway known for motor control and planning, namely C3, C4, F3 and F4. The artifact-free EEG signals of fifteen (15) minutes duration was selected for spectral analysis using Fast Fourier Transformation (FFT) algorithm in MATLAB platform and PSD (power versus frequency) was plotted. The PSD revealed significant peak at frequency of 50Hz and smaller or none at 100Hz, for all healthy subjects; but in case of SCP children, peak at 100Hz were prominent and at 50Hz it was found to be quite low, prior to r-TMS. After 20 sessions of r-TMS therapy; a shift in the PSD frequency peak in SCP was observed in all SCP children at 50Hz similar to that of normal children and at 100Hz the peak was smaller in magnitude. The result of this study demonstrates that r-TMS combined with ST enhances motor ability in SCP children over ST alone in limited number of sessions by stimulating the motor neurons of the brain as evident from the PSD of EEG signal post r-TMS.

Disclosures: **D. Bhatia:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Department of Science and Technology, Government of India. **B.L. Rajak:** None. **M. Gupta:** None. **S. Paul:** None. **A. Mukherjee:** None. **T.K. Sinha:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.18/O14

Topic: C.05. Neuromuscular Diseases

Title: Establishment of TDP-43 cryptic exon as a new ante-mortem functional biomarker for Inclusion Body Myositis

Authors: *K. E. BRAUNSTEIN[1], J. P. LING[1], T. E. LLOYD[2], P. C. WONG[1];
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Abstract: Inclusion body myositis (IBM) is the most common acquired myopathy occurring in adults aged older than 50 years. Abnormal cytoplasmic accumulations of TDP-43, a heterogenous ribonucleoprotein first linked to ALS and FTD, have been also consistently described in myofibers in cases of IBM. We previously reported that TDP-43 represses nonconserved cryptic exons, a critical role that is compromised in ALS and FTD. We hypothesized that such loss of TDP-43 function could underlie degeneration of skeletal muscle in cases of IBM exhibiting TDP-43 pathology. Using RNA-sequencing analysis, we show in myoblast cell culture lacking TDP-43 the incorporation of nonconserved cryptic exons within a series of RNA targets that are flanked by UG-rich elements. Using reverse transcription-polymerase chain reaction analysis, we further reveal the presence of cryptic exons in IBM myofibers exhibiting mislocalization of TDP-43, but not in those of control cases. Thus, these results establish TDP-43 cryptic exon as a novel functional ante-mortem biomarker for the diagnosis of IBM, information that will be critical for assessing target engagement and following efficacy of therapeutic strategy in clinical trials.

Disclosures: **K.E. Braunstein:** None. **J.P. Ling:** None. **T.E. Lloyd:** None. **P.C. Wong:** None.

Poster

313. Neuromuscular Disease

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Topic: C.05. Neuromuscular Diseases

Support: Supported by the National Science Foundation

National Institutes of Neurological Disease and Stroke

Title: High-throughput automated time-lapse imaging of neuron degeneration within a live animal using robotic microscopy

Authors: *J. LINSLEY[1,2], S. FINKBEINER[1,2];
[1]GIND, Gladstone Inst., San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Understanding the cellular changes that destine a neuron to live or to die is a fundamental challenge to understanding neurodegenerative disease. Robotic microscopy, an imaging platform that uses automated identification and tracking of millions of individual neurons over set time intervals provides the ability to quantitatively relate intermediate changes within a neuron to it's fate. Yet, while robotic microscopy facilitates longitudinal single cell analysis of neurons in culture, it has not yet been adapted for use in intact tissue, limiting the ability to study neurodegenerative diseases that have cell non-autonomous phenotypes. Taking advantage of the ability to perform time lapse imaging on immobilized zebrafish larvae, we have created a high-throughput, time-lapse, confocal imaging platform combined with automated image analysis software for single cell analysis of genetically defined subsets of neurons within larvae. To demonstrate this technology, genetically encoded nitroreductase was selectively expressed in sensory neurons or motor neurons, and time-lapse imaging was performed before and after exposure to the prodrug Metronidazole. We believe this technology will be easily adaptable to current zebrafish models of neurodegenerative disease, providing both longitudinal single cell analysis as well as a potential drug screen platform.

Disclosures: J. Linsley: None. **S. Finkbeiner:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

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Program#/Poster#: 313.20/O15

Topic: C.05. Neuromuscular Diseases

Title: Clinical investigation of manihot esculenta's cyanogenic glycosides induced neurological conditions,treatments and the effect of Netfussion innovated device on reducing chemical tuber food poisoning in Nigeria and third world countries.

Authors: *A. EKWERIKE[1,2,3,4,5], C. A. DIKEUKWU[6], K. MUFORO[6], H. IGBONAGWAM[7], V. C. OSUOHA[8], R. OKEA[3];
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Abstract: Introduction: This study is aimed at finding means of reducing the increasing incidents of neurological conditions caused by cassava's cyanide poisoning. The studied legend, Manihot Esculenta (Cassava) is a major staple food in Africa, high in Carbohydrates and contains Linamarin (cyanogenic glycoside) which causes Cerebellar Ataxia, Tropical Ataxic Neuropathy and konzo paralytic conditions among the consumers. Netfussion is our innovated device that extracts this toxic cyanide from the raw or poorly processed manihot esculenta (Cassava) and serves as a therapeutic for reducing human systemic and tuber raw food cyanide. Method: 80 known consumers (20 per group) were selected as control and Netfussion test groups. Divided into four groups namely Control (Non net fussed),and three Net fussed test groups; Raw, Fermented & Dry. All groups were neurologically diagnosed & showed acute cyanide poisoning signs of Stomachaches,diarrhea,vomiting,irregular blood pressure,rapid pulse & respiration,headache,twitching,convulsion,mental confusion,etc and differential symptoms of cerebellar ataxia, tropical ataxic neuropathy or Konzo paralysis respectively which affirmed the cyanogenic glycoside poisoning, clinically. Test: Netfussion was employed to extract the Linamarin (cyanogenic glycoside) from the raw cassava tuber, water fermented grade (Fufu), and the dried granule (Gari) before consumption..All groups were subjected to their drug prescriptions, physical therapy and net fussed meals daily except the Control mixed group that were not netfussed. 90 days per batch study was recorded, though still ongoing. Result:Netfussion processed cassava meals administered three groups,(Raw, Fermented and Dry) showed significant improvements more than the non net fussed Control mixed group. Within the net fussed test groups, the Fermented and Dry groups showed excellent improvements more than the raw group. Conclusion: Combination of medical, physical therapies and the novel netfussion toxic cyanide extracting device can reduce the incidents of cerebellar ataxia, tropical ataxic neuropathy and konzo neurological disorders in Africa. This advises against the consumption of poorly processed & raw cassava,and also shows that Netfussion mechanism of extracting bitter cassava's content of Linamarin (cyanogenic glycoside) is very effective and efficient in producing cyanide free & safe cassava meals.

Disclosures: A. Ekwerike: None. **C.A. Dikeukwu:** None. **K. Muforo:** None. **H. Igbonagwam:** None. **V.C. Osuoha:** None. **R. Okea:** None.

Poster

313. Neuromuscular Disease

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Program#/Poster#: 313.21/O16

Topic: C.05. Neuromuscular Diseases

Support: Departmental startup grant

Title: Aggregation of the disease-causing mutants of cysteine string protein-alpha via Fe-S cluster binding

Authors: *N. NASERI[1], B. ERGEL[1], Q. HUANG[2], R. HUANG[2], G. A. PETSKO[1], M. SHARMA[1];
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Abstract: Disease-causing point mutations in cysteine string protein-α (CSPα) lead to aggregation of the protein in adult onset neuronal ceroid lipofuscinosis (ANCL), a lysosomal storage disease that accompanies neurodegeneration. However, the mechanism of aggregate formation remains unclear. Here we show that the cysteine-string region of CSPα, which is normally palmitoylated, lacks efficient palmitoylation in ANCL mutants, allowing oligomerization via Fe-S cluster interaction. In primary mouse neurons, oligomers of mutant CSPα incorporate wild-type CSPα molecules, suggesting a dominant-negative mechanism for ANCL pathogenesis. This aggregation is significantly rescued by pharmacologically chelating iron in primary neurons.

Disclosures: N. Naseri: None. **B. Ergel:** None. **Q. Huang:** None. **R. Huang:** None. **G.A. Petsko:** None. **M. Sharma:** None.

Poster

313. Neuromuscular Disease

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Program#/Poster#: 313.22/O17

Topic: C.05. Neuromuscular Diseases

Support: CONACYT SALUD-2012-01- 181611

Title: Screening of dysferlinopathies by whole blood flow cytometry

Authors: *L. SANCHEZ-CHAPUL[1], M. DEL ANGEL MUÑOZ[2], L. RUANO-CALDERÒN[5], A. B. LUNA-ANGULO[2], R. M[6], J. MAGAÑA[3], O. HERNÀNDEZ-HERNÀNDEZ[3], R. E. ESCOBAR-CEDILLO[4], A. LÒPEZ-MACAY[3], S. VARGAS[7];
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Abstract: Background. Muscular dystrophies are genetic disorders in which muscle strength is progressively lost with replacement of muscle tissue by fibroadipose tissue. Dysferlinopathies are underdiagnosed muscular disorders caused by mutations in dysferlin encoding gene. Three main phenotypes have been reported: Miyoshi Myopathy (MM); Limb Girdle Muscular Dystrophy type 2B (LGMD 2B), and Distal Myopathy with Anterior Tibial onset (DMAT), among other clinical variants. It is the second most frequent cause of muscular dystrophy in different parts of the world including Mexico. Whole Blood Flow Cytometry (WBFC) has been proposed as a new specific, suitable, and fast alternative for dysferlinopathies prior to molecular diagnosis. This work aimed to make a quantitative analysis of the expression of dysferlin in peripheral blood monocytes (PBM) by calculating the Mean Fluorescence Intensity (MFI) by flow cytometry. **Objective.** To establish a simplified algorithm based on likelihood ratios of dysferlin MIF to allow us to classify individuals with a clinical suspicion of dysferlinopathy prior genetic analysis. **Material and Methods.** We obtained blood samples from 183 healthy individuals and 29 patients diagnosed with dysferlinopathy on the basis of clinical study and skeletal muscle immunofluorescence. PBM were doubled immunolabeled using anti-dysferlin and anti-CD14 monoclonal antibodies. Samples were run on a FACSCalibur™ Flow Cytometer, and analyzed by FlowJo software. We screened cDNA for *DYSF* gene mutations. The relative quantity of dysferlin was expressed as MFI. Performance of WBFC diagnostic test was assessed by calculating likelihood ratios at different MFI cut-off points **Results.** We reported quantitative levels of dysferlin in CD14+ PBM of healthy individuals and compared these with the levels expressed in patients with dysferlinopathy. We established a simplified algorithm based on likelihood ratios that allowed us to classify individuals with a clinical suspicion of the disease into four groups. We found 4 mutations in the *DYSF* gene that were reported previously in our population and two novel mutations. **Conclusion.** The algorithm established give us a range of diagnostic probability a) to guide the physician in applying molecular confirmation by sequencing the *DYSF* gene, b) to distinguish primary dysferlin deficiency from a secondary decrease in other muscular dystrophies and c) to be able to conduct clinical re-evaluation of a case that is not a dysferlinopathy.

Disclosures: L. Sanchez-Chapul: None. **M. del Angel Muñoz:** None. **L. Ruano-Calderòn:** None. **A.B. Luna-Angulo:** None. **R. M:** None. **J. Magaña:** None. **O. Hernàndez-Hernàndez:** None. **R.E. Escobar-Cedillo:** None. **A. Lòpez-Macay:** None. **S. Vargas:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.23/O18

Topic: C.05. Neuromuscular Diseases

Support: NS054154

Title: Translational profiling of motor neurons in two mouse models of Charcot-Marie-Tooth disease Type 2D

Authors: *E. L. SPAULDING[1,2], R. W. BURGESS[1];
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Abstract: Charcot-Marie-Tooth disease (CMT) is a collection of debilitating peripheral neuropathies caused by mutations in over 80 genes. The heterogeneity of the disease, as well as the technical challenge of studying the mammalian peripheral axon *in vivo*, both contribute to the lack of a cure. Dominant mutations in glycyl tRNA synthetase (*GARS*), cause an axonal form of the disease, Charcot-Marie-Tooth Type 2D (CMT2D). How mutations in *GARS* cause CMT2D is unclear, but a toxic gain-of-function is suspected. We are using an *in vivo*, cell type- and compartment-specific approach to profile translation in motor neurons of two mouse models of CMT2D. Impaired translation has recently emerged as a potential gain-of-function mechanism based on work with *Drosophila* models of CMT2D. Because five other tRNA synthetases are also linked to CMT, impaired translation is an attractive disease mechanism to test in a mammalian system. To profile translation in motor neurons we are using two *in vivo*, cell type-specific techniques; non-canonical amino acid-tagging (NCAT) provides the location, identity, and quantity of newly translated proteins, and ribosome-tagging catalogs ribosome-associated RNA. We are separately profiling motor neuron cell bodies from the spinal cord and axons from the sciatic nerve, providing additional cell compartment-specificity. Although local translation in adult, mammalian axons is not established, our preliminary data show that ribosomes are present and associated with mRNA in motor axons of the sciatic nerve. Local translation in axons also occurs during regeneration after injury and our initial studies using NCAT suggest that CMT2D motor axons show a regenerative phenotype. Therefore, we are also profiling regenerating wild-type motor neurons after sciatic nerve crush for comparison against mutant *Gars* samples. Our approach will create translational profiles of CMT2D, healthy wild-type, and regenerating wild-type motor neuron cell bodies and axons. These data will uncover new motor neuron biology that can be used for future study of other peripheral neuropathies or motor neuron diseases. In addition, our study will help to elucidate the disease mechanism of CMT2D and possibly other tRNA synthetase-linked forms of peripheral neuropathy.

Disclosures: **E.L. Spaulding:** None. **R.W. Burgess:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

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Program#/Poster#: 313.24/P1

Topic: C.05. Neuromuscular Diseases

Support: FWO Grant 1111513N

Title: Progranulin overexpression attenuates TDP-43^{A315T} mediated neurodegeneration

Authors: **S. BEEL**[1], S. HERDEWYN[2], L. VAN DEN BOSCH[1], W. ROBBERECHT[1], *P. VAN DAMME[2,1];
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Abstract: TAR DNA binding protein 43 (TDP-43) positive inclusions are the main pathological hallmark of both amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Up to 40% of the patients with FTLD show a familial history for neurodegenerative disease, but only in very rare cases a mutation in the *tardbp* gene can be identified. Much more frequent are mutations in *progranulin* that explain approximately 20% of familial FTLD patients. Interestingly, these patients have neuronal ubiquitin positive inclusions that are most of the time also TDP-43 positive. In total, about 50% of all FTLD patients show similar TPD-43 pathology. While mutations in the *tardbp* gene explain only 3 - 5% of familial ALS cases, a loss of nuclear TDP-43 and cytoplasmic TDP-43 positive aggregates are found in the upper and lower motor neurons and glial cells of most ALS patients. Modeling this TDP-43 pathology in rodents has been a great challenge over the years. Several knockout and (mutant) overexpression models have been generated, but the first mouse model that expresses a mutant human TDP-43 (A315T) protein under control of the mouse prion protein (Prp) promoter has been studied the most. We and others have observed that these Prp-TDP43^{A315T} mice die a sudden death due to gastrointestinal complications. We recently showed that upon prevention of this intestinal obstruction, Prp-TDP43^{A315T} mice show a progressive neurodegenerative phenotype. In the present study, we investigated whether the overexpression of human progranulin (hPGRN) could attenuate their neurodegenerative phenotype. To this end, we crossed the Prp-TDP43^{A315T} mice with hPGRN overexpressing mice and compared them to Prp-TDP43^{A315T} and non-transgenic mice that were all put on the easy to digest gel food diet to prevent intestinal obstructions. Follow up analysis showed that hPGRN overexpression significantly increases the survival and disease duration in Prp-TDP43^{A315T} mice. On a pathological level, we could also find an

increased number of large upper motor neuron axons in the lateral horn of the spinal cord of TDP-43^{A315T}xhPGRN mice compared to TDP-43^{A315T} littermate controls. These findings suggest an important role of hPGRN in attenuating the toxic effect of mutant TDP-43 overexpression in mice. The mechanism by which hPGRN mediates these effects still needs to be elucidated and will help us gain insight in the interplay between TDP-43 and PGRN.

Disclosures: S. Beel: None. **S. Herdewyn:** None. **L. Van Den Bosch:** None. **W. Robberecht:** None. **P. Van Damme:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.25/P2

Topic: E.10. Motor Neurons and Muscle

Support: NIH grant R01 NS091278-01A1

Title: Decrease of rate dependent depression of H-reflex in newborns with muscle hypertonia after antenatal hypoxia-ischemia in rabbit cerebral palsy model

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[1]Pediatrics, Northshore Univ. Hlth. Syst. Res. Inst., Evanston, IL; [2]Physiol., Northwestern Univ., Chicago, IL

Abstract: Newborn rabbit kits after global antenatal hypoxic-ischemic (H-I) injury exhibit motor deficits similar to human infants with cerebral palsy, including muscle hypertonia. Loss of supraspinal presynaptic inhibition has been implicated as a mechanism leading to the development of hypertonia and spasticity in cerebral palsy. We hypothesized that a decrease of descending corticospinal motor projections, observed in this rabbit model, will affect the rate dependent depression (RDD) of H-reflex as a measure of supraspinal inhibitory control. The secondary objective was to examine developmental changes in H-reflex during postnatal maturation. H-reflex was elicited in lightly anesthetized P1, P5, P11, P18 and adult rabbits using needle electrodes for stimulation and thin wire electrodes placed in m. flexor carpi radialis in forelimbs and m. gastrocnemius in hind limbs for recording. In some experiments H-reflex was measured on exposed nerve preparation with subsequent nerve cutting to confirm the origin of H-wave. RDD was measured as a ratio of H-wave magnitudes in control and test stimuli pairs, presented every 10 sec with 5, 2, 1, 0.5, 0.15, 0.08, 0.03 sec inter-stimulus intervals (ISI). In addition, maxH/maxM amplitude ratio was measured by varying stimulation amplitude. Newborns of naïve control and rabbit dams that underwent global fetal H-I at E25 for 40 min

were used. We were able to elicit H-reflex 85% in forelimbs and 65% hind limbs at P1, and in almost all kits at later ages. The magnitude of the test pulse decreased from 82% to 53% to 21%, at 5 ,0.5, 0.03 sec ISI respectively. There was no significant difference in RDD during postnatal development between P1 and P11 in either forelimb or hind limbs. There was also no significant difference in RDD between forelimbs and hind limbs at corresponding ages. Significant reduction of RDD was found in hypertonic kits after H-I, both in fore- and hind limbs at P1 and P11, at 0.3, 0.15.008 sec ISI: Significant increase in hypertonic kits in maxH/maxM from 0.24 to 0.42 was found only in hind limbs at P1 but not at P11.We conclude that the significant reduction of RDD in hypertonic kits suggests a decreased supraspinal inhibitory control that might be mediated by the loss of descending motor projections after fetal H-I. An increase of maxH/maxM may indicate that stronger afferent synaptic connection is retained in hypertonic kits due to delayed arrival of corticospinal projections. Subsequent decrease of maxH/maxM at later ages may indicate continued degeneration of the spinal circuit in hypertonic kits.

Disclosures: A. Drobyshevsky: None. **K. Quinlan:** None.

Poster

313. Neuromuscular Disease

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 313.26/P3

Topic: E.10. Motor Neurons and Muscle

Support: R01NS091278

Title: Serotonin sensitivity of spinal motor neurons from hypoxia-ischemia rabbit model of cerebral palsy

Authors: *K. A. QUINLAN[1], A. DROBYSHEVSKY[2];
[1]Physiol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Northshore Univ. Hlth. Syst., Evanston, IL

Abstract: Spinal motor neurons show profoundly increased excitability in the presence of 5HT. This excitability has been shown in previously published studies to underlie muscle spasms after spinal cord injury. More recently, an increased presence of serotonin in the spinal cord has been shown in the rabbit hypoxia-ischemia (H-I) model of cerebral palsy as well. Increased hypertonicity of the limb muscles was correlated with increases in serotonin immuno-positive fibers and serotonin concentration (determined by HPLC). Blocking serotonin receptors with intrathecal methysergide *in vivo* was shown to decrease the muscle stiffness of the rabbit kits affected by H-I. However, changes in gene expression levels of 5HT2 receptors and the 5HT

transporter suggest H-I affected spinal cords could be less responsive to serotonin. Thus, this work aimed to determine whether spinal motor neurons are equally responsive to serotonin (specifically 5HT2 receptor agonists) in control and H-I affected rabbit kits. Spinal motoneurons were targeted for whole cell patch clamp in transverse spinal cord slices from control (sham operated and unaffected kits) and H-I affected rabbit kits between birth and 5 days of age. Lumbar motor neurons from H-I affected kits have higher input resistance (suggestive of smaller cell size), a larger amplitude after-spike after hyperpolarization (AHP), and a smaller current amplitude at firing onset (I-on) than the lumbar motor neurons of control rabbits. Motor neurons respond to bath perfusion of 0.3 uM alpha-methyl 5HT (a 5HT2 receptor agonist) and 10 uM citalopram (a selective serotonin reuptake inhibitor) with an increase in input resistance, a reduction of I-on and an increased AHP duration. Our preliminary results show H-I affected motor neurons are less responsive to 5HT2 receptor activation than controls. However, since these properties (input resistance, I-on and AHP) are all altered in the baseline measurements of control vs H-I motor neurons, this suggests H-I motor neurons are influenced by higher basal levels of serotonin. Thus lumbar motor neurons from H-I affected rabbits show baseline properties that align with increased serotonergic tone. In conclusion, lumbar motoneurons from H-I affected rabbits show baseline properties that align with increased serotonergic tone, and are less responsive to exogenous 5HT2 agonist application. Future work will verify current results and test sensitivity of H-I affected motoneurons to inhibition of serotonergic receptors.

Disclosures: K.A. Quinlan: None. **A. Drobyshevsky:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.01/P4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: United States Public Health Service grant DA000266

Title: Inositol polyphosphate multikinase is a regulator of transsulfuration pathway

Authors: *R. TYAGI, S. H. SNYDER, B. D. PAUL;
The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Inositol polyphosphate multikinase (IPMK) is one of the members of inositol phosphate kinase family that generates inositol polyphosphates. IPMK possesses inositol phosphate kinase (IP3-kinase) as well as phosphatidylinositol kinase (PI3-kinase) activities. IPMK is a pleiotropic protein and non-catalytically regulates mammalian target of rapamycin

complex 1 (mTORC1), serum response factor, p300, and tumor suppressor protein p53. We report that IPMK regulates expression of cystathionine γ- lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST), enzymes involved in Hydrogen sulfide production. Protein levels of CSE and MST are increased in IPMK null fibroblasts as compared to wild type fibroblast cells. Regulation of CSE and MST is independent of catalytic activity of IPMK. IPMK regulates CSE expression at the transcriptional level. Since cystathionine β-synthase (CBS) is expressed at relatively lower levels as compare to CSE in fibroblasts, CSE is the major source of cysteine generation from cystathionine in mouse fibroblasts. Lysates prepared from IPMK null fibroblasts produced more cysteine as compared to wild type cells. Hydrogen sulfide levels are also increased in IPMK null fibroblasts. Expression of CSE is inducible upon condition such as lipopolysaccharides (LPS), and inflammation mediated by tumor necrosis factor α (TNF-α). We propose that IPMK acts as a repressor of CSE expression.

Disclosures: **R. Tyagi:** None. **S.H. Snyder:** None. **B.D. Paul:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.02/P5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MRC Programme Grant

ERC Consolidator Award, PAROSIN

Title: Investigating the function of TLDc proteins in the oxidative stress response and neurodegeneration

Authors: ***P. L. OLIVER**, M. J. FINELLI, K. X. LIU, Y. WU, K. E. DAVIES;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Oxidative stress is a pathological feature of many neurological disorders; therefore, utilizing proteins that are protective against such cellular insults is a potentially valuable therapeutic approach. Oxidation resistance 1 (OXR1) has been shown previously to be critical for oxidative stress resistance in neuronal cells; deletion of this gene causes neurodegeneration in mice, yet conversely, overexpression of OXR1 is protective in cellular and mouse models of amyotrophic lateral sclerosis (ALS). Interestingly, specific isoforms of OXR1 are located to the mitochondrial membrane; however, the molecular function of this protein is unclear. OXR1 contains the Tre2/Bub2/Cdc16 (TBC), lysin motif (LysM), domain catalytic (TLDc) domain, a

motif present in a family of proteins including TBC1 domain family member 24 (TBC1D24), a protein mutated in a range of disorders characterized by epilepsy, hearing loss, and neurodegeneration. The TLDc domain is highly conserved across species, although the structure-function relationship is unknown. To understand the role of this domain in the stress response, we have carried out systematic analysis of all mammalian TLDc domain-containing proteins, investigating their expression and neuroprotective properties in parallel. In addition, we have performed a detailed structural and functional study of this domain in which we identified key residues required for its activity. Our data demonstrate that the integrity of the TLDc domain is essential for conferring neuroprotection, an important step in understanding the functional significance of all TLDc domain-containing proteins in the cellular stress response and disease. We are currently investigating novel interactors common to all TLDc domain-containing proteins to understand how they regulate oxidative stress.

Disclosures: P.L. Oliver: None. **M.J. Finelli:** None. **K.X. Liu:** None. **Y. Wu:** None. **K.E. Davies:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.03/P6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: N-Acetyl-L-Cysteine attenuates Porphyromonas gingivalis-induced inflammatory response and cell death in murine brain endothelial cells

Authors: *V. CHAROENSAENSUK[1], K.-L. OU[1,2,3], L.-Y. YANG[4];
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Abstract: Several epidemiologic studies have reported that *Porphyromonas gingivalis* (*P.gingivalis*), a major pathogen of periodontal diseases, increases the risk of having cardiovascular and cerebrovascular diseases including stroke. However, the cellular and molecular mechanisms underlying the *P.gingivalis*-induced inflammatory response as well as death of endothelial cells and the relation between the periodontal diseases and the cerebrovascular diseases remain poorly understood. In this study, we investigated the effect of *P.gingivalis* infection on the inflammatory response and cell death in murine brain endothelial cells. Live *P.gingivalis* of different multiplicity of infection (MOI) was used to infect bEnd.3 mouse brain endothelial cells for 90 minutes. Twenty-four hours after the infection, cell viability

was assessed by MTT assay and Trypan Blue Dye staining. Nuclear condensation of cells was also examined by DAPI staining and the cytokine expression of cells was evaluated by Western Blot analysis. In separate experiments, bEnd.3 cells were pretreated with N-Acetyl-L-Cysteine (NAC), an antioxidant, at different concentrations for 2 hours before the *P.gingivalis* infection. Our results indicated that live *P.gingivalis* caused the death of bEnd.3 cells in an MOI-dependent manner. The DAPI staining experiment showed that brain endothelial cells after *P.gingivalis* infection underwent apoptotic cell death, manifested by the nuclear condensation following DAPI staining. *P.gingivalis* infection induced an increased expression of interleukin-1 beta (IL-1β) in an MOI-dependent manner. Pre-treatment with NAC significantly attenuated *P.gingivalis*-induced cell death, which was accompanied by the decreased expression of IL-1β. Our findings demonstrate that *P.gingivalis* causes cell death and promotes the expression of IL-1β in murine brain endothelial cells. Furthermore, antioxidant NAC attenuates the *P.gingivalis*-induced cell death and expression of IL-1β, strongly suggesting that *P.gingivalis* causes the inflammatory response and cell death through an oxidative stress pathway.

Disclosures: **V. Charoensaensuk:** None. **K. Ou:** None. **L. Yang:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.04/P7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NCTR/FDA

Title: Sevoflurane-induced changes in gene expression in developing monkey brain

Authors: ***F. LIU**, T. A. PATTERSON, M. G. PAULE, W. SLIKKER, Jr., C. WANG;
Natl. Ctr. For Toxicological Research/FDA, Jefferson, AR

Abstract: Sevoflurane, a volatile anesthetic, is frequently used during general anesthesia. However, its safety in pediatric use is of growing concern. The present study evaluated whether prolonged exposure of infant monkeys to a clinical concentration of sevoflurane is associated with any adverse effects on the developing brain using DNA microarray analysis. Infant monkeys were exposed to 2.5% sevoflurane (a concentration which produces anesthesia) for 9 hours. Afterwards, brain frontal cortex was collected and RNA was extracted from the tissue. Gene expression profiling was performed using the Agilent M. Mulatta (Rhesus) Oligo Microarray platform containing 43,663 probes. The gene expression data from 4 sevoflurane-exposed and 4 control monkeys were analyzed using GeneSpring GX with quantile

normalization. Using the inclusion criteria of greater than a 1.5 fold-change in expression (either up or down) and a *p*-value<0.05, 576 differentially expressed genes (DEGs) were identified: 303 genes were up-regulated and 273 genes were down-regulated in the sevoflurane-exposed monkey brains. These genes were loaded into an Ingenuity Pathway Analysis database for pathway, disease and biological function, and network analyses. The results indicated that the DEGs contributed significantly to nervous system development and functions; neurological diseases; cell death and survival; and cell cycling. Moreover, a considerable number of DEGs were associated with networks of lipid metabolism, molecular transport and small molecule biochemistry. Findings from the microarray analysis have provided evidence to further evaluate the mechanisms that underlie sevoflurane-induced neural damage during development (Supported by NCTR/FDA and CDER/FDA).

Disclosures: **F. Liu:** None. **T.A. Patterson:** None. **M.G. Paule:** None. **W. Slikker:** None. **C. Wang:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

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Program#/Poster#: 314.05/P8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT Grant 241655 to PDM

Title: Participation of MAPK signaling pathway in a model of neuronal degeneration in rat striatal

Authors: *R. A. SANTANA MARTINEZ[1], D. BARRERA OVIEDO[2], P. D. MALDONADO JIMÉNEZ[1];
[1]Natl. Inst. of Neurol. and Neurosurg., Mexico, Mexico; [2]Pharmacol., Facultad de Medicina, Mexico City, UNAM, Mexico

Abstract: Quinolinic acid (QUIN) is an endogenous metabolite from kynurenine pathway, which acts as a competitive agonist on NMDAr and its intrastriatal administration to rats has been used to reproduce some biochemical, behavioral and morphological alterations similar to those observed in some chronic-degenerative disorders. QUIN induces selective neuronal death on medium spiny neurons in the striatum, which has been related to an oxidative microenvironment. It has been checked that alterations of oxidative stress-depended signaling pathway are related with the physiopathology of some brain diseases. Oxidative stress activates in some cases MAPK signaling to further the survival cell, but also the activation of the same

pathway, promotes cell death. MAPK activation must be finely regulated in time and intensity, but its study in vivo is scarce. MAPK activation- dependent survival cell has been related to suitable levels of neurotrophic, whose post-mortem deficit has been associated to death cell in the striatum. In this study, we evaluate the participation of MAPK activation in the cell death in the striatum induced with QUIN. Animals were intrastriatally infused with QUIN (30, 60, 120 and 240 nmol/µl). Right striatum was dissected at 2 h, 24 h and 7 days after QUIN injection. MAPK levels were detected by western blot and IHC. Histological analysis was done by H&E and FJ-B at 7 days after QUIN injection. Motor evaluation was done 6 days after operation. In all experiments SSI was used as vehicle in the control group. We found that QUIN (120 and 240 nmol/µl) significantly increased the activation of pathways related to death cell (p-JNK y p-p38) at 7 days. Also, QUIN decreased the activation of pathways that promote survival cell (p-ERK1/2). The sustained activation of p-JNK with QUIN 120 nmol from 2 h to 7 days could be involved in the onset of morphological alterations observed up to 7 days. Moreover, cell death in the striatum could also be due to decreased levels of BDNF, which regulates the function of adult neurons. The decrease of preserved cells may be the cause of the deficit in motor evaluation with higher doses with QUIN. These data suggest that activation of MAPK related to cellular death could be participating in the mechanism of damage of QUIN in the striatal cells of rats.

Disclosures: R.A. Santana Martinez: None. **D. Barrera Oviedo:** None. **P.D. Maldonado Jiménez:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

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Program#/Poster#: 314.06/P9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit Review Award 5l101BX001117

NIH Grant 5R01NS050730

NIH Grant R01NS088058

Title: NADPH oxidase-2 and inflammasomes after traumatic brain injury

Authors: *M. W. MA[1,3], J. WANG[1,3], K. M. DHANDAPANI[2,3], D. W. BRANN[1,3]; [1]Neurosci. and Regenerative Med., [2]Neurosurg., Med. Col. of Georgia - Augusta Univ., Augusta, GA; [3]Charlie Norwood VA Med. Ctr., Augusta, GA

Abstract: Traumatic brain injury (TBI) is the leading cause of death in young adults and contributes to nearly a third of all injury related deaths. Oxidative stress and inflammation both play key roles in the pathology following TBI. The membrane enzyme NADPH oxidase (NOX) is a major contributor to the oxidative stress, and inflammasomes have been linked to the development of inflammatory events following brain injury. Though NOX inhibition reduces injury severity post-TBI, its protective mechanism is still unclear. Our current study evaluates the role of the major NOX isoform, NOX2, in inflammasome activation after TBI. Wild type and NOX2 knockout mice underwent focal TBI. The expression of Nod-like receptor 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), and inflammasome products cleaved caspase-1 and interleukin-1β (IL-1β) were assessed in the cerebral cortex at various time points after TBI. We found that ASC protein expression increased significantly at 4 days post-TBI in the WT mice. This increased ASC expression was attenuated in NOX2 KO mice and with NOX2 inhibitor (apocynin) treatment. Furthermore, ASC and IL-1β mRNA expression was also significantly reduced in the NOX2 KO mice as compared to WT mice. Immunostaining of injured WT mice also showed increased cleaved caspase-1 and NLRP3 expression, which was attenuated in NOX2 KO mice. These results suggest a key role for NOX2-dependent oxidative stress in the induction of inflammasomes after TBI.

Disclosures: **M.W. Ma:** None. **J. Wang:** None. **K.M. Dhandapani:** None. **D.W. Brann:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.07/P10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of diferuloylmethane on egg-laying behaviour in hydrogen peroxide-exposed *Caenorhabditis elegans*

Authors: M. MENDOZA-MAGAÑA, M. MALDONADO-RUBIO, M. GALLEGOS-SAUCEDO, S. NERY-FLORES, A. CASTILLO-ROMERO, M. RAMIREZ-HERRERA, G. CAMARGO, *L. HERNANDEZ;
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Abstract: Background. High levels of reactive oxygen species (ROS) produces Oxidative stress (OS) which cause injuries in cell components (lipids, proteins and DNA). Thus, the OS has been associated with several diseases, including neurodegenerative and psychological diseases. Particularly, depression and anxiety disorders, linked to Serotonin and Dopamine levels, could

be associated with OS. It has been proposed that the damage associated with ROS, might be improved by interventions that increase resistance to OS. The use of naturally occurring substances, such as vitamins, phenols and essential oils, might be a cost-effective option. In this sense, a useful approach could be the phytochemical Diferuloylmethane (DFM), a polyphenolic compound with well documented antioxidant properties against certain reactive oxygen species such as hydroxyl groups, peroxides and superoxide radicals. Several studies in relation to oxidative stress have been conducted in the nematode *Caenorhabditis elegans* (*C. elegans*). The *C. elegans* is one of the simplest organisms with a nervous system that performs many functions featured by the nervous systems of more complex organisms and often is used as a model to help understand the basic mechanisms which complex behaviours are built. Particularly, *C. elegans* has the components of the serotonergic and dopaminergic systems and their activity can be studied through the stereotyped egg-laying behavior. Additionally, the *C. elegans* possess cellular and molecular mechanisms of oxidative stress response similar to those existing in humans. **Objective.** We investigated the effect of hydrogen peroxide on Serotoninergic and Dopaminergic system through egg-laying behavior, and we evaluated if this noxious effect could be counteracted by DFM. **Methodology:** WT (Bristol N2) strain of *C. elegans*, adult age synchronized and cultured in NMG-agar plates seeded with E. coli OP50, were used in this study. They were transferred to 12-well plates and were exposed to different concentrations of H_2O_2. Then, worms were relocated to Agar-NMG plates with food, and after 1 hour, the eggs laid were counted in each group of worms. This protocol was repeated by adding DFM as antioxidant. **Preliminary Results and Conclusion:** H_2O_2 exposure decreased the egg-laying in C. elegans, so probably the oxidative damage by H_2O_2 caused a dysfunction of serotonergic activity. For its part, DFM partially reverted the damage caused by the H_2O_2 exposure. These results suggest that DFM, at least in part, protected against damage oxidative induced to neurotransmission systems involved in *C. elegans* egg-laying, mainly the serotonergic pathway.

Disclosures: **M. Mendoza-Magaña:** None. **M. Maldonado-Rubio:** None. **M. Gallegos-Saucedo:** None. **S. Nery-Flores:** None. **A. Castillo-Romero:** None. **M. Ramirez-Herrera:** None. **G. Camargo:** None. **L. Hernandez:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.08/P11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Crispr/Cas9 knockout demonstrates a key role for Bid as a molecular link between paradigms of ferroptosis and mitochondrial death pathways in neuronal cells

Authors: *A. JELINEK, S. NEITEMEIER, L. HOFFMANN, G. GANJAM, C. CULMSEE; Inst. of Pharmacol. and Clin. Pharm., Philipps-University of Marburg, Marburg, Germany

Abstract: Targeted genome engineering by CRISPR/Cas9 is an evolving tool for generating specific knockout cell lines by DNA cleavage and introduction of indel mutations. In this study, we exploited this tool to generate a Bid (BH3-interacting domain death agonist) knockout cell line in neuronal HT-22 cells. Bid determines regulated cell death in paradigms of oxidative glutamate toxicity (oxytosis) in neurons, where its activation and mitochondrial translocation mediates mitochondrial damage, subsequent release of Apoptosis inducing factor (AIF) and cell death. In the present study we generated a Bid CRISPR/Cas9-knockout cell line to elucidate the potential role of Bid in a model of ferroptosis in HT-22 cells. Ferroptosis has recently been characterized as an iron-dependent form of oxidative stress induced cell death and involves cystine/glutamate antiporter (X_c^-) inhibition, impairment of GpX4 (glutathione peroxidase 4) activity and subsequently increased lipid peroxidation. Until today, however, the key mechanisms mediating cell death as a consequence of ROS accumulation and potential involvement of mitochondrial death pathways in paradigms of ferroptosis remain to be investigated.

We induced ferroptosis with erastin in wild-type cells and analyzed the effects of the established inhibitors ferrostatin-1, liproxstatin-1 and the Bid inhibitor BI-6c9 on paradigms of mitochondrial parameters compared to the effects in Bid-knockout cells. BI-6c9 inhibited erastin-induced morphological changes and also prevented cell death. Similar protective effects were achieved in a concentration-dependent manner with ferrostatin-1 and liproxstatin-1. FACS analysis demonstrated that the applied inhibitors abolished lipid peroxide formation and reduced mitochondrial ROS production in conditions of erastin-induced ferroptosis and glutamate-induced oxytosis. Confocal imaging of dsRed-Bid and mito-GFP transfected cells further demonstrated the inhibitory effect of both, BI-6c9 and ferrostatin-1 on Bid translocation to the mitochondria in this model system. Investigating the CRISPR/Cas9-Bid-knockout HT-22 cell line revealed that Bid knockout prevented lipid peroxidation and the impaired mitochondrial respiration as well as mitochondrial ROS formation and loss of mitochondrial membrane potential, and cell death upon X_c^--inhibition by erastin.

In conclusion, the present study exposes the mitochondrial transactivation of Bid as a key molecular link between oxidative stress and mitochondrial pathology in the model of ferroptosis considerably resembling major characteristics of cell death in paradigms of oxytosis in neuronal cells.

Disclosures: **A. Jelinek:** None. **S. Neitemeier:** None. **L. Hoffmann:** None. **G. Ganjam:** None. **C. Culmsee:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.09/P12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The pro-survival pathway of sigma-1 receptor-zinc finger protein 179 guards against hydrogen peroxide-induced cellular damage.

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Abstract: The accumulation of reactive oxygen species (ROS) is associated with several diseases such as neurodegenerative disorders, traumatic brain injury and stroke. For this reason, reducing the accumulation of ROS in neurons is crucial in the treatment of those diseases. This study aimed to investigate the neuro-protective functions of sigma-1 receptor (Sig-1R), a chaperon residing at the endoplasmic reticulum, and the zinc finger 179 (Znf179), a brain protein whose function is mostly unknown. We established the model in which the ROS accumulation was triggered by hydrogen peroxide (H_2O_2) and the resultant apoptosis signaling pathway initiated by H_2O_2 was also verified. We found that the Sig-1R agonists, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), greatly reduced the activation of apoptotic pathways. By performing protein-protein interaction assays and shRNA knock-down of Sig-1R, we identified that the Znf179 is a downstream target of Sig-1R. Further, overexpression of Znf179 produced a neuroprotective effect just like that caused by DHEA and likewise being mediated the antioxidant mechanism. Henceforth, we examined the amount of peroxiredoxin 3 (Prx3) and superoxide dismutase 2 (SOD2) in the hippocampi of wild-type and Znf179 knockout mice, and found that both enzymes are reduced in the knockout when compared to wild-type mice. In conclusion, this study demonstrated a novel role of Znf179 as a downstream mediator of the neuro-protective action of Sig-1R against neurodegenerative and neuro-traumatic diseases.

Disclosures: **T. Su:** None. **P. Lee:** None. **S. Yeh:** None. **T. Hsieh:** None. **S. Chou:** None. **J. Hung:** None. **W. Chang:** None. **Y. Lee:** None. **J. Chuang:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.10/Q1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACyT Grant 241655

Title: Evaluation of the Nrf2 activation in a no canonical pathway. Participation of DPP3 protein

Authors: *C. A. SILVA[1], P. D. MALDONADO[2];
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Abstract: The transcription factor Nrf2 is the master regulator of the cellular antioxidant responses and the cells counteract the oxidative stress by activation of Nrf2. The canonical pathway of Nrf2 activation involves the oxidation of the some Keap1 cysteins residues (protein that negative regulates Nrf2 by retains Nrf2 in the cytoplasm), phosphorylation of serine 40 in Nrf2, subsequent dissociation of the complex Keap1-Nrf2 and the Nrf2 translocation to the nucleus. However, a not canonical pathway has been reported in vitro recently. This pathway involves the disruption of Keap1-Nrf2 complex by direct interaction of some proteins with Keap1 or Nrf2, like p62, DPP3, p21 and others; however, there have been few studies *in vivo*. It has been reported that quinolinic acid (QUIN) is a selective agonist of NMDAr is capable to induce an oxidative/nitrosative state in the cell, so its intrastriatal administration has been used as an excitotoxic/pro-oxidant model to study both events. Unexpectedly, we found that QUIN administration increase the Nrf2 activation 30 min after to injury without increase of ROS production. In this work we propose to evaluate the effect of different doses of QUIN on Nrf2/Keap1 interaction, and study the participation of some proteins DPP3 and p62 on this interaction, in the rat striatum in an in vivo model. We administrated 1 μL of isotonic saline or QUIN (15, 30, 60, 120 and 240 nmol) in the right striatum of male Wistar rats (260-300 g) and then we sacrificed the animals at 30 min after injection. The Nrf2, Keap1, p62 and DPP3 levels was measured by western blot, the oxidative stress level was evaluated by the GSH/GSSG ratio finally we evaluated the Keap1 and DPP3 protein interaction by IP. The total amount of p62 and DPP3 proteins shows no change with all doses of QUIN, whereas total Keap1 increased 30 min after the QUIN administration in a dose-response manner. The GSH/GSSG ratio showed not changes with all doses of QUIN however the interaction of Keap1 and DPP3 increases in a dose-response manner. These results suggest that at 30 min, the activation of Nrf2 could be associated with the Keap1-Nrf2 disruption by DPP3 and oxidative stress independent.

Disclosures: C.A. Silva: None. **P.D. Maldonado:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.11/Q2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: by Ege University Scientific Research Foundation 13/TIP/003

Title: Manganese induced toxicity can be attenuated by VIP in NE 4C cell line

Authors: *T. DAGCI[1], G. ARMAGAN[2], S. BORA[3], A. ERDOGAN[3];
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Abstract: Vasoactive intestinal peptide (VIP), a peptide neurotransmitter released by GABAergic interneurons in the dentate gyrus, has a role in modulating neural stem/progenitor cells. VIP has beneficial effects in murine models of inflammatory diseases including septic shock, rheumatoid arthritis, multiple sclerosis and Crohn's disease. Manganese (Mn) is an essential metal in the organism. It has many roles in metabolic processes in the biological system. Increased concentration of Manganese in the brain is known to be associated with excitotoxicity and neuroinflammation.
The aim of this study was to examine the effects of VIP against manganese toxicity in NE-4C cell line. Neural stem cells are considered to be an appropriate study model to assess toxic effects of various agents since they are cells present in both developing nervous systems and also in the adult brain. The cytotoxicity was measured by lactate dehydrogenase assay and apoptotic/antiapoptotic protein levels were analyzed using Western Blot techniques following treatments.
In this study, significant reduction in LDH release was observed following VIP treatments when compared with Mn-treated cells. Similarly, proapoptotic protein bax and ROS production significantly decreased in cells after incubation with VIP in the presence of Mn ($p<0.001$). However, an increase in bcl-2 levels that was not statistically significant was observed in the group that was treated with Mn and VIP. Our study provides the evidence that VIP may exert protective effects via modulating oxidative stress in Mn induced neurodegeneration in NE-4C cells. The assessment of the impact of potential neuroprotective compounds against Mn toxicity would be important for novel drug discovery.

Disclosures: **T. Dagci:** None. **G. Armagan:** None. **S. Bora:** None. **A. Erdogan:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.12/Q3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Genetic loss of CYLD exerts neuroprotective effects against RIP-1 mediated necroptosis *In vitro* and after experimental traumatic brain injury

Authors: *N. A. TERPOLILLI[1,2], G. K. GANJAM[3], S. DIEMERT[3], I. EISENBACH[3], L. HOFFMANN[3], C. REUTHER[3], N. PLESNILA[2], C. CULMSEE[3];
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Abstract: Background
The tumor suppressor deubiquitinase CYLD is as a key regulator of NF-kB activity, a transcription factor that promotes neuronal survival after brain injury. In addition, CYLD was recently linked to pathways of death receptor-mediated necroptosis, a form of programmed cell death. In the present study we sought investigated the role of CYLD in a model of glutamate-induced oxidative damage *in vitro* and in an model of experimental traumatic brain injury (TBI) *in-vivo*.
Results
CYLD depletion enhances receptor interacting protein 1 (RIP1)-ubiquitination after induction of oxidative stress in HT 22-cells and primary cultured neurons thereby preventing the formation of the RIP1-RIP3 necroptosome formation. mitochondrial damage andcell death. In vivo, genetic loss of CYLD significantly attenuated secondary lesion expansion and intracranial hypertension 24h after trauma. Brain water content was significantly reduced by 39% in the traumatized hemisphere of CYLD knock-out animals as compared to wild type littermates at this time point. 7 days after trauma lesion volume was reduced by trend in CYLD transgenic mice, neurological outcome, however, was not altered.
Summary and conclusion
Our results suggest that CYLD is a mediator of RIP1/RIP3-dependent necroptosis in paradigms of oxidative stress, acting upstream of mitochondrial demise. The present findings imply that detrimental CYLD activation occurs independently of death receptor stimulation thus suggesting a novel pathway of CYLD-dependent necroptosis triggered by oxidative stress. As genetic loss of CYLD resulted in a significant reduction of posttraumatic brain damage, CYLD dependent RIB1/RIP3 dependent necroptosis seems to play an important role after TBI; CYLD may therefore represent a novel target for the development of neuroprotective strategies applicable in acute brain trauma.

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Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.13/Q4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of copper on the activity of kynurenine pathway enzymes in rat cortex slices

Authors: *D. RAMÍREZ ORTEGA[1], I. I. LUNA PRIETO[2], D. F. GONZALEZ ESQUIVEL[2], B. PINEDA[3], C. RIOS[2], V. PEREZ DE LA CRUZ[2];
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Abstract: Copper is a heavy metal and an integral component of various enzymes. It is necessary for some biological functions and mitochondrial respiration; however, excess copper is neurotoxic and has been implicated in neurodegenerative diseases as Alzheimer. This metal has consequences in cellular state, for example modifies the cellular redox environment which in turn, influences various cellular functions. On another hand, tryptophan degradation through kynurenine pathway (KP) is also modulated by the redox environment and some KP metabolites possess redox properties as L-kynurenine (L-KYN), kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK) which are produced by indolamine 2-3 dioxigenasa (IDO), kinurenine aminotransferase (KAT) and kynurenine 3-monoxygenase (KMO), respectively. The imbalance in the production of these kynurenines is related with aging and some pathologies. The aim of this study was to evaluate the effect of copper on IDO, KAT and KMO activities and its correlation with oxidative stress parameters. To accomplish these objectives, we first evaluated copper toxicity (0-500 uM) through MTT reduction, reactive oxidative species (ROS) production, lipid peroxidation (LP) and cell death on rat cortex slices. Then, IDO, KAT and KMO activities were evaluated in rat cortex slices after 2h of incubation with copper (0-500 uM). Our results showed that $CuSO_4$ decreased MTT reduction in a concentration-dependent manner, increased ROS, LP and cell death. After 2 h of incubation, $CuSO_4$ drastically abolished IDO activity, and KAT activity also was reduced; while increased KMO activity. Our data suggest that copper affects redox balance and alters KP metabolism, which is very significant in diseases with common factors as copper, KP metabolites and oxidative damage.

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Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.14/Q5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG044817

NIH Grant AG051266

Alzheimer Association Grant ZEN-14-283969

BrightFocus Foundation Grant A2015275S

Title: Pro-inflammatory and oxidative stress mechanisms elicited by Aβ in astrocytic/glial cells: their impact for the integrity of the neurovascular unit

Authors: *A. A. ROSTAGNO, S. COCKLIN, J. GHISO;
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Abstract: Vascular deposition of amyloid (cerebral amyloid angiopathy, CAA) is an almost universal feature in Alzheimer's disease and the primary cause of cerebral haemorrhage in early onset familial cases bearing specific mutations in the Aβ peptide. CAA narrows vessel lumen and changes vessel architecture, impairing cerebral blood flow and generating hypoxic/ischemic conditions that negatively impact in the metabolic regulation and function of the neurovascular unit. In this line, current findings highlight a previously unrecognized functional and pathogenic synergy between neurons, glia and vascular cells, providing a new framework to reevaluate how alterations in cerebral blood vessels contribute to the neuronal dysfunction. The complexity of the cellular mechanisms elicited by amyloid in astrocytic/glial cells as well as their relationship to the induction of pro-inflammatory conditions capable of affecting microvessel function/permeability remain to be fully elucidated. We aimed to provide insight into the compromised molecular pathways and identify potential new targets for therapeutic intervention. Astrocytic/glial cells were challenged with wild-type Aβ and the AβE22Q Dutch vasculotropic variant associated with cerebrovascular deposition and a hemorrhagic clinical phenotype. A combination of FACs-analyzed bead arrays, ELISA, zymography, and confocal studies were employed to evaluate the production of pro-inflammatory cytokines, activation of MMPs, and ROS generation whereas the vitamin-E analog Trolox was tested for prevention/amelioration of these detrimental cellular pathways. Oligomeric Aβ triggered elevated production of the pro-inflammatory mediators IL-6, IL-8, and IFN-γ, enhanced activation of MMP-2, exacerbated ROS production, and cell death. In all cases, challenge with AβE22Q translated into a more pronounced response, in agreement with the high oligomerization tendency of the variant and the

aggressiveness and early onset of the clinical phenotype. Trolox not only inhibited ROS production and MMP-2 activation, but also preserved cellular integrity and viability, highlighting the primary role of ROS in the initiation of amyloid-induced cell death pathways. Our data emphasizes the detrimental role of astrocyte/glia-initiated Aβ-mediated pro-inflammatory pathways and suggests that targeting oxidative stress is a potential complementary therapeutic strategy to preserve the integrity of the neurovascular unit.

Disclosures: **A.A. Rostagno:** None. **S. Cocklin:** None. **J. Ghiso:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.15/Q6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

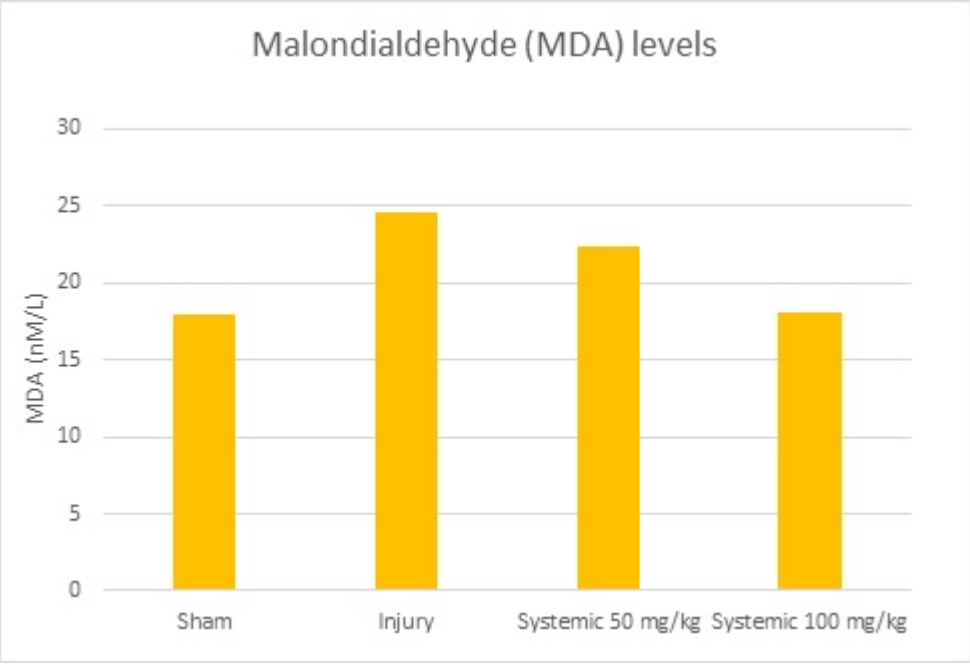
Support: American University in Cairo research grant.

Title: Anti-oxidant effects of Curcumin following spinal cord injury in rats

Authors: Y. A. ABDULLAH, S. A. EL SAYED, S. W. AZIZ, *A. A. ABDELLATIF; Biology, SSE, American Univ. in Cairo, New Cairo, Egypt

Abstract: Spinal Cord Injury (SCI) is considered a major challenge to healthcare systems. Primary mechanical injury initiates a "cascade of biological events" which constitutes the secondary injury which contributes to neurological damage and dysfunction. Lipid peroxidation is a critical process in the pathogenesis of secondary damage following SCI. In the process of lipid peroxidation, reactive oxygen species scavenge electrons from lipids. This process causes great damage to the cells as lipids are a main component of the plasma membranes, and therefore results in extensive cell damage.
Curcuma longa commonly known as Turmeric is a spice widely used in India in Ayurvedic medicine as a treatment for inflammatory conditions. The main primary active constituent of the plant is curcumin which gives it its characteristic yellow color. Curcumin has been studied for multiple potential medical uses such as, anti-cancer, antioxidant, antibacterial and anti-inflammatory (Aggrawal, 2007).
The aim of this work is to explore the antioxidant activities of Curcumin and its therapeutic potential in reducing oxidative stress in SCI model in rats.
Material & Methods: All experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals. Adult female Sprague-Dawly rats weighing approx. 150 grams were divided into the following groups: normal, sham control receiving only skin and

muscle incision with no injury to the cord tissue, an injury group that received right side hemi-section of the spinal cord at T 9-10 level, and treatment groups receiving SCI at T 9-10 level followed by the treatment with Curcumin either orally mixed with food or locally at the injury site or systemic injection of increasing doses of 50-300 mg/kg.
Total Antioxidant Capacity (TAC) & Malondialdehyde (MDA) were estimated in the cord tissue at 1 and 2 weeks post injury.
Results: Treatment with Curcumin showed a dose dependent increase in the Total Antioxidant Capacity (TAC) as well as reduction in Malondialdehyde (MDA) activity in the treatment groups when compared with control groups.



Disclosures: **Y.A. Abdullah:** None. **S.A. El Sayed:** None. **S.W. Aziz:** None. **A.A. Abdellatif:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.16/Q7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: An expanded palette of redox-sensitive fluorescent proteins with enhanced sensitivity and brightness

Authors: *B. CAMPBELL, C. LIU, G. PETSKO;
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Abstract: Oxidative stress is a ubiquitous and chronic pathological feature of neurodegenerative diseases (NDDs), but the technology available for visualizing this process is limited. Redox-sensitive fluorescent proteins (roFPs) are the most promising tools available for quantitative, long-term, and compartment-specific reporting of the intracellular redox state. However, after more than a decade since their inception, they are still restricted primarily to the green emission channel and exhibit poor photostability and low brightness. To advance this technology, we used rational and semi-random mutagenesis to produce novel cyan, green, and yellow redox sensors that surpass the capabilities of roGFP, the current gold standard. Our genetically encoded biosensors display the largest dynamic range yet reported for a roFP and exhibit enhanced maturation, brightness, and photostability. Because they are ratiometric by excitation, they can be used for quantitative analysis in living cells independent of probe expression levels, illumination intensity, and bleaching. We are currently developing orange and red variants to complement this palette, and are using these sensors to investigate the role of mitochondrial dysfunction in NDDs using multiplex imaging.

Disclosures: **B. Campbell:** None. **C. Liu:** None. **G. Petsko:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.17/Q8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of pyrophosphate thiamine on the nerve conduction speed in patients with diabetic polyneuropathy

Authors: *I. IBARRA VALDOVINOS[1], R. G. RESENDIZ GUTIERREZ[1], B. UGALDE VILLANUEVA[1], J. C. SOLIS SAINZ[1], P. GARCIA SOLIS[1], M. RAMOS GOMEZ[2], L. S. GALLARDO VIDAL[3], H. L. HERNANDEZ-MONTIEL[1];
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Abstract: Diabetic polyneuropathy pathophysiology is multifactorial and largely caused by the alteration of metabolic pathways that compromise the structural and functional integrity of peripheral nerves. These metabolic pathways include glycosylation of proteins with accumulation of advanced glycosylation products, sorbitol and hexosamines pathways, and protein kinase C. Chronic hyperglycemia increases the production of superoxide radicals, causing tissue damage. Although different drugs have been employed to inhibit pathways involved in this damage, a true benefit for this pathology has not been shown. Therefore, a good control of blood glycose is still a key factor to prevent or delay tissue damage. This work aims to evaluate the use of thiamine pyrophosphate to prevent the damage associated to diabetic polyneuropathy. Thiamine pyrophosphate belongs to a new group of powerful therapeutic agents that activate the enzyme transketolase, thus decreasing the activation of pathophysiologic metabolical pathways. The studied population will be adult patients between (30 to 60 years of age) with type 2 diabetes mellitus (5 to 10 years of evolution) with a diagnosis of diabetic polyneuropathy in lower limbs clinically determined by The Michigan Neuropathy Screening Instrument. Our hypothesis is that thiamine pyrophosphate will improve the nerve conduction velocity (NCV) by improving metabolic glycemic control as well as oxidative balance. Thus, the NCV in the same patient before the treatment will be its own control. Only patients treated with oral hypoglycemic agents that agree to participate in the project. This project has been approved by the Committee of Bioethics of the Autonomous University of Queretaro, and follows the guidelines of Helsinki. Patients will be assessed before and after the treatment by monitoring blood glycose, thiamine pyrophosphate, thiamine, fructosamine, insulin, glycosylated hemoglobin, C-peptide, endogenous antioxidant enzymes (SOD, CAT and GSH), pro-inflammatory cytokines and NCV in lower limbs. NCV will be analyzed with the international Kimura standards, by determining the change for each patient before and after treatment; thus using each subject as its own control.

Disclosures: I. Ibarra Valdovinos: None. **R.G. Resendiz Gutierrez:** None. **B. Ugalde Villanueva:** None. **J.C. Solis Sainz:** None. **P. Garcia Solis:** None. **M. Ramos Gomez:** None. **L.S. Gallardo Vidal:** None. **H.L. Hernandez-Montiel:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.18/Q9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of an endogenous interferon inducer and a promoter of energetic metabolism on the viability of a islets of langerhans hepatic portal graft in a murine model of type 1 diabetes mellitus: a new approach to neurodegeneration?

Authors: *B. UGALDE VILLANUEVA[1], I. IBARRA VALDOVINOS[1], R. G. RESÉNDIZ GUTIÉRREZ[1], E. A. LÓPEZ ARVIZU[1], M. D. ABURTO FERNÁNDEZ[1], M. RAMOS GÓMEZ[2], H. L. HERNÁNDEZ MONTIEL[3];
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Abstract: Type 1 diabetes mellitus (T1D) is a metabolic disease caused by an autoimmune reaction that destroys the pancreatic beta cells, gradually decreasing the ability to produce insulin, leading to the development of hyperglycemia. It is estimated that in 2015, around the world 542,000 children under 15 were suffering from T1D, and it is estimated an incidence of 86,000 new cases per year. In Mexico, the reported incidence up to 2010 was 6.2%, and according to the International Diabetes Federation, in our country around 13,500 children under 15 were suffering T1D in 2015. T1D treatment is based on exogenous insulin administration; however, glycemic control is often difficult due to the constant dose adjustment based on weight, making it difficult to prevent chronic complications. This has led to the constant search for new treatments to afford a better control of glycemia. One of the most promising alternatives is the graft of islets of Langerhans. This approach is used in humans, and patients have achieved independence of exogenous insulin administration for up to 5 years. However, the use of these techniques have some technical limitations as the process of islet isolation, insufficient amount of islets available for grafting, the adverse effect on the use of immunosuppressive agents and gradual loss of function as well as destruction of grafted islets, all of which limit their efficiency. In this project we will analyze the beneficial effect on the functionality of the islets of Langerhans graft by using thiamine pyrophosphate as a promoter of energetic metabolism, and an inducer of endogenous interferon in a model of T1D induced with streptozotocin (STZ). The graft will be applied through the portal hepatic vein, and our hypothesis is that the treatment will induce an improvement in the glycemic metabolic control and in the graft survival. The graft of islets will be obtained from non-treated rats through a Ficoll separation column, and the islets will be grafted into diabetic rats via hepatic portal vein. In order to confirm the induction of diabetes, glycose and C-peptide will be measured in STZ-treated rats before grafting. To determine the degree of functionality of the islets of Langerhans graft, glycose, C-peptide, antioxidant enzymes (CAT, SOD, GSH) and a histological and immunohistochemical evaluations will be performed. The use of adjuvant treatments could have a future impact on the use of grafted islets in the cerebral ventricular system in models of neurodegeneration such as Alzheimer's disease to determine the effect of insulin central administration.

Disclosures: B. Ugalde Villanueva: None. **I. Ibarra Valdovinos:** None. **R.G. Reséndiz Gutiérrez:** None. **E.A. López Arvizu:** None. **M.D. Aburto Fernández:** None. **M. Ramos Gómez:** None. **H.L. Hernández Montiel:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.19/Q10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inflammation and oxidative stress risk factors for neurodegenerative diseases in a population of patients with metabolic syndrome from queretaro, mexico.

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Abstract: Metabolic syndrome (MS) has showed an increased incidence in Mexican adult population, positioning itself as a serious health problem. According to the International Diabetes Federation (IDF), affects at least a quarter of the world's population. In Mexico, according to the latest National Health and Nutrition Survey of 2012 (ENSANUT), the prevalence of MS was 45%. At present, it is considered that insulin resistance is perceived like the main cause of MS pathophysiology, which is related to the progression and development of subsequent metabolic diseases. Other related factors are obesity, hypertension, hyperglycemia and dyslipidemia. At a cellular level, it has been shown that chronic low-grade inflammation and oxidative stress participate as risk factors in the development of MS, diabetes and several neurodegenerative disorders. This has highlighted the relationship between MS and neurodegenerative disorders, especially implicating hipoinsulinemia and insulin resistance within the nervous system. Conditions such as Alzheimer's disease, Parkinson's disease and depression have been shown to share pathophysiological disturbances with MS. Considering the increase in MS prevalence, this relationship implies a parallel increase in the number of new cases of neurodegenerative diseases. The aim of this project is to determine the presence of risk factors for neurodegenerative diseases in adult patients with MS (according to NCEP ATP III criteria), in addition to the measurement of anthropometric assessment, proinflammatory cytokines (TNF-alpha, IL-6) and endogenous antioxidant enzymes (CAT, SOD and GSH). This project will be developed according to the guidelines of the Bioethics Committee of our University and the Helsinki declaration. Our purpose is to develop a neurodegenerative disease risk profile in the MS population.

Disclosures: **R.G. Resendiz Gutierrez:** None. **B. Ugalde Villanueva:** None. **I. Ibarra Valdovinos:** None. **P. García Solís:** None. **M. Ramos Gómez:** None. **L.S. Gallardo Vidal:** None. **H.L. Hernández Montiel:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

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Program#/Poster#: 314.20/Q11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Consejo Nacional de Ciencia y Tecnología. CB.2012/183867

Title: Quinolinic acid toxicity involves mitochondrial dysfunction as an independent manner of NMDA receptor activation in astrocytes primary cultures

Authors: *J. G. REYES OCAMPO[1], A. SALAZAR-RAMIRO[2], D. GONZÁLEZ-ESQUIVEL[3], B. PINEDA[2], C. RIOS[3], V. PÉREZ-DE LA CRUZ[3];
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Abstract: Quinolinic acid (QUIN), a neuroactive metabolite of the kynurenine pathway, is normally present in nanomolar concentrations in human brain and cerebrospinal fluid (CSF) and is often implicated in the pathogenesis of a variety of human neurological diseases. QUIN is an agonist of NMDA receptor and is able to produce oxidative stress leading to cell death. The aim of this study was to evaluate whether the mitochondrial dysfunction induced by QUIN was depending of ROS production or NMDA receptor activation. We evaluated ROS levels, mitochondrial membrane potential and cellular function in astrocytes primary culture exposed to QUIN (100 μM) and/or MK-801 (1mM). QUIN induced intracellular ROS production in a time- and concentration dependent manner in astrocytes cultures. The pre-incubation with MK-801 abolished the ROS production induced by QUIN. QUIN decreases both mitochondrial membrane potential, evaluated by JC-1, and mitochondrial function evaluated by MTT reduction. However, MK-801 does not have any effect on these parameters. Considering the results obtained in astrocytes primary cultures, we evaluated the effect of QUIN on succinate dehydrogenase (SDH) activity on isolated mitochondria from different brain regions. After 1 h of incubation, QUIN (100 and 500 μM) decreased almost 50% of SDH activity in mitochondria from all regions. Our data showed that QUIN affects SDH activity, which can be related with the mitochondrial dysfunction observed in astrocytes and it is independent of NMDA receptor activation. All these evidence suggests that mitochondrion is an important target of QUIN toxicity and could be that

QUIN has a transport mechanism by which enter into cell and directly interacts with the mitochondrion.

Disclosures: **J.G. Reyes Ocampo:** None. **A. Salazar-Ramiro:** None. **D. González-Esquivel:** None. **B. Pineda:** None. **C. Rios:** None. **V. Pérez-de la Cruz:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG043970

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Title: Regulation of mitochondrial oxidative stress and protein aggregation in dopaminergic neuron degeneration in Parkinson's disease

Authors: ***H. SHI**[1], K. PARK[1], N. MILLER[1], J. R. MAZZULLI[2], Y.-C. MA[1];
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Abstract: Mitochondrial dysfunction and protein aggregation have been suggested as major contributors to midbrain dopaminergic (DA) neuron degeneration in Parkinson's disease, but the underlying mechanism remains largely unknown. In this study, we investigated a novel gene expression program controlled by DA neuron-specific transcription factor Foxa2 in regulating mitochondrial oxidative stress and protein degradation. In a mouse genetic model of Parkinson's disease, we found that loss of Foxa2 transcription factor activity contributes to DA neuron degeneration and mitochondria functional impairment. To further investigate intracellular functions modulated by Foxa2 in DA neurons, we performed ChIP-Seq analysis using induced pluripotent stem cell (iPSC)-derived DA neurons and identified novel targets of Foxa2 transcriptional regulation in mature DA neurons. Our ChIP-seq study revealed a novel gene expression program that regulates mitochondrial reactive oxygen species (ROS) production, autolysosome formation, lysosome biogenesis, and ubiquitin-mediated protein degradation. These findings suggested a critical role of Foxa2 in regulating mitochondrial function and protein degradation, two of the most critical pathways associated with the pathogenesis of Parkinson's disease.

Disclosures: **H. Shi:** None. **K. Park:** None. **N. Miller:** None. **J.R. Mazzulli:** None. **Y. Ma:** None.

Poster

314. Oxidative Stress and Cell Death

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Program#/Poster#: 314.22/Q13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DIP-251

Title: Neuron releases α-synuclein during hypoxia causes neuroinflammation and exacerbate neurodegeneration through Mac1/NOX2 pathway.

Authors: ***V. JAIN**[1], S. B. SINGH[2];
[2]Neurobio., [1]Defence Inst. of Physiol. and Allied Sci., North Delhi, India

Abstract: Cellular responses to hypoxia can be acute or chronic. Acute responses mainly depend on oxygen-sensitive ion channels, whereas chronic responses rely on the transcription factors, which up-regulate the expression of enzymes, transporters, and growth factors. Chronic hypoxia known to causes neuronal cell death in mixed neuronal culture but the role of glial cells in this pathology is still an enigma. To better understand the pathogenic mechanisms and role of glial cell, we evaluated neuronal damage, calcium homeostasis, neuroinflammation and possible signalling involved. Results from present study revealed that hypoxia exposure resulted in a delayed accumulation of intracellular calcium coupled to a decrease in the rate of calcium clearance from the cell which further causes neurodegeneration. Additionally these neurodegenerative cells exacerbate microglial activation and damage-associated molecular patterns (DAMPs) level as evident from increases level of α-synuclein level. This increased DAMPs further enhance level of integrin receptor Mac1 (also known as CD11b/CD18, complement receptor 3 [CR3], or aMb2) its downstream effector NADPH oxidase (NOX2) which further contribute to maintaining chronic neuroinflammation. This study revealed the crucial role of Mac1/NOX2 signaling in maintaining neuroinflammation and progressive neurodegeneration during chronic hypoxia stress.

Disclosures: **V. Jain:** None. **S.B. Singh:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5P20MD006988

Title: Inhibition of palmitic acid-induced cell death by blocking apoptosis, necroptosis and inducing autophagy

Authors: *M. L. MONTERO[1,2], J.-W. LIU[3], M. DE LEON[3];
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Abstract: Lipotoxicity (LTx) is triggered by lipid overload that leads to oxidative stress, cellular dysfunction and cell death. This cellular phenomenon has been associated with metabolic syndrome, obesity, and type II diabetes. Nerve growth factor differentiated pheochromocytoma 12 (NGFDPC12) cells serve as a neuronal model to study this process. Autophagy is a survival mechanism induced by starvation, growth factor deprivation, hypoxia and oxidative stress by which the cell rids itself of protein aggregates, oxidized lipids, carbohydrates and even dysfunctional organelles with the intent to maintain adequate energy levels contributing to cellular homeostasis. Previous work in our laboratory showed that high concentrations of Palmitic Acid (PA) induces cell death mainly by apoptosis. When apoptosis is inhibited by caspases inhibitor, cell death still goes on in a caspase independent manner known as necroptosis. In this study, we found that PA-LTx also decreased the expression and activation of the vesicle docking protein LC3 (Atg8) that is crucial for autophagosome formation during autophagy. Therefore, using a caspase inhibitor, ZVAD-fmk, in conjunction to a necroptosis inhibitor, Necrostatin-1, and an autophagy inducer, Rapamycin, we are able to increase cell viability during PA-LTx. The apoptotic process can be measured using the split pattern of nuclear proteins Topoisomerase I (Topo I, 100kDa) and Poly (ADP-ribose) polymerase (PARP, 110kDa) by Western blots. There is a specific cleavage product catalyzed by caspases of 70kDa and 85 kDa for Topo I and PARP, respectively. On the other hand, there are specific cleavage products catalyzed by cathepsins; 70 and 45 kDa for TopoI and 50 kDa for PARP. Using these markers we identify the cell death mechanism taking place in our experimental conditions. Our laboratory has also demonstrated that docosahexaenoic acid (DHA) reverses PA-LTx-induced cell death in NGFDPC12 cells. Our results show that DHA inhibits caspases activation and the 70kDa and 85kDa split products of Topo I and PARP respectively. Moreover, DHA activated the autophagy cascade. We found increase mRNA levels of autophagy related genes like Atg7 and Atg12. Also DHA increased the active form of LC3 as measured by Western blot. Our data suggest that DHA rescuing of PA-LTx-induced cell death is through inhibition of apoptosis

and/or necroptosis. At the same time induction of autophagy gives the cell the capacity to cope with excess formation of ROS, lipid peroxidation and protein malfunction and provides energy sources for ATP formation.

Disclosures: **M.L. Montero:** None. **J. Liu:** None. **M. De Leon:** None.

Poster

314. Oxidative Stress and Cell Death

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Program#/Poster#: 314.24/R1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH RO1-NS076772

Title: Multiple sources contribute to extracellular H_2O_2 dynamics in the striatum

Authors: ***S. PANDA**, L. R. WILSON, A. C. SCHMIDT, L. A. SOMBERS;
Chem., North Carolina State Univ., Raleigh, NC

Abstract: Oxidative stress has been implicated as a key player in various neuropathologies such as Parkinson's disease and Alzheimer's disease. A variety of cellular processes are involved in the generation and accumulation of extracellular reactive oxygen species, such as hydrogen peroxide (H_2O_2), in the striatum; however, the extent to which each of these sources contributes to extracellular H_2O_2 dynamics in striatal tissue remains unknown. Potential sources of H_2O_2 in the striatum include mitochondrial activity, and the biosynthesis and metabolism of dopamine (DA). The goal of this project is to quantitatively investigate key contributors to striatal H_2O_2 fluctuations using fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes. Pharmacological agents are used to manipulate (1) DA synthesis (L-DOPA, 100 mg/kg), (2) DA metabolism (pargyline, 75 mg/kg), and (3) the mitochondrial electron transport chain (local infusion of rotenone, 197 pg; or sodium succinate, 243 pg). Striatal H_2O_2 dynamics are voltammetrically quantified in real time using a dual microelectrode device. This device consists of two carbon fiber microelectrodes, one of which is coated with an m-phenylenediamine (mPD) membrane - a size exclusion membrane that enables selective detection of H_2O_2. The uncoated microelectrode is used to simultaneously monitor the effects of these drugs on local dopamine dynamics. The results indicate that each of these processes is contributing to the generation of extracellular H_2O_2 in the striatum. Overall, this work sheds light on the potential for these pathways to contribute to oxidative stress in this critical brain region.

Disclosures: **S. Panda:** None. **L.R. Wilson:** None. **A.C. Schmidt:** None. **L.A. Sombers:** None.

Poster

314. Oxidative Stress and Cell Death

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 2R23GM060507

NIH Grant 5P20MD006988

Title: Structure and function analysis of epidermal fatty acid binding protein in nerve growth factor-differentiated PC12 cells

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Abstract: Epidermal fatty acid-binding protein (E-FABP/FABP5/DA11) binds and transports long-chain fatty acids in the cytoplasm and protects neuronal cells from reactive oxygen species (ROS) and cell death after palmitic acid-induced lipotoxic injury (PAM-LTx). However, the mechanism of protection from PAM-LTx remains unclear. Current understanding suggests E-FABP functions as an antioxidant protein against ROS through covalent modification of Cys-120 residue. Additionally E-FABP could function by binding free fatty acids in the cytoplasm rendering fatty acids unavailable for lipid peroxidation. The amino acids responsible for binding of fatty acids in EFABP binding pocket are Arg^{109}, Arg^{129}, and Tyr^{131}; furthermore, Arg^{129} forms a salt bridge with the carboxylate group of fatty acids that may be essential for stabilizing fatty acid binding. Therefore, we sought to create a mutant E-FABP that significantly decreased binding affinity to palmitic acid and examine whether the mutant retained protection against ROS in nerve growth factor-differentiated PC12 cells (NGFDPC12 cells). The use of this mutant will allow for the examination of E-FABP antioxidant properties that are dependent or independent of fatty acids binding to E-FABP. We hypothesize mutant E-FABP-R129A will significantly decrease binding affinity to palmitic acid while retaining antioxidant function. Isothermal titration calorimetry demonstrates that recombinant rat E-FABP-R129A clearly exhibits significantly reduced binding to palmitic acid verses wild type E-FABP. Furthermore, we found NGFDPC12 cells are protected against ROS when recombinant E-FABP-R129A is delivered by BioPORTER® QuikEase™ Protein Kit. These findings suggest that E-FABP antioxidant function is not dependent on fatty acid binding, and support roles of this protein beyond functioning as intracellular fatty acid transporter.

Disclosures: A. Duran: None. **J. Liu:** None. **M. Deleon:** None.

Poster

314. Oxidative Stress and Cell Death

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 314.26/R3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Anti-inflammatory actions of heme oxygenase mediated by binding and down- regulating nitric oxide synthase

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Abstract: Nitric oxide (NO) and carbon monoxide (CO) are synthesized from L-arginine and heme by the catalytic actions of NO synthase (NOS) and heme oxygenase (HO), respectively. NO and CO similarly activate soluble guanylate cyclase but also display notable differences. It is unclear whether the two gasotransmiters converge or exhibit reciprocal feedback regulation. Macrophage-inducible NOS generates NO to kill other cells via nitrosative burst, whereas HO1 synthesizes bilirubin, which exerts antioxidant cytoprotective effects and also provides cytoprotection by facilitating iron extrusion from cells. We observe coordinated reciprocal pro- and anti-inflammatory activity in macrophage cells stimulated by LPS/IFN-γ. A sudden surge of cyclooxygenase 2 activity occurs (as early as 4 h post treatment) followed by inducible NOS expression (6 h post treatment) ultimately activating heme oxygenase 1 (12 h post treatment) to rescue the uncontrolled inflammatory insult. HO1 induced by NO interacts with and inhibits iNOS by 12 h post treatment. While iNOS binds strongly with HO1 in the ectopically expressed HEK cells, the binding is not affected by mutating the substrate binding residue in HO1 (H25A). Ectopically expressed HO1 shuts off iNOS enzymatic action, while its own activity is increased by binding to iNOS. Mapping the binding domain sites for HO1 indicates that its N-terminal 40 amino acids are indispensable for binding with iNOS.

Disclosures: S. Chowdhury: None. **S. Snyder:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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The Indian Medical Research Council, New Delhi, Govt of India

The University Grants Commission, New Delhi, India

Title: Sleep deprivation aggravates brain pathology in Alzheimer's disease. Enhanced neuroprotection with co-administration of nanowired 5-HT6 receptor antagonist SB-399885 and cerebrolysin

Authors: *A. SHARMA[1], D. F. MURESANU[2], J. V. LAFUENTE[3], A. NOZARI[4], A. OZKIZILCIK[5], R. TIAN[6], R. PATNAIK[7], H. MOESSLER[8], H. S. SHARMA[9];
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Abstract: Sleep deprivation (SD) in military leads to often decline in cognitive and higher mental functions. Since a close link between SD and onset of Alzheimer's Disease (AD) is established, it appears that SD may worsen brain pathology in AD. Previously, we showed that 48 or 72 h SD alters serotonin (5-hydroxytryptamine, 5-HT) metabolism and induces brain pathology that is significantly reduced by 5-HT3 receptor antagonist ondansetron. Recent reports suggest that treatment with 5-HT6 receptor antagonists has also beneficial effects in attenuating behavioral and cognitive functions in AD.
We found that Cerebrolsyin, a balanced composition of several neurotrophic factors and active peptide fragments when delivered through TiO2-nanowired-technology results in superior neuroprotective effects on brain pathology in AD. Thus, we examined whether AD brain pathology is aggravated in SD and nanodelivery of 5-HT6 receptor antagonist SB-399885 together with Cerebrolsyin may have synergistic enhanced therapeutic effects in AD in combination with SD.
Male Wistar rats (age 20 to 25 weeks) were subjected to 72 h SD using an inverted flowerpot model placed in a pool of water maintained at 1 cm below the surface so that animals are deprived of restful sleep. After 72 h of SD these animals were administered amyloid-beta peptide

(AβP, 250 ng/10 µl, i.c.v.) in the left lateral ventricle once daily for 4 weeks to develop AD like symptoms and brain pathology. Control group received 0.9 % saline instead of AβP.
Our observation shows that AβP infusion in SD rats resulted in marked exacerbation of brain pathology (2 to 3 fold higher) after 4 weeks in terms of AβP deposition in the brain, neuronal damages in cortex, hippocampus and cerebellum, blood-brain barrier (BBB) breakdown and edema formation as compared to identical AβP infusion in control rats. These SD rats also showed much worse behavioral performances on Rota-Rod treadmill, inclined plane angle test, and water maze apparatus.
TiO2-nanowired delivery of 5-HT6 receptor antagonist SB-399885 (3 mg/kg) together with Cerebrolsyin (2.5 ml/kg) intravenously once daily for 2 weeks starting from 1 week after the onset of AβP infusion resulted in marked neuroprotection in AD brain in SD group as compared to tehse drugs either given alone or without nanotechnology under identical conditions. Interestingly, nanowired delivery of drugs in combination also improved behavioral function remarkably in SD rats after AβP infusion. These observations are the first to show that a combination of 5-HT6 receptor antagonist with Cerebrolsyin using nanodelivery has superior neuroprotective effects in AD induced brain pathology in SD, not reported earlier.

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Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

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The University Grants Commission, New Delhi, India

Title: Nanowired cerebrolysin potentiates neuroprotective effects of histamine H3 receptor inverse agonist and antagonist with partial H4 agonist in Alzheimer's Disease

Authors: *R. PATNAIK[1], A. SHARMA[2], D. F. MURESANU[3], S. D. SKAPER[4], R. J. CASTELLANI[5], A. NOZARI[6], A. OZKIZILCIK[7], R. TIAN[8], J. V. LAFUENTE[9], H. MOESSLER[10], H. S. SHARMA[2];
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Abstract: Alzheimer's disease (AD) inflicts over 40 millions people aged 65 and older Worldwide and roughly 5 million Americans are living with the disease that involves huge burden on the society and families as well. Thus, exploration of novel therapeutic measures is needed to contain the disease and improve the quality of life of the victims.
Previous reports from our laboratory showed that amyloid beta peptide ($A\beta P_{1-40}$) infusion induced AD like symptoms developed in rats with regard to brain pathology is significantly reduced by drugs modulating histamine receptors. Thus, histamine H3 receptor inverse agonist BF 2649 hydrochloride and one selective H3 receptors antagonist with partial H4 agonist properties Clobenpropit dihydrobromide when given after 1 week of AβP infusion for 3 weeks resulted in significant reduction in AβP deposits in the brain along with neuronal damage and glial activation. These drug treatments also attenuated breakdown of the BBB to Evans blue albumin and radioiodine in cortex, hippocampus, hypothalamus and cerebellum. Interestingly, Clobenpropit showed superior effects than BF2649 in reducing brain pathologies in AD. However, when these drugs were administered 2 weeks after the onset of AβP for 2 weeks, the magnitude of neuroprotection was severely diminished. This suggests that histamine receptor modulation could reduce AD pathology in a narrow therapeutic window. Since TiO2-nanowired delivery of cerebrolysin- a multimodal drug comprising a balanced composition of several neurotrophic facto and active peptide fragments administered (2.5 ml/kg, i.v.) after 1 week of AβP infusion for 3 weeks also induced neuroprotection, in this investigation we studied the co-administration of cerebrolysin and histaminergic drugs in our AD model on brain pathology. AD like pathology was induced by intraventricular administration of ($A\beta P_{1-40}$, 250 ng/10 µl) once daily for 4 weeks. In separate group of rats BF 2649 (1 mg/kg, i.p.) or Clobenpropit (1 mg/kg, i.p.) was administered once daily for 2 weeks after onset of βbP administration accompanied with TiO2 nanowired cerebrolysin (2.5 ml/kg, i.v.). Our results showed a significant reduction in AbP deposits in the brain along with neuronal damage and glial activation. Breakdown of the BBB and brain edema formation was also absent in these treated AD rats. Interestingly, a combination of cerebrolysin and Clobenpropit showed superior effects in reducing brain pathology in AD. Taken together, our observations are the first to show that

cerebrolysin potentiate the neuroprotective effects of H3 receptor blocker and stimulation of H4 receptors is in AD pathology even initiated 2 weeks after AβP infusion.

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Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

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Program#/Poster#: 315.03/R6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Impact of cellular stress produced by acidic pH and growth factor withdrawal on neuronal differentiation of human hippocampal neural precursor cells (hHippNPCs): implications in neurodegenerative diseases.

Authors: *M. CARDENAS-AGUAYO[1,2], L. GOMEZ-VIRGILIO[3], G. LOPEZ-TOLEDO[4], U. GARCIA[4];
[1]Physiol., UNAM, Ciudad DE Mexico, Mexico; [2]Physiol., Sch. of Medicine, Natl. Autonomous Univ. of Mexico, Mexico, Mexico; [3]Mol. Biomedicine, [4]Physiology, Biophysics and Neurosci., Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst. (CINVESTAV-IPN), Mexico, Mexico

Abstract: Background: NPCs are the most primordial and less committed and pluripotent cells of the nervous system. They give rise to three principal phenotypes in CNS: neurons, glia and oligodendrocytes. Neurotrophic factors had a crucial role in proliferation; differentiation and survival of NPCs. Alzheimer's disease is characterized by alterations in growth factor levels and a stress environment. It is known that chemical stimulus could reprogram somatic cells. Neurodegenerative diseases are characterized by a reduction in neurogenesis and by impairment in protein recycling by autophagy or proteasome, that results in protein aggregation. **Methods**: To elucidate the impact of stress on neural differentiation, recycling systems and tau protein, we cultured embryonic hHippNPCs in the presence or absence of 5 ng/ml FGF-2 for 2 to 7 days or treat them for 25 minutes at pH5.7 and subsequently culture them in the presence of FGF-2 for 2 to 7 days. We analyze by Western blot and immunocytochemistry the expression of NPCs, proliferation, glial, neuronal and synaptic markers and total, phosphorylated and oligomeric tau. We evaluate cell viability, autophagy and proteasome activation and electrophysiological properties. **Results:** We found significant decrease in Nestin, Sox-2, Lin28, PCNA and GFAP expression at 7 days in cells cultured in absence of FGF-2 and in cells exposed to pH5.7 in the

presence or absence of FGF-2. Synaptic markers: PSD95, Arc and neuronal differentiation markers: DCX, NF and NeuN, were increased. Tau expression was significantly increased in cells cultured in the absence of growth factors for 2 and 7 days or in acidic conditions for 7 days. Tau oligomers were significantly increased at 2 days without growth factors or pH5.7 in the presence of FGF-2. Tau phosphorylation, autophagy and proteasome markers were significantly increased at 7 days in the absence of growth factors and in acidic conditions. At 2 days in acidic conditions with or without FGF-2, ROS where significantly increased and Nrf-2 activated. hHippNPCs cultured in the absence of FGF-2 or in acidic conditions showed outward potassium currents and action potentials when differentiate for 28 days. **Conclusions**: We conclude that both growth factor withdrawal and acidic environment induce neuronal differentiation of hHippNPCs, promote tau expression, phosphorylation and aggregation and induce activation of major recycling systems as a response to cellular stress that activates signal transduction and regulates gene expression. Both growth factor withdrawal and acidic environment constitute a stress condition similar to a neuropathology where growth factors are scarce and acidosis could exist.

Disclosures: M. Cardenas-Aguayo: None. **L. Gomez-Virgilio:** None. **G. Lopez-Toledo:** None. **U. Garcia:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.04/R7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC

Alberta Innovates/Alzheimer Society

Title: Prion protein suppresses seizure-induced c-fos expression, a marker for neural activity in zebrafish

Authors: *R. KANYO, P. L. A. LEIGHTON, W. T. ALLISON;
Biol. Sci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Introduction: Prion protein (PrP) has been linked to Alzheimer’s disease (AD), the most prevalent dementia. PrP directly interacts with amyloid-β oligomers, and PrP-knockout mice are more susceptible to seizures and excitotoxicity, compelling PrP as a promising target for drug therapy. Zebrafish enable potent high-throughput drug screening platforms that

faithfully represent the complexity of the central nervous system. Using our engineered mutants, two PrP homologues expressed in zebrafish are investigated here to identify screenable phenotypes related to neuroprotection.
Methods: *Prp1, Prp2* and compound mutant (*Prp1/2*; *prp1* and *prp2* are disrupted) fish were engineered by targeted mutagenesis. Seizure susceptibility was compared in mutant and wild type (WT) embryos via treatment with convulsant pentylenetetrazol (PTZ). Neural activity was measured using *c-fos* expression as a proxy, and quantified using real-time PCR. mRNA expressing zebrafish *prp1*, *prp2* or mouse homologue *prnp* were injected to query specificity of the knockout phenotype and to investigate the conserved role of PrP.
Results: Neural activity (*c-fos* mRNA levels) was increased in *Prp* mutants compared to WT when treated with PTZ. *Prp1* had 2.2-fold higher *c-fos* mRNA levels than WT, *Prp2* 1.68-fold, and *Prp1/2* 2.61-fold. In *Prp1/2* mutants, the knockout-mediated enhancement of *c-fos* expression was suppressed/rescued by delivery of mRNA expressing *prp* variants. Mouse *prnp* mRNA suppressed *c-fos* expression by 51 % almost down to WT levels. Zebrafish paralogs of *prp* moderately supressed *c-fos* expression, but were less effective than mouse *prnp.* Further experiments will titrate the dose of the rescuing mRNA.
Conclusion: A seizure-susceptibility phenotype previously established using behaviour and electrophysiology in our *Prp2* mutant (Fleisch *et al.* 2013) was confirmed here using molecular phenotyping, and exacerbated in compound mutants when *prp1* was also disrupted. Our findings provide a route towards a screenable phenotype and support that PrP is neuroprotective, including during seizure, by regulating neuron excitability (channels). Differences amongst zebrafish and mouse PrP revealed herein prompt new hypotheses regarding which protein domains mediate the ancient protective function of PrP. Disrupting PrP is emerging as a therapeutic target in AD, thus identifying modulators of PrP neuroprotective activity is expected to have an impact beyond the socioeconomic consequences of prion diseases in cattle and cervids.

Disclosures: **R. Kanyo:** None. **P.L.A. Leighton:** None. **W.T. Allison:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.05/R8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Medicinska Fakulteten, Umeå Universitet

Fundación Canaria Dr. Manuel Morales

JC Kempes Minnes Akademiska Fond

Title: Neuroprotection against Alzheimer's disease-related amyloid-β toxicity: sigma-1 receptor modulation of VDAC-1 channels

Authors: *C. FERNÁNDEZ ECHEVARRÍA, T. MEDIAVILLA, D. MARCELLINO;
Integrative Med. Biol., Umeå Univ., Umeå, Sweden

Abstract: Investigation into the mechanisms underlying neurodegenerative disease has been one of the fastest growing areas of research in recent decades. This is especially true in the case of Alzheimer's disease (AD), whose revalence continues to increase dramatically. Recent research has revealed proteins, such as plasmalemmal VDAC-1, to contribute to the toxicity of amyloid-β, the main component of amyloid plaques found in the brains of Alzheimer patients. Furthermore, sigma-1 receptors (SIG1R) have been proposed as putative targets to offer neuroprotection against neurodegenerative disease. We propose that one of the neuroprotective mechanisms of SIG1R activation may involve the regulation of VDAC-1 related toxicity. Establishing a link between SIG1R and VDAC-1 is a matter of great importance for the development of novel pharmacological strategies to treat disease. We have specifically investigated the possible modulation of VDAC-1 by SIG1R in cellular models of Alzheimer-related toxicity. In addition, the putative neuroprotective effects of sigma-1 receptor ligands were evaluated with regard to its ability to reduce Aβ-induced neuronal toxicity.

Disclosures: C. Fernández Echevarría: None. **T. Mediavilla:** None. **D. Marcellino:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

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Program#/Poster#: 315.06/R9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH (RCMI-BRIDGES)

Title: Neuroprotection by a N-terminal fragment and hexapeptide core sequence of beta amyloid

Authors: *N. ALFULAIJ;
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Abstract: Amyloid-beta (Aβ) is the primary component of plaques associated with Alzheimer’s disease (AD). We previously demonstrated that the presence of nicotinic receptors (nAChRs), an identified neuromodulatory target for Aβ, sensitizes neurons to Aβ neurotoxicity. A N-terminal

Aβ fragment, $A\beta_{1-15}$, also has neuromodulatory activity but is not toxic. We showed specifically that picomolar levels of $A\beta_{1-15}$ enhances Ca^{2+} signaling, fear memory and long-term potentiation (LTP). Within $A\beta_{1-15}$, we further identified a hexapeptide core sequence ($A\beta_{core: 10-15}$), YEVHHQ, which is as or more effective as $A\beta_{1-15}$.
In view of the absence of neurotoxicity, we conjectured that $A\beta_{1-15}$ and $A\beta_{core}$ would be neuroprotective against full-length Aβ toxicity. Using a reactive oxygen species (ROS) assay as a measure of toxicity, we demonstrated that $A\beta_{1-15}$ or $A\beta_{core}$ blocks full-length Aβ neurotoxicity (100nM – 3 days) in a neuroblastoma cell line expressing nAChRs. Combination treatments were done using different timing and dosages of the peptide fragments to evaluate their action and potency. With co-incubation, both $A\beta_{core}$ and $A\beta_{1-15}$ blocked full-length Aβ-induced ROS and nuclear disintegration in cultures expressing nAChRs, while rescue (post Aβ treatment) with the peptides was partially effective.
To confirm our findings in a physiologically relevant model, we have utilized primary hippocampal neuron cultures and slice cultures subjected to full-length Aβ compared to combination treatment of $A\beta_{1-42}$ and $A\beta_{1-15}$ or $A\beta_{core}$ with various timing and dosage regimens. To further elucidate the molecular pathways affected by Aβ, $A\beta_{1-15}$, $A\beta_{core}$ or combinations, cell and slice cultures were treated with the aforementioned peptides and then changes in pJNK and pERK were examined, as both of these MAP kinases have been implicated in Aβ toxicity. Based on our previous work showing $A\beta_{1-15}$ to be protective against full-length Aβ-induced LTP inhibition in wild-type acute hippocampal slices, we are also examining the impact of $A\beta_{core}$ in synaptotoxicity. These studies include testing the $A\beta_{core}$ as well as $A\beta_{1-15}$ against acute full-length Aβ application in wild-type hippocampal slices as well as slices from 5xFAD (APP/PS1 expressing transgenic mouse model) mice which rapidly accumulate Aβ.
The action of the N-terminal fragment $A\beta_{1-15}$ and the $A\beta_{core}$ indicates that they may serve a competitive, neuroprotective role at the synapse in the context of accumulating Aβ in AD. Furthermore, the neuroprotective action of the $A\beta_{core}$ suggests the possibility of using this core sequence as a scaffold for optimization of a potential biologic for protection against Aβ-induced toxicity.

Disclosures: N. Alfulaij: None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.07/R10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NMSS Grant RG 4046

NIH NS093134

Neuropathology Training Grant T32 NS007098

Title: Extensive axonal damage is observed in $Gas6^{-/-}Axl^{-/-}$ double-knockout (DKO) mice following exposure to cuprizone.

Authors: *B. SHAFIT-ZAGARDO[1], A. RAY[1], R. C. GRUBER[2], J. WILLIAMSON[1], J. DUBOIS[1];
[2]Pathology, [1]Albert Einstein Col. Med., Bronx, NY

Abstract: Multiple sclerosis (MS) is characterized by inflammation, axonal damage and demyelination. Growth arrest-specific protein 6 (Gas6) and members of the Axl, Tyro3 and MerTK (TAM) family of receptor tyrosine kinases important for the innate immune response, efferocytosis and homeostasis are aberrantly expressed in MS lesions. Both Gas6 and ProS1 signal through the TAMs but only Gas6 directly activates Axl. We generated $Gas6^{-/-}Axl^{-/-}$ DKO mice to ensure complete deletion of the Gas6/Axl signaling axis while leaving ProS1/Tyro3/Mertk signaling intact. This eliminates the possibility that ProS1 can activate Axl via receptor heterodimerization with Tyro3 or MerTK in the absence of Gas6. Naïve DKO and WT mice have equivalent numbers of Olig2+ and Olig1+ oligodendrocytes, normal-appearing myelin, and equivalent numbers of myelinated axons in the corpus callosum as determined by electron microscopy. DKO mice show significantly fewer Olig2+ oligodendrocytes in the corpus callosum at 5 weeks cuprizone treatment, and little to no remyelination apparent at 6 weeks, indicating a lag in remyelination, while WT mice begin to remyelinate by 6 weeks. After 6-week-cuprizone exposure and 3-week withdrawal, female and male DKO mice had significant axonal damage including swollen axons with autophagolysosomes and multivesicular bodies, and fewer myelinated axons relative to WT mice. There was no difference in the number of $Iba1^{+}/4D4^{+}$ microglia between both groups of mice. These data demonstrate an important role of Gas6/Axl signaling in maintaining axonal integrity and remyelination. This warrants further studies for therapeutic intervention in mouse models and perhaps individuals with MS.

Disclosures: B. Shafit-Zagardo: None. **A. Ray:** None. **R.C. Gruber:** None. **J. Williamson:** None. **J. DuBois:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

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Program#/Poster#: 315.08/R11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS-019108

4UH-2 TR000918-03

3UH3TR00918-03S1

The University of Chicago Innovation Fund

Title: *In vivo* tracking confirms dendritic cell exosomes enter the brain and may travel along CSF pathways

Authors: *A. D. PUSIC, D. DUKALA, R. P. KRAIG;
Univ. of Chicago, Chicago, IL

Abstract: Exosomes from IFNγ-stimulated dendritic cells (SDC-Exos) contain miRNAs which promote myelination, reduce oxidative stress (OS), and preferentially enter oligodendrocytes in brain slice cultures (1). When nasally administered to naïve animals, SDC-Exos improve remyelination following lysolecithin-induced demyelination (an *in vivo* model of multiple sclerosis) and inhibit spreading depression (SD) (2). SD is the likely cause of migraine with aura, and a well-established experimental model of migraine that triggers increased OS and transient demyelination (3). Collectively, these experiments show that nasal administration of SDC-Exos effectively improve brain function. However, little is known about the route of entry, timing and distribution of nasally delivered SDC-Exos. We nasally administered mCLING-labelled SDC-Exos and tracked their entry into brain. mCLING (membrane-binding fluorophore-cysteine-lysine-palmitoyl group) labels plasma membranes and is taken up by endocytosis (4), making it ideal for organelle tracking. The dye remains detectable in membranes after fixation and exhibits switching between bright and dark states under continuous excitation (flickering), allowing for its use in ground-state depletion microscopy (GSD; with ~20 nm resolution). Using wide field, confocal and GSD microscopy, we showed that exosomes enter and widely distribute throughout the brain. They appear to follow interstitial pathways from the olfactory bulb, with a gradient from the pial surface into the parenchyma consistent with CSF initial distribution. A rostral-caudal gradient was also noted. Preliminary data suggest nasally delivered exosomes travel along perivascular spaces. GSD images confirm fluorophore flickering was only seen with labelled exosomes (using a running median filter; 5). Our results provide evidence that SDC-Exos enter brain, distribute throughout the parenchyma, and have a functional impact. This data further supports use of SDC-Exos as a novel therapeutic for neurodegenerative disorders.
1) Pusic A, et al, Neuroimmunol, 2014; 2) Schumer J et al, Neurosci Abst, 2015; 3) Pusic A et al, Exptl Neurol, 2015; 4) Revelo NH et al, J Cell Biol, 2014; 5) Hoogendoom E et al., Sci Rep, 2014.

Disclosures: **A.D. Pusic:** None. **D. Dukala:** None. **R.P. Kraig:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.09/R12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit review grant

Title: Delayed treatment of 7, 8-Dihydroxyflavone protects axonal injury and demyelination in an animal model of Multiple Sclerosis

Authors: T. K. MAKAR[1,2,3], V. NIMMAGADDA[1,2], P. R. GUDA[1], *D. TRISLER[1,2,3], C. T. BEVER, Jr[1,2,3];
[1]Dept Neurol., Univ. Maryland Sch. Med., Baltimore, MD; [2]VA Med. Ctr., Baltimore, MD; [3]VA Multiple Sclerosis Ctr. of Excellence East, Baltimore, MD

Abstract: Axonal pathology is a key regulator to long-term disability in multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system, but the mechanisms that underlie axonal pathology in MS remain elusive. Evidence suggests that axonal pathology is a direct consequence of demyelination, as we and others have shown using animal model (EAE) of MS.7, 8-Dihydroxyflavone (DHF), a TrkB agonist, is a neuro-protective agent used for different animal models of neurodegenerative diseases.We recently reported (Makar.et.al. 2016 journal of Neuroimmunology 292 : 9-20) that axonal protection is enhanced when we treated the EAE mice from the onset of the disease. Furthermore, treatment with DHF (5mg/kg, I.P, daily for 30 days) in EAE mice inhibited clinical severity and inflammation when we treated the EAE mice from the onset of disease. Treatment with DHF increased TrkB activation after EAE. Improvement in EAE, suggests a pivotal role of TrkB signaling in anti-inflammatory performance after EAE. A potential action of DHF on delayed treatment is still active or not is not clear because delayed treatment is more effective for MS patients. Now we treated EAE mice with the same dose of DHF at the severity of the disease and interestingly we found that delayed treatment is also effective to inhibit the clinical severity, axonal damage, demyelination and inflammation. Since progressiveness of MS is associated with axonal degeneration associated with demyelination, which is an important factor for the development of neurodegeneration in MS/EAE. These results set a precedent for the therapeutic use of DHF in EAE and perhaps MS during progressive stages.

Disclosures: **T.K. Makar:** None. **V. Nimmagadda:** None. **P.R. Guda:** None. **D. Trisler:** None. **C.T. Bever:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.10/R13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R21NS075645

kent state university

Title: Neuronal hemoglobin expression and its relevance to multiple sclerosis neuropathology

Authors: *K. ALKHAYER, N. K. SINGHAL, J. MCDONOUGH;
biological sciences and school of biomedical sciences, Kent State Univ., Kent, OH

Abstract: Multiple sclerosis (MS) is characterized by demyelination and progressive neurological disability. Previous studies have reported defects to mitochondria in MS including decreased expression of nuclear encoded electron transport chain subunit genes and inhibition of respiratory complexes. We previously reported increased levels of the hemoglobin β subunit (Hbb) in mitochondrial fractions isolated from postmortem MS cortex compared to controls. In the present study, we performed immunohistochemistry to determine the distribution of Hbb in postmortem MS cortex and identified proteins which interact with Hbb by liquid chromatography tandem mass spectrometry (LC-MS/MS). We found that Hbb was enriched in pyramidal neurons in internal layers of the cortex and interacts with subunits of ATP synthase, histones, and a histone lysine demethylase. We also found that Hbb is present in the nucleus and that expression of Hbb in SH-SY5Y neuroblastoma cells increased trimethylation of histone H3 on lysine 4 (H3K4me3), a histone mark that regulates cellular metabolism. These data suggest that Hbb may be a part of a mechanism linking neuronal energetics with epigenetic changes to histones in the nucleus and may provide neuroprotection in MS by supporting neuronal metabolism.

Disclosures: K. Alkhayer: None. **N.K. Singhal:** None. **J. McDonough:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

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Program#/Poster#: 315.11/R14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: T32 Neuroendocrinology training grant

Title: Estriol preserves axonal integrity and cortical volume in experimental autoimmune encephalomyelitis

Authors: *C. E. MEYER[1], H. JOHNSONBAUGH[2], S. LEPORE[2], N. ITO[2], R. VOSKUHL[2], A. MACKENZIE-GRAHAM[1];
[1]Neurol., [2]UCLA, Los Angeles, CA

Abstract: Despite wide symptomatic variability in multiple sclerosis (MS), grey matter atrophy has consistently emerged as a strong indicator of clinical disability. In fact, evidence suggests progressive loss of grey matter correlates with physical and cognitive dysfunction better than many other commonly occurring hallmarks of MS, including enhancing lesions and lesion burden. Current treatment options are primarily designed to reduce inflammation and have had only modest success at slowing grey matter atrophy. Treatment with the sex hormone, estriol, has been shown to both reduce relapses and preserve grey matter in MS and in its most commonly used mouse model, experimental autoimmune encephalomyelitis (EAE). In this study, we sought to elucidate the mechanism by which estriol treatment preserves grey matter. Our investigation involved implanting either an estriol or placebo pellet subcutaneously in female THY1-YFP+ mice prior to EAE induction with myelin oligodendrocyte glycoprotein (MOG) peptide. *In vivo* Magnetic Resonance Images were collected 20 days after disease induction. Following image acquisition, the mice were sacrificed and Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel (CLARITY) was performed on the brain and spinal cord. Volumetry analysis revealed preservation of cortical volume in estriol-treated mice with EAE when compared to vehicle-treated mice with EAE. CLARITY images demonstrated that estriol treatment resulted in preservation of cortical layer V pyramidal neurons and reduced formation of axonal ovoids and end bulbs in the spinal cord. Through cross-modality analysis, our results shed light on the relationship between cortical atrophy and axonal transection while also suggesting a mechanism by which estriol treatment preserves grey matter in EAE.

Disclosures: C.E. Meyer: None. **H. Johnsonbaugh:** None. **S. Lepore:** None. **N. Ito:** None. **R. Voskuhl:** None. **A. MacKenzie-Graham:** None.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.12/R15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sensitivity of mri measures to axonal and/or myelin repair in the presence of inflammation: a quantitative assessment of *In vivo* mtr, dti, and post-mortem immunohistochemistry in a rodent model of spontaneous remyelination

Authors: *B. A. HOOKER, R. RAJAGOVINDAN, M. J. VOORBACH, C. H. SCHROEDER, J. D. BEAVER;
ZR13, AbbVie, North Chicago, IL

Abstract: Background: Magnetization Transfer (MT) imaging and Diffusion Tensor Imaging (DTI) are increasingly utilized in clinical trials of novel multiple sclerosis therapies. While measures derived from these techniques are sensitive to changes in white matter pathophysiology, they are also believed to be influenced by confounding factors such as changes in edema, typical in MS lesions, and thus limited in specificity. We investigated the sensitivity and specificity of *in vivo* MT ratio (MTR) and DTI measures to quantitative changes in myelin and axonal density in the presence of inflammation and edema assessed by immunohistochemistry (IHC).
Methods: Focal demyelination was induced in Sprague Dawley rats (n=24) using stereotactic injection of 3uL of 1% lysophosphotidylcholine into the corpus callosum. DTI (380x380x500 μm, b~700 s/mm^2, 30 directions) and MTR (250x250x500 μm, 9 μT Gaussian RF pulse, 2 kHz off-resonance) at 4.7T were acquired in three cross-sectional cohorts of animals at 14, 28, 56 days post injection (dpi), and sacrificed following image acquisition. Fractional anisotropy (FA), axial (AD), radial (RD), mean diffusivity (MD), T2 signal intensity and MTR maps were derived from the MR images. Brains were serially-sectioned and stained to assess myelin density (MBP), axonal density (NF1), inflammation (Iba1) and edema (IgG). Quantitative endpoints were derived from lesions manually delineated on MRI and IHC.
Results: Focal lesions were detected in all animals. Myelin and axonal pathology were highest at 14 dpi followed by spontaneous repair evidenced by improvement in MBP and NF1 staining ($p<0.05$) by 56 dpi. In line with the IHC findings, increase in MTR, FA and reduction in MD, RD and T2 were observed 56 dpi relative to 14 and 28 dpi ($p<0.05$). MTR and FA increased by 3 and 30%; MD, RD and T2 decreased by 4, 10 and 17% at 56 dpi relative to 14 dpi. The outcome of univariate and multivariate analysis to elucidate the magnitude of influence each underlying aspect of pathophysiology (myelin, axon, inflammation and edema) had on each MRI outcome measure will be reported along with strategies to minimize the influence of confounding factors in the interpretation of MRI outcome measures.

Conclusions: The findings provide improved understanding of the pathophysiological sensitivity and specificity of MRI measures, aiding in the choice of optimal measures and their precise interpretation in clinical trials of remyelinating agents.

Disclosures:
All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Disclosures: **B.A. Hooker:** A. Employment/Salary (full or part-time): AbbVie. **R. Rajagovindan:** A. Employment/Salary (full or part-time): AbbVie. **M.J. Voorbach:** A. Employment/Salary (full or part-time): AbbVie. **C.H. Schroeder:** A. Employment/Salary (full or part-time): AbbVie. **J.D. Beaver:** A. Employment/Salary (full or part-time): AbbVie.

Poster

315. Neuroprotective Mechanisms: Alzheimer's Disease and Multiple Sclerosis

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 315.13/R16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Delayed treatment of experimental autoimmune encephalomyelitis with glibenclamide promotes M2 polarization of macrophages and reduces neuroinflammation

Authors: *T. K. MAKAR[1,2,3], V. GERZANICH[4], V. NIMMAGADDA[1,2], P. R. GUDA[1], A. B. MORRIS[1], D. TRISLER[1,2,3], C. T. BEVER, Jr[1,2,3], M. SIMARD[4];
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Abstract: We studied therapeutic effect and anti-inflammatory mechanisms of glibenclamide in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Recently, we showed that treatment with Sur1 antagonist glibenclamide (Glib) starting from the onset EAE attenuates neuroinflammation and clinical severity of disease (Makar.et.al. 2015 Neuroinflammation Vol: 12 P-210.). Here we report new data with delayed Glib treatment during chronic stage of EAE. Treatment (20 μg Glib/day/mouse) starting at 12 and 24 days after onset of the disease significantly improved clinical symptoms and reduced inflammation in lumbar spinal cords. Similar to the reported findings with treatment at the onset of EAE, delayed treatment with Glib inhibited infiltration of the CD45+ leukocyte cells and CD11b+ macrophages. Additionally, Glib treatment significantly reduced expression ($p<0.02$) of Endothelin-1 (ET-1). Progression of the EAE was associated with significant infiltration into the

parenchyma of spinal cords macrophages with M1 activation state. Treatment with Glib reduced the number of M1 and increased the number of M2 activation state macrophages. This resulted in increased labeling for M2 markers CD163 and IL-10. The polarization of macrophages towards M2 active state in Glib treated mice was associated with decrease of the pro- inflammatory molecules IFN-γ, TNF-α, MCP-1 and ET-1. The results of the study revealed novel cellular mechanisms involved in anti-inflammatory effects of Glib. The beneficial effect of Glib observed in EAE strongly indicates that treatment with Sur1 antagonists have promising therapeutic potential as disease modifying approach at chronic stages of MS.

Disclosures: **T.K. Makar:** None. **V. Gerzanich:** None. **V. Nimmagadda:** None. **P.R. Guda:** None. **A.B. Morris:** None. **D. Trisler:** None. **C.T. Bever:** None. **M. Simard:** None.

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Air Force Office of Scientific Research (EOARD, London, UK),

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Title: Concussive head injury at simulated high altitude exacerbates blood-brain barrier breakdown, edema formation and cellular injury. Neuroprotection by nanowired delivery of cerebrolysin with mesenchymal stem cells

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Abstract: Military personnel are highly vulnerable to concussive head injury (CHI) during combat operation either at ground level or at high altitude mountains. High altitude induced l

brain edema development and alterations in cognitive dysfunctions are well known. However, effects of head injury at high altitude as compared to sea level are still not known. In this investigation we examined CHI at laboratory-simulated condition of high altitude and compared the results on identical head injury at normal laboratory conditions.
Rats were exposed to simulated high altitude (HA) equivalent of 5000 m in an Altitude chamber (Hypobaric chamber) 11.2 % O2 at 0.53 Atm for 6 h daily for 1 week. The temperature of the hypobaric chamber was maintained at 21±1 °C. The humidity (45 to 50%) and airflow was maintained at 4 liter per hour. Control rats kept at room temperature at standard laboratory conditions. Control or HA arts were provided food and water ad libitum before experiment. CHI was inflicted in control and HA rats under Equithesin anesthesia (3 ml/kg, i.p.) by dropping an Iron tapered cylinder (114.6 g) through a guide tube from 20 cm height over the exposed right parietal skull inducing an impact of 0.224 N over the skull surface without making any fracture. The method simulates counter coup injury and results in profound cellular damage in the left uninjured hemisphere as compared to the injured side 12 to 24 h after the primary insult. In these rats blood-brain barrier (BBB) breakdown to Evans blue albumin and radioiodine was examined together with edema formation using brain water content. Nissl stain on paraffin sections was used to evaluate neuronal injuries. Our results showed that CHI in HA rats resulted in 250 % exacerbation of BBB breakdown, 3-to 4- fold higher brain edema development and 2- to 2.5 fold greater neuronal injuries in the cerebral cortex, hippocampus and cerebellum as compared to rats kept at room temperature.
Co-administration of Cerebrolsyin 2.5 or 5 ml/kg, i.v. with mesenchymal stem cells (MSCs, 1 million) 4 to 6 h after trauma was able to induce profound neuroprotection in CHI arts at room temperature. However, this dose was only slightly effective in reducing brain pathology following CHI in HA rats. On the other hand when TiO2 nanowired cerebrolysin (2.5 ml) was co-administered with 10^6 MSCs 4 or 6 h after trauma significant reduction in the BBB breakdown, edema formation and neuronal injuries were seen in HA rats. Taken together our observations are the first to point out that CHI in HA results in exacerbation of brain pathology and under such situations nanodelivery of suitable drugs are needed to achieve better neuroprotection, not reported earlier.

Disclosures: **H.S. Sharma:** None. **D.F. Muresanu:** None. **J.V. Lafuente:** None. **A. Ozkizilcik:** None. **Z.R. Tian:** None. **A. Nozari:** None. **R. Patnaik:** None. **H. Moessler:** None. **A. Sharma:** None.

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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India-EU Research Program Neuroscience Research

University Grants Commission, Govt. of India

Medical Research Council, Govt. of India

Title: Nicotine exacerbates closed head injury induced brain pathology at hot environment

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Abstract: Nicotine exposure in military is associated with mental dysfunction. Whether stress related to combat or trauma results in more nicotine consumption is still unclear. Thus, further investigations on nicotine use in TBI are needed to explore outcome of brain pathology. Since substance abuse is affected by hot and cold environment, in present investigation nicotine exposure at hot environment was examined in relation to TBI in model experiments.
Previous reports from our laboratory showed that nicotine administration (9 mg/kg/day s.c. for 1 week) in rats resulted in breakdown of the blood-brain barrier (BBB) permeability, brain edema formation and neuronal injury that was exacerbated by cold environment (+8 °C). Thus, it would be interesting to see whether subthreshold dose of nicotine (4 mg/kg, s.c. per day for 1 week) could aggravate TBI induced brain pathology at normal room temperature or at hot environment (38 °C). Since closed head injury (CHI) is quite common in military personnel during combat related TBI, we examined effects of CHI in nicotine treated groups.
Male Wistar rats (age 16 to 18 weeks of age) were administered nicotine (4 mg/kg, s.c.) per day either at room temperature (21±1°C) or reared at cold ambient temperature (8 °C) for 1 week. In a separate group rats exposed to nicotine at room or cold temperatures CHI was produced by inflicting 0.224 N impact on the exposed right parietal skull under Equithesin anesthesia using a an iron cylinder tapered towards impact side (weight 114.6 g) dropped from 20 cm height using a guide tube. Control CHI group were administered 0.9 % saline instead of nicotine. After 24 h of CHI, BBB breakdown to Evans blue albumin (EBA) and radioiodine ($^{[131]}$-Iodine) was examined together with brain edema formation using brain water content. Neuronal injuries were evaluated

on paraffin sections (3-µm) with Nissl or Haematoxylin and Eosin (H&E) staining.
Our results showed that CHI in nicotine exposed rats exacerbated BBB breakdown by 150 to 200 % with 2-3 fold higher brain edema formation and 2- to 4-fold greater neuronal injuries at room temperature. Interestingly, CHI in nicotine treated rats at hot environment further exacerbated brain pathology by 250 to 300 % as compared to identical CHI in nicotine treated animals at room temperature.
Taken together our observations are the first to point out that nicotine exposure prior to TBI results in greater brain pathology and these effects are further aggravated when CHI was inflicted at hot environment with nicotine intoxication, not reported earlier.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Society for Study on Neuroprotection & Neuroplasticity (SSNN) Cluj-Napoca, Romania

SAIOTEK, and IT/491/10 (Basque Government.

Swedish Strategic Research Foundation, Stockholm, Sweden

Swedish Medical Research Council (Nr 2710-HSS),

IT 794/13 (JVL), Govt. of Basque Country and UFI 11/32 (JVL)

Title: Focal blast brain injury induces rapid edema formation, blood-brain barrier breakdown and intensive cellular damage. Neuroprotective effects of a multimodal drug cerebrolysin

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Abstract: Blast brain injury (bBT) is a serious problem in military personnel during combat or peacekeeping operations. The primary and secondary pressure waves created by the blast induces intense brain damage depending on the magnitude and severity and the distance between blast and the victim. Few reports using compressed-air or gas induced blast in the shock tube in the magnitude of 150 to 250 kPa showed unilateral brain damage e.g., neuronal loss, activation of astrocytes and distortion of axonal and synaptic connections after 2 to 12 h bBT. However, studies on drugs modifying bBT induced brain pathology are still lacking.

Previously we have shown that cerebrolysin- a multimodal drug when given alone or using TiO2 nanowired delivery induces marked neuroprotection following brain injury caused by trauma, hyperthermia or concussion. Thus, we examined effects of cerebrolysin following bBT in a rat model.

Equithesin anesthetized Male Wistar rats (age 25 to 30 weeks) were placed in a compressed air driven blast shock tube with head facing. The torso was wrapped to protect from the blast waves. Rats were exposed to blast waves of either 150±5 kPa or 250±8 kPa (measured using a pressure transducer fixed into the shock tube) and allowed to survive 8 or 24 h. Brain edema, blood-brain barrier (BBB) breakdown and cellular injuries were examined using standard protocol.

Our results showed breakdown of the BBB to Evans blue albumin and radioiodine ([131]-I) in the cerebral cortex, hippocampus and cerebellum by 4 to 8-fold from the normal control rats depending on the magnitude of the blast waves. The contralateral side also showed 2 to 4 folds higher BBB leakage after 150 or 250 kPa shock waves, respectively. The brain water content elevated by 6 to 10 fold on the right hemisphere, whereas 4 to 6-fold increase in water content was seen on the left side. Nissl staining showed pronounced neuronal loss, damage and cell death in the above brain areas that was most prominent on the right side.

Cerebrolysin (2.5 or 5 ml/kg, i.v.) administered 2 and 4 h after bBT (250 kPa) significantly reduced brain pathology following 8 h. However, multiple injections of high doses of cerebrolysin (5 ml/kg, 2,4,6,8 and 10 h after injury) are needed to induce neuroprotection 12 h after bBT. TiO2 nanowired delivery of cerebrolysin (2, 4 and 8 h after bBT) significantly reduced brain pathology at 12 h. These results for the first time show that cerebrolysin if given in multiple doses is able to thwart bBT induced brain pathology and TiO2-nanowired cerebrolysin has superior neuroprotective effects, not reported earlier.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Society for study on Neuroprotection and Neuroplasticity, Romania

Defence Research & Development, Swedish Govt.

Title: Concussive head injury induced brain ischemia and oxidative stress are thwarted by nanodelivery of Chinese traditional medicine DL-3-n-butylphthalide (DL-NBP)

Authors: *L. FENG[1], A. SHARMA[2], A. NOZARI[3], D. F. MURESANU[4], J. V. LAFUENTE[5], A. OZKIZILCIK[6], R. TIAN[7], H. S. SHARMA[2];
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Abstract: Concussive head injury (CHI) often known as closed head injury is the most prominent traumatic brain injuries in military personnel during combat operation resulting in life time disabilities. Thus, exploration of suitable therapeutic measures to improve the quality of life and rehabilitation of CHI victims is the need of the hour. Our laboratory is engaged in exploring novel therapeutic measures for the treatment of CHI to reduce brain pathology at the cellular and molecular levels using a variety of drugs using nanotechnology. Previously, we reported nanodelivery of traditional Chinese medicine DL-3-n-butylphthalide (DL-NBP) in CHI that was most effective in reducing brain pathology in CHI if given 2 and 4 h after an 8 h injury or 8 and 12 h after 24 h trauma. Since CHI induces severe brain ischemia and oxidative stress, in this investigation we examined the effects of TiO2-DL-NBP on regional cerebral blood flow (CBF) and oxidative stress in our rat model of CHI.

The CHI was induced by dropping a weight of 114.6 g on the right parietal skull bone over a distance of 20 cm in anesthetized rats resulting an impact of 0.224 N on the skull surface. This impact induces severe brain pathology over 4 h to 24 h. In separate groups of CHI we administered TiO2-nanowired-NBP (40 or 60 mg/kg, i.p.) 2h and 4 h after injury in 8 h survival group and 8 h and 12 h after trauma in 24 h survival group. In tehse untreated and treated groups regional CBF (rCBF) in the cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum and brainstem was examined using [125]-Iodine labeled microspheres (15±0.6 μm o.d.). We also measured Luminol, Malondialdehyde, Myeloperoxidase, and Glutathione in the above brain regions using standard biochemical procedures. Untreated CHI resulted in a progressive increase in brain Luminol, Malondialdehyde and Myeloperoxidase (+20 to 50 %), and a decrease in Glutathione levels (-25 to -40 %) after 8 h and 24 h CHI respectively. These changes in oxidative parameters correlated well with the reduction in the rCBF (-30 to -50 %) and development of brain edema formation. TiO2-NBP resulted in significant improvement in rCBF in all brain areas examined and thwarted the oxidative stress in CHI in all brain regions. Interestingly, normal NBP requires 80 to 100 mg/kg, dose to have comparable reduction in rCBF and oxidative stress in CHI. These observations clearly suggests that nanodelivery of NBP has superior neuroprotective effects in CHI due to its anti-ischemic and antioxidant properties. Further research is needed to understand the dose and time related neuroprotective effects in CHI, a feature that is currently being investigated in our laboratory.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development and Rehabilitation Research and Development)

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Title: Treatment with Nrf2 and p53 transcription factor modulators in an *In vitro* mild TBI model

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Abstract: In the United States each year, it is estimated that 1.7 million people sustain a traumatic brain injury (TBI). In our military members and athletes, in particular, TBI remains a major cause of morbidity and mortality. The majority of TBIs are mild and these can result in deleterious cognitive effects for which there is currently no effective treatment. We have demonstrated improved outcomes in both *in vitro* and *in vivo* models of brain injury following treatment with tert-butylhydroquinone (tBHQ). tBHQ is an activator of the inflammatory responsive transcription factor, Nrf2, and has been shown to induce the production of several neuroprotective factors. A recent study has also identified pifithrin-α oxygen (PFT-α (O)), a p53 inactivator, as demonstrating neuroprotective effects in an *in vitro* excitotoxicity model and an *in vivo* open head injury model. Our study utilizes an *in vitro* "injury in a dish" model to evaluate the effects of a repetitive physical injury on a neuronal cell line and evaluate the potential protective effects of treatment. tBHQ plus the sulfur containing inhibitor, PFT-α, produced significantly improved neuronal function measured with an MTS assay (compared to either treatment alone) while tBHQ plus SP600125, a Jun-N terminal kinase inhibitor, was not additionally protective. We have investigated treatment with tBHQ, PFT-α (O) and a combination of the two to determine whether simultaneous activation of Nrf2 and inhibition of p53 would provide a synergistic neuroprotective effect in terms of neuronal survival and neurite health. Observations indicate that treatment with tBHQ plus PFT-α (O) improves overall neuronal survival and neurite health, as measured by neurite length per cell, when compared to vehicle treated cells or those that received only one treatment. This suggests that simultaneous activation of Nrf2 and inhibition of p53 do produce enhanced neuroprotective effects in response to neuronal injury and could represent a novel treatment in response to head injury. Overall, the goal of our group is to help identify therapeutic approaches that would benefit individuals suffering mild traumatic brain injury.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Society for Study on Neuroprotection & Neuroplasticity (SSNN), Cluj-Napoca, Romania

Ministry of Science & Technology, Govt. of India

Ministry of Science & Technology, People Republic of China

Title: Co-administration of nanowired mesenchymal stem cells with antioxidant H-290/51 ameliorates exacerbation of spinal cord pathology following trauma in hypertensive rats

Authors: *A. NOZARI[1], A. SHARMA[2], L. FENG[3], P.-O. SJOQUIST[4], D. F. MURESANU[5], J. V. LAFUENTE[6], R. PATNAIK[7], A. OZKIZILCIK[8], R. TIAN[9], H. S. SHARMA[2];
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Abstract: Military personnel during combat situations are often prone to traumatic spinal cord injuries (SCI). About 11 % of combat related casualty was reported in US armed forces during 2005 to 2009 due to SCI. Combat stress in armed forces also results in hypertension. Thus, it would of great interest to investigate whether SCI in hypertension aggravates pathophysiological reaction of the cord, and if so do we need additional therapeutic strategies to treat such cases. Accordingly, to deal with these kinds of co-morbidity, exploration of novel therapeutic strategies area required.

Since hypertension alone could result from oxidative stress and SCI induces lipid peroxidation, in this investigation we explored pathophysiology SCI in chronic hypertension. Furthermore used mesenchymal stem cells together with a potent antioxidant compound H-290/51 either alone or using TiO2-nanowired technology to thwart spinal cord pathology in hypertension. Rats were made hypertensive using 2-kidney 1 clip (2K1C) technology where a silver clip was placed on the right renal artery (0.5 mm od) to constrict the vessel for renal hypertension that develops after 4 to 6 weeks (mean arterial blood pressure MABP 180±10 torr). In these hypertensive rats a focal SCI was inflicted under Equithesin anesthesia over the right dorsal horn of the T10-11 segment (1.5 mm deep and 4 mm long). The animals were allowed to survive 24 h after the primary insult.

Our results showed 150 to 230 % increase in the breakdown of the blood-spinal cord barrier

(BSCB) to Evans blue albumin and radioiodine in the T4, T9 and T12 segments in hypertensive group after SCI as compared to normotensive injured rats. Spinal cord edema was 2- to 4 –fold higher in hypertensive rats after SCI than normal injured group. Neuronal injury, activation of glial cells as seen using glial fibrillary acidic protein (GFAP) immunoreactivity and myelin degradation was 120 to 180 % higher in the T9 and T12 segments in hypertensive rats after SCI than injured normotensive animals. Co-administration of TiO2 nanowired commercial mesenchymal stem cells (MSCs, 10^6 cell, i.v.) together with H-290/51 (50 mg/kg, i.p.) 4 to 6 h after trauma significantly attenuated exacerbation of SCI pathophysiology in hypertensive rats and induced remarkable neuroprotection. In normal injured animals co-administration of MSCs and H-290/51 without nanodelivery was also effective when administered 4 to 8 h after SCI. These observations are the first to point out that hypertension exacerbates SCI-induced pathophysiology and in such situations nanodelivery of MSCs and antioxidant compound H-290/51 is needed to achieve neuroprotection, not reported earlier.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future planning Grant (2014M3C1B2048632)

Title: Differential changes of protein expression on Heat shock proteins in a rat model of contusive spinal cord injury

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Abstract: K. Yang and K. Lee are equally contributed to this work.
Heat Shock Proteins (Hsps), one of the molecular chaperone proteins, are induced by stress or damage such as spinal cord injury (SCI). Recently, Hsps, especially Hsp70 have been found to play an important role for neuroprotection relevance to cell death in SCI but there is a paucity of

study about the role of Hsp27, and 90 in the secondary injury after SCI. In this study, we investigated the change of spinal Hsp27, 70, and 90 expression patterns after SCI. Spinal cord injury was made at T10 level by free-weight dropping a 10 g from a 12.5 mm height using a New York University (NYU) impactor under isoflurane anesthesia. And to assess the temporal changes of Hsp27, 70, and 90 expressions was performed by western blotting in the injured lesion, epicenter at 4h, 8h, 12h, 24h, 3d, and 7d after SCI. Hsp27 expression was significantly increased from 4h to 7days after SCI. Hsp70 was increased immediately after injury but it returned around basal level 7 days after SCI. Interestingly, HSP 90 expression was decreased less than basal level until 7 days after SCI. These results demonstrated that the Hsp27, 70, and 90 were differentially expressed in injured lesion with a different time-course following SCI. This result will be provide a scientific information for further study to explore the role of Hsp27, 70, and 90 on neuroprotection and the secondary injury mechanisms after SCI.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Program#/Poster#: 316.08/S7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Research Foundation of Korea (NRF) grant 2013R1A1A2013440

Title: The neuroprotective role of peroxisome proliferator activated receptor-gamma (PPAR-γ) after spinal cord injury in rats

Authors: Y. KIM[1,2], J. OH[1], J. KIM[2,3], *Y. W. YOON[1];
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Abstract: Traumatic spinal cord injury (SCI) causes deficit of physiological functions below the damaged spinal segment. Pathways in biosynthesis of eicosanoids which result from membrane breakdown extremely increase immediately after SCI. Products of those are the ligands of peroxisome proliferator activated receptors (PPARs), which are a nuclear receptor to regulate gene transcription related to glucose and lipid metabolism. PPARs are classified into three isotype (α, β/δ, γ) in the spinal cord. Above all, PPAR-γ is well studied as regulation of inflammation and neuroprotection in various neurological degenerative diseases. Thus, we

investigated how the PPAR-γ has a neuroprotective role after SCI in rats. The spinal cord of the 10th thoracic vertebra level was contused using NYU device (10 g weight, 12.5mm height) after laminectomy under anesthesia with ketamine/rompun mixture (1:4) in male Sprague-Dawley rats. We examined protein expression of PPAR-γ in the injured epicenter, rostral, caudal and L4-5 region of the spinal cord at various times after SCI (6h, 12h, 24h, 3d, 1w, 3w and 5w). Pioglitazone (PPAR-γ agonist), G3335 (PPAR-γ antagonist) or vehicle (100% DMSO or saline) were administered by intrathecal injection in the early or the late phase after SCI. We assessed locomotor behavior using a BBB rating scale, and analyzed mRNA expression of inflammatory mediators using real-time PCR after the drugs administration. The protein expression of PPAR-γ was excessively increased in all of the spinal segments from 6 hours to 3 days after injury, and then that was gradually returned to basal level. Intrathecal administration of pioglitazone in the early phase improved the joint movement, however, no difference after that. On the other hand, G3335 reduced general locomotion recovery after SCI. mRNA expression of inflammatory mediators and chemoattractant were increased after G3335 treatment in the late phase than in the early phase. These results demonstrated that additionally elevated PPAR-γ may not have benefits to recover motor function anymore, however, deficit of PPAR-γ activity affects to exacerbate it through enhancement of inflammatory reaction.

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Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Program#/Poster#: 316.09/S8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Role of miR 711 in neuronal cell death after spinal cord injury

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Abstract: Spinal cord injury (SCI) triggers secondary injury processes, including neuronal cell death and axonal loss, which contribute to neurological dysfunction. Unfortunately, no current therapeutic strategies are effectively targeting these mechanisms. It is increasingly clear that focusing on one molecule or pathway at a time is unlikely to bring about functionally meaningful neuroprotection and axonal growth. MicroRNAs (miRs) are small (20-23 nucleotide) non-protein-coding RNAs that negatively regulate target gene expression at the posttranscriptional

level by binding to their mRNAs and inducing its degradation and/or inhibiting translation. As single miRs can simultaneously modulate the expression of various proteins in multiple pathways, miRs are attractive candidates as upstream regulators of the secondary SCI progression. Although miRs have been implicated in the pathophysiology of central nervous system (CNS) disorders, few have been directly evaluated at a mechanistic level in traumatic SCI. In the present study, changes in miRs in injured spinal cord after moderate contusion in mice were examined using miR arrays and qPCR. One selected miR (711) was examined with regard to its regulation and relation to neuronal cell death and axonal growth; effects of miR-711 modulation were evaluated after SCI and using *in vitro* primary cortical neurons. Levels of miR-711 were increased in the injured spinal cord early after SCI. Increases coincided with downregulation of the pro-survival protein Akt, a target of miR-711, with sequential activation of glycogen synthase kinase 3 (GSK3)α/β and pro-apoptotic BH3-only molecules PUMA (Bcl2-binding component 3). Central administration of the miR-711 hairpin inhibitor after SCI increased Akt expression and attenuated apoptotic pathways. This treatment increased neuronal survival and axonal myelination, and improved recovery of motor function and coordination. *In vitro*, neurite outgrowth was inhibited in primary cortical neurons transfected with a miR-711 mimic. Together, our data suggest that trauma-induced miR-711 elevation contribute to neuronal cell death after SCI, in part by inhibiting Akt, and may serve as a novel therapeutic target. **Key words:** MicroRNA 711, spinal cord injury, neuroprotection, axonal growth, locomotor function.

Disclosures: **J. Wu:** None. **B. Sabirzhanov:** None. **B.A. Stoica:** None. **J. Matyas:** None. **M. Coll-Miro:** None. **L. Yu:** None. **A.I. Faden:** None.

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 316.10/S9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NEI R01 EY020913

Title: The kruppel like factor gene target dusp 14 regulates axon growth and regeneration

Authors: *J. GALVAO[1], K. IWAO[2], A. APARA[3], Y. WANG[3], M. ASHOURI[3], T. N. SHAH[3], D. L. MOORE[4], M. BLACKMORE[5], N. J. KUNZEVITZKY[6], J. L. GOLDBERG[6]; [1]Ophthalmology, Stanford Univ., Palo Alto, CA; [2]Kumamoto Univ., Kumamoto 860-8556, Japan; [3]Shiley Eye Ctr., La Jolla, CA; [4]Bascom Palmer Eye Inst., Miami, FL; [5]Marquette Univ., Milwaukee, WI; [6]Ophthalmology, Byers Eye Inst., Palo Alto, CA

Abstract: Central nervous system (CNS) neurons in adult mammals are unable to regenerate their axons after injury. Retinal ganglion cells' (RGCs') intrinsic ability to regenerate and extend their axons is limited after birth. Krüppel-like transcription factor (KLF) family members regulate intrinsic axon growth ability both *in vitro* and *in vivo*, but mechanisms downstream of these transcription factors are not known. Here we identify KLF9 as an axon growth suppressor upregulated 250-fold in RGC development, and find that knocking down KLF9 in adult animals promotes long-distance optic nerve regeneration *in vivo*. By screening genes regulated by KLFs in RGCs that promote (KLF7), inhibit (KLF9 and KLF16) or have no effect in axonal growth (KLF11), we identify dual-specificity phosphatase 14 (Dusp14) as a key player limiting axon growth and regenerative ability downstream of KLF9. We demonstrate the KLF9-Dusp14 pathway inhibits the activation of mitogen-activated protein kinases (MAPK), critical to neurotrophic signaling of RGC axon elongation. Decreasing Dusp14 expression or function in RGCs increased axon growth *in vitro* and survival and regeneration after optic nerve crush *in vivo*. These results link intrinsic and extrinsic regulators of axon growth and shed light into the KLF9-Dusp14 pathway. Targeting this pathway could improve regeneration in the adult CNS after injury.

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

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: SHRF

NSERC

Title: Neuroprotective properties of BK channel modulators in an acute spinal cord injury model

Authors: M. JACOBSEN, J. BARDEN, K. LETT, M. KARNITSKY, *J. A. BUTTIGIEG; RIC rm 218, Univ. of Regina, Regina, SK, Canada

Abstract: Spinal cord injury results in significant neuronal and glial cell death resulting in impaired neurological and motor function. While there are often surviving axonal tracts, these are frequently dysmyelinated or demyelinated. Key to this pathology is uncontrolled Ca^{2+} entry,

resulting in excitotoxicity and cell death. We propose that by activating mechanisms attenuating global depolarization and limiting Ca^{2+} entry, we can reduce neuronal death and significantly improve neurological and motor function. To this effect, we previously demonstrated that the large-conductance, voltage and Ca^{2+}-activated K^+ (BK or Maxi K^+) channel is altered after SCI, and may play a part in the neuropathology of SCI. In this study, we set out to explore whether the use of a BK channel activator, Isopimaric acid (ISO), is neuroprotective post spinal cord injury. *Wister* rats received a 25-g clip compression injury at the T7 levels for 1 min. One hour post-surgery, they were administered ISO (120 μM), Ibeurotoxin (IbTx; 100nM), or a vehicle control via mini-osmotic pump. Loss of myelination, and cell death were assessed using immunohistochemistry (IHC). Compound Action Potentials (CAP) and high frequency conduction experiments were performed on spinal cords 8 weeks post surgery. Range of motion and joint velocity of knee, ankle and toe joint segments were assessed using two-dimensional motion capture on a weekly basis for 8 weeks. Animals treated with ISO had preservation or re-establishment of axonal tracts and myelination. Compound action potentials of SCI animals treated with ISO were significantly greater than in untreated animals ($p<0.01$), as was the velocity of the CAP ($p<0.001$), however, there were no differences in refractory period or spike train duration, suggesting that there was no difference in function in the surviving neurons. Finally, animals treated with ISO has significantly greater range of motion and joint velocity of their knee and ankle joints, compared to untreated controls ($p<0.001$), and were not significantly different to lamenectomised animals.
In conclusion, activation of the BK channel during acute SCI may be a novel therapeutic target for acute spinal cord injury. Further studies are required to determine if modulation of the BK channel in chronic injury is beneficial for recovery, and to understand the mechanisms by which this ion channel may be working during spinal cord injury repair.

Disclosures: **M. Jacobsen:** None. **J. Barden:** None. **K. Lett:** None. **M. Karnitsky:** None. **J.A. Buttigieg:** None.

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Program#/Poster#: 316.12/S11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Omega three fatty acids and cooling mitigate altered structural properties of cortical neurons following mechanical injury

Authors: *P. CINTORA[1], Y. J. LEE[1], C. BEST-POPESCU[2];
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Abstract: According to the center for disease control and prevention (the CDC), an estimated 1.7 million brain trauma cases occur each year in the United States (US), and Traumatic Brain Injury (TBI) contributes to a third of all injury-related deaths in the US. Brain trauma can cause immediate, or insidious and persistent, changes in a person's physical, emotional and cognitive functioning. Mild Traumatic Brain Injury (mTBI), or low impact force-induced concussion, is one of the most common causes of brain injury experienced by contact sport athletes and civilians today. In recent years, increasing scientific evidence has emerged strongly suggesting a link between long- term damage of the nervous system and recurrent, low impact force-induced concussions. Concussive forces harm neurons by a variety of mechanisms including: - stretching and shearing of axons; free oxygen radical production; excitotoxic neurotransmitter release; and the release of immune and inflammatory mediators. These consequences of injury may ultimately lead to neuronal degeneration, a break in communication between neurons and subsequent neurodegeneration (i.e. cell death). Using standard cell survival assays, in combination with a novel quantitative form of microscopy Spatial Light Interference Microscopy(SLIM), a cell trauma device (AMScien Instruments) and *in vitro* murine neuronal networks, we characterize and quantify changes in cell survival, neuronal structural integrity, and membrane dynamics after cell culture exposure to mechanical impact (10-14, and 32 psi) and repetitive low force impact. Importantly, we also evaluate how specific physical (e.g. temperature) and chemical reagents (e.g. omega-3 fatty acids and growth factors) modify the impact-induced cellular and network stress response. This *in vitro* injury model promises to broaden our understanding of the injury mechanism of cell- induced trauma and has direct implications in terms of testing effective neuroprotective prevention and treatment strategies.

Disclosures: P. Cintora: None. **Y.J. Lee:** None. **C. Best-Popescu:** None.

Poster

316. Neuroprotective Mechanisms: Brain or Spinal Cord Injury

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Repeated topical application of Acure compound AP-173 enhances neuroprotective efficacy and early functional recovery following spinal cord trauma. A comparative study using systemic dexamethasone treatment in the rat

Authors: *A. K. PANDEY[1], A. SHARMA[2], T. LUNDSTEDT[3], E. SEIFERT[3], A. NOZARI[4], D. F. MURESANU[5], J. V. LAFUENTE[6], R. PATNAIK[7], H. S. SHARMA[2];
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Abstract: Spinal cord injury (SCI) is a devastating disease causing lifetime disability for which no suitable therapeutic strategies are available except use of high doses of corticoids. Although, corticoids in high dose have serious side effects and improvements in functional and/or pathological outcomes are controversial during long-term treatment. Thus, a possibility exists that a combination of dexamethasone with other potent neuroprotective drugs in SCI will have better therapeutic value.
Our previous studies show that topical application of Acure compound AP173 having affinity to melanocortin-4 receptors that binds to both adrenergic receptors α-2a and I-2 receptors showed pronounce neuroprotective effects in SCI on functional and pathological parameters at 5 h. Thus, the possibility that repeated topical application of Acure compound AP173 either alone or in combination with dexamethasone would enhance the therapeutic value in SCI was examined in a rat model. SCI was produced by making a longitudinal incision into the right dorsal horn of the T10-11 segments under equithesin anesthesia and AP173 (10 µg in 20 µl) was administered topically at 5 min, 60 min or 120 min after injury. The rats were allowed to survive 12 h. In other group, dexamethasone (30 mg/kg) was administered intravenously at 5 min, 60 min and 120 min injury. In addition, a combination of AP173 topically and dexamethasone intravenously were given in another group of rats at identical time periods after SCI. Topical application of AP173 alone 5 min after SCI markedly attenuated behavioral dysfunction following 2 to 4 h after injury that was extended to 5 h if repeated application were made at 60 min and 120 min after trauma. On the other hand, beneficial effects of dexamethasone were seen on Tarlov scale at 9 to 12 h if administered either at 5 min, 60 min and/or 120 min after injury repeatedly. However, a combination of AP173 and dexamethasone either given at 5 min, 60 min or 120 min or their repeated administration (5 min+ 60 min +120 min) did not improve the Tarlov scale after trauma. Likewise, the effects of AP173 on spinal cord edema and BSCB permeability were most

marked when it was administered either at 5 min or repeatedly at 5 min, 60 min and 120 min after injury. Interestingly, identical administration of dexamethasone only slightly reduced the edema formation and BSCB permeability. A combination of dexamethasone and AP173 did not improve the neuroprotective effects of either compound in SCI. These results suggest that AP173 if administered repeatedly during early hours of SCI may have better therapeutic efficacy during early phase of spinal pathology and functional outcome as compared to dexamethasone alone.

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Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.01/S13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Protective effect of the L-type calcium channel on the survival of rat cortical cells

Authors: ***T. TAKADERA**[1], N. OKUMURA[2];
[1]Hokuriku Univ., Kanazawa-Shi, Japan; [2]Fukui Univ., Fukui, Japan

Abstract: Calcium ions mediate a variety of physiological neuronal responses, including cell death or survival. The purpose of this study is to examine whether the L-type calcium channel (LTCC) is involved in the survival of rat cortical cells.
Cultures of rat cortical cells were prepared from an embryonic day 18 rat neocortex. Apoptosis was quantified by scoring the percentage of cells that exhibited an apoptotic nuclear morphology at the single cell level. Intracellular calcium levels were measured using the Ca^{2+}-reactive fluorescent probe Fluo4/AM.
Dihydropyridine-type drugs, such as nifedipine, specifically interact with the LTCC and inhibit calcium influx. After being cultured for 8-10 days, the rat cortical cells were subjected to in vitro nifedipine treatment for 48 h, which induced apoptotic cell death. The cell death was accompanied by caspase-3 activation. *N*-methyl-D-aspartate (NMDA), an agonist of the NMDA receptor, protected the cells from nifedipine-induced apoptosis. Calcium influx via the NMDA receptor contributed to protecting the cells from nifedipine-induced apoptosis. On the other hand, inhibitors of the NMDA receptor, such as MK801, ketamine, alcohol, and memantine, induced apoptosis in the rat cortical cells. LTCC agonists, such as FPL64176, protected the cells from NMDA antagonist-induced apoptosis. FPL64176 increased the cortical cells' calcium levels, whereas nifedipine reduced their calcium levels.

Treating the cells with LY294002, an inhibitor of phosphatidylinositol-3 kinase (PI-3K), also induced apoptosis. The addition of NMDA or FPL64176 to the cells protected them from LY294002-induced apoptosis. Insulin-like growth factor I (IGF-I) acts via PI-3K to protect neurons from apoptosis. IGF-I inhibited NMDA receptor inhibitor- and LTCC inhibitor-induced apoptosis. In addition, the neural caspase-dependent apoptosis induced by MK801, nifedipine, and LY294002 was abrogated by glycogen synthase kinase-3 inhibitors. It is suggested that the LTCC and the NMDA receptor both contribute to the survival of cortical neurons, and hence, ensure that neurogenesis progresses normally.

Disclosures: T. Takadera: None. **N. Okumura:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

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Program#/Poster#: 317.02/S14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Regione Autonoma della Sardegna, Italy, L.R. 7/2007-CRP10810/2012

Title: Antidepressants protect mouse HT22 hippocampal cells from apoptosis through activation of the lysophosphatidic acid receptor LPA_1.

Authors: *M. C. OLIANAS, S. DEDONI, P. ONALI;
Biomed. Sciences, Sect. Neurosci., Univ. of Cagliari, Monserrato, Italy

Abstract: Antidepressants have been shown to decrease neuronal vulnerability to a variety of stressors through mechanisms not completely elucidated. The bioactive phospholipid lysophosphatidic acid (LPA) has been shown to modulate neurogenesis, axonal growth, neuronal differentiation and emotional-like behaviour through specific G protein-coupled receptors termed LPA_{1-6}. Recently, we have reported that in CHO fibroblasts different antidepressants trigger growth factor receptor transactivation and cell proliferation by activating LPA_1 receptor. In the present study we show that in immortalized mouse hippocampal HT22 cells ERK1/2 phosphorylation induced by either the tricyclic antidepressant imipramine or the atypical antidepressants mianserin and mirtazapine was antagonized by the selective LPA_1 antagonist AM966 and by Ki16425, which preferentially blocks LPA_1 and LPA_3. ERK1/2 activation induced by either antidepressants or LPA was attenuated by cell treatment with pertussis toxin and blockade of fibroblast growth factor receptor (FGF-R) tyrosine kinase activity with PD173074. Withdrawal of serum induced apoptosis of HT22 hippocampal cells, as indicated by increased annexin V staining, activation of caspase 7 and 3, and stimulation of poly-(ADP

ribose) polymerase cleavage. Both antidepressants and LPA counteracted the apoptotic cell death and this effect was antagonized by blockade of LPA_1 with AM966 and Ki16425 or by inhibition of FGF-R by PD173074. These data indicate that LPA_1 coupled to growth factor receptor transactivation is a novel molecular target of the neuroprotective action of antidepressants

Disclosures: **M.C. Olianas:** None. **S. Dedoni:** None. **P. Onali:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.03/T1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 ES024756

Title: Arundic acid increases the expression and function of human excitatory amino acid transporter 1 (EAAT1) via ERK, Akt and NF-κB pathways

Authors: P. KARKI, *E.-S. Y. LEE;
Physiol., Meharry Med. Col., Nashville, TN

Abstract: Glutamate is the major excitatory neurotransmitter in the brain and excess extracellular glutamate has to be instantly removed from the synapse to prevent excitotoxic neuronal death. Astrocytes play a critical role in maintaining glutamate homeostasis in the brain since two major glutamate transporters- excitatory amino acid transporter (EAAT) 1 and 2 are predominantly expressed in astrocytes. The reduced expression and function of these astrocytic glutamate transporters act as a contributing factor to the pathogenesis of multiple neurological disorders. The dysfunction of EAAT1 has been linked to Alzheimer's disease, ataxia, and traumatic brain injuries, and also to ophthalmic disorders such as glaucoma. Pharmacological compounds that enhance the expression and function of EAAT1 could be potential therapeutics against these diseases. Arundic acid is an astrocyte modulating agent that inhibits the synthesis of neurotoxic astrocytic protein, S100β and with its potent neuroprotective properties this compound is in the process of clinical development for acute ischemic stroke, amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases. Interestingly, arundic acid has been shown to increase EAAT1 expression but the mechanism remains to be elucidated. In this report, we investigated the mechanism of arundic acid-induced upregulation of EAAT1 at transcriptional level and found that NF-κB plays a critical role in this process. In human astrocytic H4 cells, arundic acid-induced increase in EAAT1 promoter activity, mRNA/protein levels and glutamate uptake were blocked by QNZ, a pharmacological inhibitor of NF-κB.

Further, the mutation on NF-κB consensus sites of EAAT1 promoter abolished arundic acid-induced EAAT1 expression. Arundic acid also increased NF-κB reporter activity and induced the nuclear translocation as well as bindings of NF-κB to EAAT1 promoter. Arundic acid activated the Akt and ERK signaling pathway and treatment with LY294002, an Akt specific inhibitor, and U0126, an ERK inhibitor blocked arundic acid-induced increase in EAAT1 expression. Arundic acid also increased EAAT1 promotor activity, mRNA and protein levels in human astrocytes via Akt, ERK and NF-κB pathways. Arundic acid attenuated manganese-induced decreases of EAAT1 promoter activity, mRNA/protein levels and glutamate uptake by suppressing Mn-activated Ying Yang 1 (YY1) expression. These results demonstrate that arundic acid increases the expression and function of EAAT1 via the Akt, ERK and NF-κB signaling cascades and also prevents EAAT1 repression caused by neurotoxins such as manganese via repressing YY1 activation.

Disclosures: P. Karki: None. **E.Y. Lee:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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endowment for the William C. Friday Chair.

Title: Paraoxon effects in hippocampal explants and adult rats: synaptotoxicity and protection through an endocannabinoid enhancement avenue

Authors: S. MCEWAN[1], K. L. G. FARIZATTO[1], H. W. ROMINE[1], M. F. ALMEIDA[1], C. LONG[1], C. MUNDELL[1], A. BYRD[1], V. NAIDOO[2], V. SHUKLA[3], S. NIKAS[3], A. MAKRIYANNIS[3], *B. A. BAHR[1];
[1]Biotech. Res. and Training Ctr., Biotech Ctr. / William C. Friday Lab., Pembroke, NC; [2]Univ. of Cape Town, Cape Town, South Africa; [3]Northeastern Univ., Boston, MA

Abstract: The anticholinesterase paraoxon (Pxn) is related to military nerve agents and, here, was studied in rat hippocampal slice cultures. Low dose treatment (50 μM) caused a slow, time-dependent loss of GluR1 over 3 days, and high dose Pxn (200 μM) resulted in faster decline in synaptic markers. Evidence in the slice cultures indicated the synaptic compromise is transient for low-dose Pxn, but not for the high-dose insult. Pxn was then studied in vivo, and injected adult rats exhibited a pathogenic cascade initiated by seizure events. Subsequent signs of damage were evident by higher levels of brain proteins labeled with an antibody against 4-hydroxynonenal (4-HNE), an indicator of oxidative stress. Loss of synaptic markers was also found in samples of frontal cortex and hippocampus, regions implicated in learning and memory. Next, we tested a fatty acid amide hydrolase (FAAH) inhibitor (AM5206) for protective effects in Pxn rats since such FAAH modulation has been found beneficial for offsetting seizure-related pathology (Naidoo et al. 2011-12: J Mol Neurosci 43:493, Neurotherapeutics 9:801). The ip injected AM5206 reduced Pxn-induced seizure levels by 86% ($p<0.001$), and it improved balance and coordination measured 24 h post-insult ($p<0.01$). In addition to the seizure and functional protection, evidence shows that AM5206 reduced the 4-HNE indicator of oxidative stress, and the results were in conjunction with synaptic protection. These results support the endocannabinoid enhancement pathway for neuroprotection, in which negative modulation of FAAH slows the breakdown of endogenous cannabinoids, thus allowing them to persist, activating CB1 receptors and repair mechanisms linked to the reduction in excitotoxic progression. FAAH inhibition is an area of research focused on exploiting signaling pathways to prevent and treat neurological damage.

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Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AG 022550

NIH AG 027956

NIH T32 AG 020494

Title: Androgen receptor-independent mechanisms for dihydrotestosterone protection in rodent astrocytes

Authors: ***N. K. KUBELKA**, N. RYBALCHENKO, M. SINGH;
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Abstract: Dihydrotestosterone (DHT) is known to exert protective effects in the central nervous system through activation of the intracellular androgen receptor (AR). However, recent studies suggest that DHT may also exert protective effects by way of alternate mechanisms that include prior conversion to 5-alpha androstane-3,17 beta diol, (3beta-diol), a metabolite that can bind to and activate estrogen receptors. Using rodent-derived astrocytes, which lack classical androgen receptor expression, we found that DHT protected against the cytotoxic effects of iodoacetic acid (IAA) toxicity, a metabolic and pro-oxidant stressor. The protective effects of DHT, as assessed by the Calcein-AM assay, were blocked by the co-application of the non-selective estrogen receptor (ER) antagonist, ICI-182,780. This finding implicated ERs as mediators of DHT-induced cytoprotection. Using a complementary viability assay, the MTT assay, which is not only a surrogate marker for cell viability/number, but also an index of mitochondrial respiration, we found that 3beta-diol was also protective against IAA. Interestingly, while the effects of 3beta-diol, the presumptive mediator of the effects of DHT, were blocked by ICI-182,780, they were not blocked by the ER isoform-selective antagonists MPP, against ER alpha, and PHTPP, against ER beta. Collectively, these data support our hypothesis that DHT is protective against cytotoxicity in rodent astrocytes, which lack classical androgen receptor expression, and that the metabolite of DHT, 3beta-diol, may be an important mediator of DHT's effects. Our results also implicate the capacity to convert DHT to 3beta-diol as a relevant factor in conferring protection in postmenopausal women, since in the postmenopausal period, circulating estrogen and progesterone levels have diminished, but androgen levels persist.

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Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.06/T4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Knock-down of targets in the rat CNS using Antisense Oligonucleotides: Kinetics of distribution and pharmacodynamics in the CNS after an intrathecal dose

Authors: *F. KAMME[1], B. POWERS[2], C. MAZUR[2], D. A. WOLF[3], J. M. SULLIVAN[4], D. A. NORRIS[2], A. VERMA[3], E. SWAYZE[2];
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Abstract: Antisense Oligonucleotides (ASOs) reduce the amount of a target protein by hybridizing to the cognate RNA. The ASO-RNA hybrid is detected by RNAseH1 and leads to scission and subsequent nonsense-mediated decay of the RNA. In turn, this leads to decreased expression of the target protein. ASO drugs are used clinically, and several are in late stage clinical development, for systemic as well as CNS indications. This study was undertaken to characterize distribution and pharmacokinetics of ASOs in the rat CNS after an intrathecal dose. We imaged ASO distribution in rat brain as a function of time and dose. Imaging was performed semi-quantitatively using an ASO-specific antibody, immunofluorescent detection and high-throughput laser scanning. Furthermore, ASO distribution in live rats was measured by ^{125}I-labelled ASO and whole body SPECT/CT. Down regulation of the target mRNA was measured with qRT-PCR and protein downregulation measured with immunofluorescence and laser based scanning. Finally, ASO tissue concentrations were measured using ELISA. SPECT/CT showed rapid distribution of the dosed ASO from the site of injection in the lumbar cord, to the brain. ASO-staining showed early diffusion of ASO from CSF in the subdural space into brain parenchyma. At 6-8 h post dosing, ASO redistributed rapidly from what appeared to be the extracellular space, into cell bodies. At 24 h, ASO staining showed widespread distribution throughout the rat CNS, including hippocampus, striatum and brainstem. After 24h, changes in ASO distribution were subtle, with an overall gradual decrease in staining intensity until the end of the time course, 16 weeks. RNA knock down was maximal at 7 days after dosing, and maintained at >50% mRNA reduction until week 8. Immunostaining showed a concomitant reduction in protein expression throughout the brain. We show widespread distribution of an intrathecally delivered ASO, into rat brain and spinal cord. Target mRNA and protein knock-down was efficient and sustained. ASO technology is a versatile and powerful platform for CNS research that is rapidly translatable into clinical applications.

Disclosures: **F. Kamme:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **B. Powers:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **C. Mazur:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **D.A. Wolf:** A. Employment/Salary (full or part-time): Biogen Inc. **J.M. Sullivan:** A. Employment/Salary (full or part-time): inviCRO LLC. **D.A. Norris:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals. **A. Verma:** A. Employment/Salary (full or part-time): Biogen Inc. **E. Swayze:** A. Employment/Salary (full or part-time): Ionis Pharmaceuticals.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Natural Science Foundation of China (No. 31171022)

Title: The dysfunction of glutamate transporters on the endothelial cells induced by A2AR decreases intracranial glutamate efflux across the blood brain barrier

Authors: *W. BAI, N. YANG, X. CHEN, Y.-L. NING, P. LI, Y. PENG, R.-P. XIONG, Y. ZHAO, Y.-G. ZHOU;
Mol. Biol. Center,State Key Lab. of Trauma,Burn and Combined Injury, Res. Inst. of Surgery and Daping Hosp., Chongqing City, China

Abstract: Besides of the re-uptake by nerve cells, the outward transport by endothelial glutamate transporters is an important mechanism to ensure the intracranial glutamate maintaining at a low level. Our previous study found that the increased level of intracranial glutamate after traumatic brain injury (TBI) was associated with the change of blood glutamate level, suggesting that there was an disturbance of glutamate outward transport across the blood brain barrier (BBB). In this study, we found that Adenosine A2A receptor (A2AR) selective agonist CGS21680 significantly decreased the efflux of intracranial H^3-glutamate, thus lead to the increase of intracranial glutamate level. While inhibiting A2AR activity, either by applicating antagonist ZM241385 or A2AR genetic knockout, could recover the reduction of intracranial glutamate outflow caused by TBI. Furthermore, in an oxygen glucose deprivation (OGD) model of monolayer Bend.3 cells ,we found that A2AR activation increased the expression of glutamate transporters (GLAST/GLT-1) but at the same time reduced their transport efficiency from intracellular to extracellular, resulting in a increased glutamate level in culture medium which even higher than the initial level, indicating that A2AR activation might also cause the reverse transport of glutamate with GLAST/GLT-1. We further explored that A2AR activation could decrease the PKA-dependent interaction of Na^+/K^+-ATPase α1 subunit and phospholemman (FXYD1), thus decreasing the Na^+/K^+-ATPase activity and the Na^+/K^+ distribution inside and outside the cells, leading to rapid decreasing of Na^+-dependent GLAST/GLT-1 efficiency. Consistent with these results, these effects were all inhibited by administration of A2AR antagonist ZM241385. Taken together, the above results showed that A2AR may play an important role in regulating intracranial glutamate homeostasis, and the dysfunction of GLAST/GLT-1 induced by A2AR activation contributes significantly for the sharp rise of intracranial glutamate level after TBI and other brain injuries, which provides a potential therapeutic target for the treatment of brain injury.

Disclosures: **W. Bai:** None. **N. Yang:** None. **X. Chen:** None. **Y. Ning:** None. **P. Li:** None. **Y. Peng:** None. **R. Xiong:** None. **Y. Zhao:** None. **Y. Zhou:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.08/T6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: N-docosahexaenoylethanolamine enables robust optic nerve regeneration after injury

Authors: *H. KWON, H.-Y. KIM;
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Abstract: *N*-docosahexaenoylethanolamine (synaptamide), a structural analog of the cannabinoid receptor ligand anandamide, is an endogenous metabolite of docosahexaenoic acid (DHA). Our previous studies have shown that synaptamide stimulates neurite growth, neurogenesis and synaptogenesis. In this study, we investigated the effect of synaptamide on axon growth and regeneration after injury. For *in vitro* studies, mouse P0 mouse primary cortical neurons were plated on an axon chamber and axotomy was performed on 7DIV when axons were sufficiently grown into the other side of the axon chamber. To investigate axon regeneration *in vivo*, mice at 12-14 week of age were injected with synaptamide (10-25 mg/kg) intravitreally after optic nerve crush (ONC). Synaptamide at nM concentrations promoted axon outgrowth *in vitro* through the activation of G protein coupled receptor (GPCR) 110/cAMP/ protein kinase A (PKA) signaling. Significant increases in axon growth were observed when synaptamide was added to either soma or axon side of the chamber. Axon growth after axotomy was also promoted by synaptamide addition to either side of the chamber. Using bodipy-synaptamide and GPR110 antibody, direct physical interaction of synaptamide and GPR110 in primary cortical neuron was demonstrated, and this interaction was crucial for the observed axon outgrowth. Knocking out *gpr110* abolished synaptamide effects on axon outgrowth and regeneration, indicating that GPR110 is required for synaptamide-mediated axon regeneration. In the ONC animal model, axon regeneration was promoted after synaptamide injection. Moreover, electroretinogram (ERG) and visual evoked potentials (VEP) measured at 3 months following optic nerve injury indicated significant improvement of visual function with the synapatamide injection. Collectively, these findings suggest synaptamide as a potential therapeutic agent for functional recovery after nerve injury.

Disclosures: **H. Kwon:** None. **H. Kim:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.09/T7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Howard Hughes Medical Institute

Roche Postdoctoral Fellowship, RPF 311

Title: Efficient protein-based genome editing by local delivery in the brain.

Authors: *B. T. STAAHL[1], M. BENEKAREDDY[4], C. COULON-BAINIER[4], A. GHOSH[4,5], J. A. DOUDNA[2,6,7,8,3];
[2]Mol. and Cell Biol., [3]Dept. of Chem., [1]Univ. of California, Berkeley, Berkeley, CA; [4]F. Hoffmann-La Roche Pharma Res. and Early Develop., Basel, Switzerland; [5]E-Scape Bio, San Francisco, CA; [6]Howard Hughes Med. Inst., Berkeley, CA; [7]Innovative Genomics Initiative, Berkeley, CA; [8]Physical Biosci. Division, Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: RNA-guided genome editing triggered by the CRISPR-Cas9 DNA endonuclease has the potential to cure the underlying cause of a genetic disease. This therapeutic strategy will require tissue-specific delivery of the editing molecules *in vivo*, a goal that is particularly challenging in the brain. Here we show the Cas9 ribonucleoprotein (RNP) complexes can be used to edit post-mitotic neurons in the mouse brain. We also show that protein engineering of Cas9 increased the efficiency of *in vivo* neuronal cell editing ten-fold. Cas9 RNPs introduced locally in the brain trigger genome editing in cells including hippocampal, striatal and cortical neurons by a mechanism that involves spreading beyond the immediate site of injection. We also show that Cas9 RNP complexes are stable over 6 hours in blood plasma. These advances provide a robust technology for application of genome-editing in the brain to treat the underlying cause of neurological genetic diseases.

Disclosures: **B.T. Staahl:** A. Employment/Salary (full or part-time): University of California, Berkeley. **M. Benekareddy:** A. Employment/Salary (full or part-time): Roche Pharma Research and Early Development, Basel, Switzerland. **C. Coulon-Bainier:** A. Employment/Salary (full or part-time): Roche Pharma Research and Early Development, Basel, Switzerland. **A. Ghosh:** A. Employment/Salary (full or part-time): E-Scape Bio, San Francisco, California, USA. **J.A. Doudna:** A. Employment/Salary (full or part-time): University of California, Berkeley, California, USA., Innovative Genomics Initiative, University of California, Berkeley, California, USA.., Howard Hughes Medical Institute, University of California, Berkeley, California, USA., Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA., Department of Chemistry, University of California, Berkeley, California, USA..

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.10/T8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Inhibition of Cdc42 signaling enhances the sensitivity of cerebellar granule neurons to intrinsic apoptosis

Authors: *N. PUNESSEN, A. NGUYEN, D. A. LINSEMAN;
Univ. of Denver, Denver, CO

Abstract: Neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease are caused by loss of specific populations of neurons in the brain, brainstem, or spinal cord. Many of these diseases lack highly effective treatments, as most current therapeutics only provide symptom relief but do not alter the underlying cell death mechanisms that contribute to disease progression. One of the most significant contributors to neurodegeneration is excessive programmed cell death, also known as apoptosis. Rho family GTPases, including Rho, Rac and Cdc42, are critical regulators of neuronal growth cone dynamics, axonal outgrowth, and dendritic spine morphogenesis. In addition, these GTPases are also key modulators of neuronal survival and apoptosis. A substantial body of literature has demonstrated a largely pro-apoptotic role for Rho in neurons, while Rac typically plays a pro-survival role. In contrast to the well described antagonistic roles of Rac and Rho in regulating neuronal survival, relatively little is known regarding the effects of Cdc42 on this process. Here, we investigated the role of Cdc42 in cerebellar granule neuron (CGN) survival by testing specific inhibitors of Cdc42 function or one of its downstream effectors. CGNs require serum and depolarizing extracellular potassium (25K healthy medium) for their survival in culture and removal of these survival signals (5K apoptotic medium) leads to intrinsic apoptosis of approximately 50% of the cells after 24 hours. Inhibition of Cdc42 with two dissimilarly structured compounds, ZCL278 and ML141, had no effect on CGN survival in 25K healthy medium but markedly potentiated apoptosis in 5K apoptotic medium. Inhibitors of the downstream Cdc42 effectors, PAK (FRAX486), ACK (AIM100), and N-WASP (Wiskostatin), each produced similar enhancement of CGN apoptosis in 5K apoptotic medium without inducing overt apoptosis in 25K healthy medium. We are currently utilizing molecular approaches (siRNA and dominant negative constructs) to confirm the results obtained with these chemical inhibitors. Overall, the data suggest that Cdc42 signaling to multiple downstream effector proteins plays a pro-survival role in CGNs. Interruption of these Cdc42 pathways is not sufficient on its own to trigger CGN apoptosis in the presence of strong survival signals like serum and depolarizing potassium. However, inhibition of Cdc42 signaling does significantly sensitize CGNs to intrinsic

apoptosis. It will be of interest in the future to determine if Cdc42 pro-survival signals are compromised in various types of neurodegenerative disease.

Disclosures: **N. Punessen:** None. **A. Nguyen:** None. **D.A. Linseman:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.11/T9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Identification of proteins that bind to LDL Receptor-related Protein-1 (LRP1) in Schwann cells and activate c-Jun *In vitro* and *In vivo*.

Authors: *A. FLÜTSCH[1], A. GILDER[2], K. HENRY[1], E. MANTUANO[2], S. L. GONIAS[2], W. M. CAMPANA[1];
[1]Dept. of Anesthesiol., [2]Departments of Pathology, UCSD, La Jolla, CA

Abstract: After injury, Schwann cells (SCs) serve as "first responders" by transdifferentiating into a repair phenotype that supports proliferation, cell migration, secretion of growth factors and extracellular matrix proteins and clearance of degraded myelin. These processes are essential for functional nerve regeneration and are referred to as activation of the SC Repair Program (Jessen *et al*. 2015 Dev Cell 34:613-20). LRP1 is up-regulated in SCs after injury and orchestrates many elements of the SC Repair Program (Campana *et al*., 2006 J Neurosci 26(43):1197-207). Cell signaling pathways activated by SC LRP1 include ERK1/2, PI3K, Rac1, and the unfolded protein response (UPR).
The transcription factor c-Jun plays an essential role in the activation of the SC Repair Program (Arthur-Farraj *et al*., 2012 Neuron 75(4):663-47), however SC signaling receptors that regulate c-Jun have not been identified. To address this question, primary SCs were treated with the known LRP1 ligands, tissue-type plasminogen activator (tPA) or the hemopexin domain of MMP-9 (PEX), for 5-30 minutes. Both LRP1 ligands robustly phosphorylated c-Jun and the response was blocked by Receptor-associated Protein (RAP), a known LRP1 antagonist. To confirm that LRP1 activates c-Jun *in vivo*, sciatic nerve injury was induced in Sprague Dawley rats. 24 hours later, when LRP1 is substantially up-regulated in SCs, tPA or PEX was injected directly into the nerves. After 15 min, both ligands significantly increased c-Jun activation as determined by immunoblotting.
To identify novel LRP1 ligands, responsible for activating the SC Repair Program, we applied a proteomics-based discovery approach. Extracts of injured sciatic nerves, prepared so that intact cells are not disrupted, were immunoprecipitated with Fc-fusion proteins containing the

extracellular ligand-binding domains of LRP1 (CCR2 and CCR4). The same samples were incubated with free Fc protein as a control. Tandem mass spectrometry (LC-MS/MS) revealed potential novel LRP1 ligands including Pacsin-1, the axonal protein NFM, and von Willebrand factor. The LRP1-interactome was computationally scored with MiST to exclude false positives and to narrow down candidates for *in vitro* binding validation. Novel LRP1 ligands involved in c-Jun activation may be used therapeutically to augment the SC Repair Program and thus, enhance functional nerve repair.

Disclosures: **A. Flütsch:** None. **A. Gilder:** None. **K. Henry:** None. **E. Mantuano:** None. **S.L. Gonias:** None. **W.M. Campana:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.12/T10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Immunohistochemistry of selected gene candidates following early life exposure of NMDA and glutamate to hippocampal neurons reveals diversity

Authors: *L. K. FRIEDMAN[1], A. SLOMKO[2];
[2]Cell Biol. and Anat., [1]New York Med. Col., Valhalla, NY

Abstract: Immature neurons can resist neurotoxicity and are capable of generating substantial preconditioning effects in response to seizures or certain excitatory amino acids (EAAs). Our recent transcriptome profiling of hippocampal neurons revealed many up- and down- regulated genes involving distinct Ca^{2+}-binding protein families, G-coupled proteins, growth factors, synaptic vesicle docking factors, neurotransmitter receptors, heat shock, oxidative stress, and anti-apoptotic Bcl-2 gene members that influence neuronal survival. In order to verify regulation of certain candidate genes involved in neuronal preconditioning neuroprotection, we used our prior protocol to expose immature hippocampal neurons to high doses of glutamate (250μM) or NMDA (100μM) for 48 hrs (5DIV), followed by immunohistochemistry with 15 specific antibodies 7 days later when cultured neurons mature (14DIV). A marked decrease in the number of Calb1 and Calm2 positive neurons was observed following NMDA but not after glutamate treatment whereas ryanodine and Cav1.2 voltage gated channel expression was steady under both conditions. A dramatic reduction in the density of $GABA_A\alpha5$ and $GABA_B$ receptor expressing neurons was also observed by prior NMDA exposure but immunodensity measurements of the survivors was unchanged as was expression of the GABA synthesizing enzyme, GAD, suggesting fast inhibitory neurotransmission and response to benzodiazapines

and $GABA_B$-mediated IPSPs may not be reduced in mature survivors despite loss of inhibitory networks. While NR1 mRNA expression was decreased in the microarrays, protein expression revealed selective loss of the NR1 C1 splice variant. Calm2 which can induce inactivation of NMDA receptors by binding tightly to C1 but not C2 regions of its NR1 subunit suggests that the C1 splice variant is co-regulated with Calm2 and that simultaneous loss of these proteins would reduce subsequent NR1 trafficking, phosphorylation, and NMDA currents following early life NMDA exposure. Selective upregulation of Chat after glutamate treatment can lead to persistent increases in cholinergic content. CNRIP mRNA and protein, CB1 interacting, was upregulated under both conditions which can inhibit excitatory neurotransmission and contribute to preconditioning. Diverse changes following overactivation of excitatory receptors of immature neurons appear permanent and are expected to have profound effects on network function and adaptive responses to further insult. Understanding immature defenses may allow translational intervention to improve histopathology and memory and attention tasks deficits that follow neurological insults in adulthood.

Disclosures: L.K. Friedman: None. **A. Slomko:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.13/T11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KAKENHI (25340104)

KAKENHI (16K00626)

SENRYAKU (2013-2017)

Title: The importance of expression of nur family genes on neurite outgrowth through the effect of p300 and histone modification

Authors: *K. SHIMOKE[1], R. YAMAZOE[1], Y. NISHIHATA[1], H. MARUOKA[2];
[1]Kanasai Univ., Suita, Osaka, Japan; [2]KURABO, Kadoma, Osaka, Japan

Abstract: Nerve cells are specialized to transduce an electric signal via the axon, thus it is necessary to construct the neuronal network by the elongation of neurites.In this process, specific genes are expressed during neurite formation. Identification and functional analyses of these genes are important in developing a new strategy for regenerative therapy.We have previously analyzed that valproic acid (VPA), a histone deacetylase inhibitor, promoted neurite outgrowth.

In addition, we have demonstrated that VPA induced the nur family, nur77 and nurr1 genes, in PC12 cells.In present study, we investigated which gene belonging to the nur family, nur77 or nurr1, was important in elongation of the neurites in the presence of VPA by knock-down experiments that suppress nur77 or nurr1 mRNA and p300 mRNA by the specific siRNAs, showing that both genes are important for neurite outgrowth through the function of p300 in the presence of VPA. We also found that epigenetic regulation via histone H3 modification was important for the VPA-induced neurite outgrowth.
These results suggest that up-regulation of nur77 and nurr1 are involved in neurite outgrowth in the presence of VPA through the effect of p300 and acetylation of histone H3 at the specific lysine residue.

Disclosures: **K. Shimoke:** None. **R. Yamazoe:** None. **Y. Nishihata:** None. **H. Maruoka:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Creighton University Health Science Strategic Initiative Award

NIAID R15AI118550

Title: Investigation of intracellular signaling and non-coding RNA underlying microglial polarity Induced by neuronal damage.

Authors: S. YACKLEY[1], C. MEYER[1], E. WHITEFORD[1], M. TAPPATA[1], *A. SHIBATA[2]; [1]Biol., [2]Biol. Dept., Creighton Univ., Omaha, NE

Abstract: Activated microglia, the resident immune cells in the CNS, can trigger neurotoxic inflammatory responses or promote neurogenesis and neuronal survival. Microglia exhibit functional polarization becoming pro-inflammatory (M1) or anti-inflammatory (M2) depending upon the activating stimulus. The underlying mechanisms of microglia polarity are still not fully understood. We hypothesize that traumatic injury to cortical neurons polarizes microglia to an M2-like state to promote neurogenesis. We utilize an *in vitro* model system to examine how neuronal damage influences microglial polarization. Immunocytochemical, immunoblot, FLOW and RT-PCR analyses suggest that microglia responding to damaged neurons acquire an M2-like phenotype expressing higher levels of M2 markers CD206 and Arg1 and lower levels of M1

markers CD45 and CD86. A significant decrease in the neurotoxic cytokines IFN-γ and TNF-α and a 23 ± 2.5% increase in the neurogenic cytokine MCP-1 was measured by ELISA in media from microglia co-cultured with damaged neurons as compared to media from microglia co-cultured with undamaged neurons ($p<0.05$). RT-PCR analysis confirms a reduction of IFN-γ and TNF-α mRNA and an increase in MCP-1 mRNA in microglia co-cultured with damaged neurons. Reactive nitrogen species production by microglia co-cultured with damaged neurons was significantly lower (1.26 ±0.09 mM) than that produced by LPS-stimulated M1 microglia (6.05 ±0.06 mM, $p<0.05$). Transition to a neurogenic M2-like microglial phenotype may involve regulation of protein expression via non-coding RNAs. RT-RCR analysis of microRNA (miR) in microglia co-cultured with neurons revealed significant increases in let-7c, miR-124, and miR-9 expression. Co-culture of microglia with undamaged neurons increased let-7c expression by 8.5 fold ± 2.0 ($p=.038$) and miR-124 expression by 9.8 ± 1.3 ($p=.002$) as compared to control microglia. Co-culture of microglia with damaged neurons increased let-7c expression by 12.2 fold ± 3.9 ($p=.009$) and miR-124 expression by 9.9 ± 0.9 ($p=.0001$) as compared to control microglia. MiR-9 expression is specifically increased (4.6± .64 fold) in microglia co-cultured with damaged neurons as compared to microglia co-cultured with undamaged neurons ($p=.037$) suggesting a specific regulatory role for miR-9 in neurogenic microglia. Ongoing immunoblot analysis suggests MAPK pathway and STAT signaling is associated with polarization of microglia following activation by neuronal damage. Understanding the immune mechanisms that drive the neurogenic phenotype of microglia will provide insight into the intrinsic neuroprotective role of immune activity in the CNS.

Disclosures: **S. Yackley:** None. **C. Meyer:** None. **E. Whiteford:** None. **M. Tappata:** None. **A. Shibata:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 317.15/T13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP

CAPES

CNPq

Title: Drugs that affect dna methylation modulate neuritogenesis in sh-sy5y cells

Authors: *R. A. CANTELMO[1], A. C. SANTOS[2], N. A. G. SANTOS[2], S. R. L. JOCA[3]; [1]Sch. of Pharmaceut. Sci. of Ribeirão Pret, Ribeirao Preto, Brazil; [2]Dept. of Toxicology, [3]Dept. of Pharmacol. Sci., Sch. of Pharmaceut. Sci. of Ribeirão Preto, Ribeirão Preto, Brazil

Abstract: Introduction: DNA methylation is an epigenetic mechanism catalyzed by DNA methyl transferases (DNMT) and associated with gene silencing. Studies suggest that it regulates the expression of genes associated with neural plasticity and might play a role in neurodegenerative diseases. However, little is known about how drugs that target DNA methylation affect neuroplasticity. The present study addresses this issue in SH-SY5Y cells, a widely used neuronal cell model. **Objective:** The aim is to evaluate the effect of two DNMT inhibitors (5-aza-cD, 5-aza-2´-deoxycytidine and RG108, n-phthaloyl-l-tryptophan) and of a metil donor (SAM, S-adenosil-metionine) on neuritogenesis in SH-SY5Y cells stimulated (or not) with retinoic acid (RA), which induces the expression of neurotrophin receptors (trkB and trkC) in these cells. **Materials and Methods:** Growth medium: F12 Ham plus 15% fetal bovine serum (FBS) and 1% antibiotics (PSN). Neurite outgrowth assays: $1x10^5$ cells/well in 24-well plates for 24h; medium was replaced by F12K plus 1% FBS with (or without) 10 μM RA and incubation for 5 days. Additions: RG108 (2000 μM, 100 μM and 4 μM), 5-aza-cD (0.5 μM and 0.005 μM) or SAM (10 nM) and incubation for 72h. Quantitation: inverted-phase-contrast microscopy and Image J open source software. Statistics: one-way ANOVA with Dunnet´s multiple comparisons test or Mann-Whitney t-test for pairs, $P < 0.05$, GraphPad Prism software. **Results:** RG108 (100 μM and 4 μM) 5-aza-cD (0.5 μM) and SAM (10 nM) increased the neuritogenesis of RA-differentiated cells; while RG108 (2000 μM) completely inhibited the neuritogenesis. RG108 (4 μM), 5-aza-cD (0.5 μM and 0.005 μM) and SAM (10 nM) also increased the neuritogenesis of non-RA-differentiated cells; while RG108 (2000 μM) decreased it. **Discussion and Conclusions:** DNA methylation regulators are able to increase the neuritogenesis in SH-SY5Y cells regardless the stimulation by retinoic acid, which suggests their ability not only to activate neurotrophin receptors, but also to induce their expression in this cellular model. Pharmacological modulation of DNA methylation might be an important target to modulate neuroplasticity and the underlying pathways should be investigated.

Disclosures: R.A. Cantelmo: None. **A.C. Santos:** None. **N.A.G. Santos:** None. **S.R.L. Joca:** None.

Poster

317. Neuroprotective Mechanisms: Signaling and Gene Expression

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 NS069835

Title: Pathological stimuli-induced HDAC1 nuclear export is dependent on calcineurin-mediated dephosporylation

Authors: *Y. ZHU, O. G. VIDAURRE, K. P. ADULA, N. KEZUNOVIC, G. HUNTLEY, P. CASACCIA;
Neurosci. Dept., Icahn school of Med. at Mount Sinai, New York, NY

Abstract: Axonal damage is a common pathological feature shared by many distinct neurodegenerative disorders, but its mechanism is only partially understood. Previous work from our lab identified HDAC1 nuclear export as one of the mechanisms contributing to impaired mitochondrial transport and axonal damage. We also reported that inhibition of nuclear export ameliorated the clinical signs related to axonal damage in animal models of demyelination. In our current study, we showed that neuronal-specific ablation of HDAC1 in specific brain regions (by crossing floxed HDAC1 mice to Camk2a-Cre or Grik4-Cre mice) partially protected axons and circuits from excitatory amino acid and cytokine induced damage. In addition, site-directed mutagenesis of serine 421 and 423 to alanine residues promoted the nuclear-cytosolic shuttling of HDAC1, suggesting that dephosphorylation of the serine residues is critical in the process. Importantly, overexpression of dephosphorylated HDAC1 in naïve neurons was sufficient to induce onset of axonal damage. Finally, since our previous studies identified intracellular calcium as an upstream mediator of HDAC1 translocation, we hypothesized that the calcium-dependent phosphatase calcineurin may also play a role in the process. Inhibition of calcineurin activity by the specific inhibitor FK506 prevented HDAC1 nuclear export as well as axonal damage in neurons treated with excitatory amino acid and cytokine. Taken together, our study provides novel insights to the molecular mechanism of HDAC1 translocation and axonal damage under pathological conditions, highlighting HDAC1 as a potential therapeutic target that can promote neuroprotection in a variety of neurodegenerative disorders.

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Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Grant FRN93603

Weston Brain Institute

Title: A time course analysis of focused ultrasound mediated glial activation and neuronal stress responses.

Authors: *J. SILBURT[1], K. MARKHAM-COULTES[3], M. A. O'REILLY[4,2], K. HYNYNEN[4,2], I. AUBERT[3,1];
[1]Lab. Med. and Pathobiology, [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [3]Brain Sci., [4]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Focused ultrasound (FUS) has been used to locally and temporarily permeabilize the blood-brain barrier (BBB), facilitating the non-invasive delivery of therapeutics from the blood to the brain. This technology can be used to deliver genes, stem cells, and therapeutics in animal models. The current safety profile of transcranial FUS-induced BBB permeability led to a clinical trial for the treatment of brain tumors. Clinical applications for neurological conditions such as Alzheimer's disease are being developed and are requiring additional information on the impact of FUS on neural responses. To date, most analysis on safety has focused on gross histology and have not analyzed in depth neuronal stress and glial activation. We have previously shown that in the cortex, following FUS treatment microglia and astrocytes are activated by 4 hours and 4 days respectively, both of which resolve to baseline by 15 days (Jordão et al, 2013). Since this study, the development of new ultrasound transducers and a feedback control system has substantially improved FUS-induced BBB permeability applications. To further assess the impact of FUS-induced BBB permeability on the brain, we evaluated glial cell activation and neuronal stress responses following FUS, up to 10 days post-treatment in a time-course experiment. Using a feedback control system, we applied FUS unilaterally to the hippocampus of C57Bl/6 mice at 3.5 months of age and subsequently analyzed the neuronal stress and glial response using immunohistochemistry. We demonstrate an activation timeline of microglia and astrocytes following FUS. Both astrocytes and microglia display a transient activation, resolving within 10 days. This activation is specific to the focal points of BBB opening. We also investigated whether FUS damages neurons, and show minimal neuronal stress and no neurodegeneration. We conclude that using the feedback control system, FUS induces a mild and transient activation of glial cells. This provides further evidence that FUS-mediated BBB permeability can be applied to the brain in a controlled manner, without inducing damage.

Disclosures: J. Silburt: None. **K. Markham-Coultes:** None. **M.A. O'Reilly:** None. **K. Hynynen:** None. **I. Aubert:** None.

Poster

318. Neuroinflammation

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Program#/Poster#: 318.02/T16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FRN93603

Weston Brain Institute

Title: Proteomic analysis of focused ultrasound-induced blood-brain barrier permeability

Authors: *M. LYNCH[1,3], M. KAWAJA[5], J. SILBURT[1,3], S. HEINEN[1], I. AUBERT[1,3], M. O'REILLY[2,4], K. HYNYNEN[2,4];
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Abstract: Focused ultrasound (FUS), in presence of microbubbles, transiently induces blood-brain barrier (BBB) permeability, allowing for the delivery of therapeutics to targeted areas of the brain. FUS has been shown to safely induce BBB permeability without causing edema, hemorrhage or red blood cell extravasation. However, the effect of FUS on the proteomic profile, particularly regarding stress proteins and molecular chaperones, remains unknown. Furthermore, most FUS safety studies have been conducted in healthy models while clinical use includes neurological disorders, such as Alzheimer's disease (AD). As such, the effects of FUS in disease states must also be explored.
Transgenic amyloidosis mice and their non-transgenic littermates underwent unilateral FUS treatment. 24-hours, 2 and 9 days after FUS, mice were sacrificed and treated/untreated hemispheres as well as hippocampal regions were collected for proteomic analysis. Briefly, samples were trypsinized and analyzed using tandem mass tag mass spectrometry. MS/MS samples were analyzed using Sequest and X! Tandem and protein identification was done using Scaffold. Using the untreated contralateral hemisphere as a control we examined the effect of FUS in non-transgenic and transgenic mice both separately and together. We searched specifically for heat shock proteins, molecular chaperones, inflammatory markers, apoptosis, transcription and translation to identify stress and damage related proteomic changes. In addition, using 2D gel electrophoresis we identified upwards of 20 unique proteins modulated by FUS. This study demonstrates that FUS-induced BBB permeability can modulate protein expression in the brain, with minimal impact on stress and damage related proteins, in a mouse model of amyloidosis and their non-transgenic littermates. As FUS is being considered for clinical trial in the treatment of Alzheimer's disease, it is important to know how this method can change protein expression in the brain, in both healthy and pathological conditions.

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Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 318.03/T17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2014R1A2A1A11050246

Title: Induction of osteopontin expression in 3-nitropropionic acid-induced neurotoxicity in rats: potential involvement in striatal neuronal death

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Abstract: Osteopontin (OPN), an adhesive glycoprotein, has recently been shown to be involved in 3-nitropropionic acid (3-NP)-induced neurotoxicity. The present study explores further the involvement of osteopontin in striatal neuronal death after 3-NP intoxication. Rats were given intraperitoneal injection of 3NP (15mg/kg/day) for 3 days. Double-labeling study using cell death markers such as Fluoro jade-B or TUNEL revealed that OPN expression was induced exclusively in dying neurons as early as 6 hours after the last injection of 3NP. The expression of OPN coincided with a decrease in DARPP-32 expression, marker for medium-sized spiny striatal neurons. OPN expression in dying neuron was evident within 6 hours after last injection of 3NP, peaked at 24 hours, and almost completely disappeared at 7 days. Instead, punctate OPN-positive profiles, most of which were co-labeled with DARPP-32, were absent in the injured striatum until 3 days after the intoxication, but progressively increased in their number and intensity by 7 days. From 3 days and onward, brain macrophages expressing OPN accumulated within the injured striatum. Immunoperoxidase and immuno-gold electron microscopy revealed two different subcellular distribution patterns in degenerating striatal neurons, with OPN protein being diffusely distributed over the somata and specifically localized within swollen mitochondria of degenerating dendrites, which corresponded to punctate profiles in light microscopy. These data suggest that after the 3-NP intoxication OPN is induced exclusively in degenerating neurons, in which OPN was differentially distributed along their somato-dendritic

domains, suggesting that OPN may be involved in the pathogenesis of striatal degeneration.
Number of Grant: NRF-2014R1A2A1A11050246.

Disclosures: **T. Riew:** None. **Y. Shin:** None. **X. Jin:** None. **J. Choi:** None. **M. Lee:** None.

Poster

318. Neuroinflammation

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Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development and Rehabilitation Research and Development)

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Florida Department of Health James and Esther King Biomedical Research Program

Title: Preventing oxidative neurodegeneration after traumatic brain injury followed by secondary smoke exposure

Authors: *B. A. CITRON[1,2], W. A. RATLIFF[1,2], K. L. KEELEY[1,2], C. G. PICK[5], M. OKUKA[3], J. C. M. TSIBRIS[3], R. F. MERVIS[6,4], J. N. CHANG[1,2];
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Abstract: Worldwide, approximately 0.5% of the population suffers a traumatic brain injury each year with most of these injuries classified as mild. Even mild injuries can have long-term consequences that interfere with the life of the patient and impose a burden on our health care system. An improved understanding of the signaling events that are responsible for this neurodegeneration will help to determine necessary treatments to reduce or eliminate neuronal loss. Additionally, while we cannot prevent all injuries, it is important to understand preventative strategies and lifestyle choices, such as cigarette smoking, that could affect the postinjury prognosis. Oxidative damage that results from injury contributes to neuronal loss and an increasing body of evidence suggests that cigarette smoke promotes neurodegeneration. We sought to determine whether exposure to cigarette smoke results in increased neurodegeneration following traumatic brain injury. Additionally, we examined antioxidant therapy that could be

beneficial following injury and could be particularly helpful when the injury is combined with a cigarette smoke insult. We investigated whether tobacco smoke amplifies oxidative stress and accelerates neurodegenerative processes after traumatic brain injury and whether treatment to elevate antioxidant transcription factor activity, Nrf2, could be beneficial with a mouse TBI and tobacco smoke exposure model. We found that postinjury exposure to second hand smoke resulted in less dendritic material in granule cells in the hippocampus. Changes in dendritic complexity in the brain, reactive astrocytosis, and Nrf2 pathway expression may provide a context for the effects of tobacco smoke exposure on traumatic brain injury recovery and whether increasing antioxidant signaling affected plasticity following injury.

Disclosures: B.A. Citron: None. **W.A. Ratliff:** None. **K.L. Keeley:** None. **C.G. Pick:** None. **M. Okuka:** None. **J.C.M. Tsibris:** None. **R.F. Mervis:** None. **J.N. Chang:** None.

Poster

318. Neuroinflammation

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Title: Endothelial cell responses in the hippocampus and cerebellum after irradiation to the young mouse brain

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Abstract: Cranial radiotherapy is essential in the treatment of malignant pediatric brain tumors, but is unfortunately associated with long-term side effects, including cognitive decline. The underlying cellular mechanisms are only partly known and the relative contribution of, for example, reduced neurogenesis, perturbed myelination, astrogliosis, neuronal cell death, and vascular dysfunction, is a matter of debate. In the current study we investigated the role of the vasculature in cranial radiotherapy. Postnatal day 14 mice received a single absorbed dose of 10 Gy whole brain irradiation (IR) and were sacrificed 6 hours, 24 hours or 7 days post IR. Endothelial cells were isolated from the hippocampus and from the cerebellum using fluorescence-activated cell sorting, followed by cell cycle analysis and gene expression profiling. Flow cytometry revealed that IR increased the percentage of endothelial cells (relative to the whole cell population) in both the hippocampus and the cerebellum, indicating that other cell types were more susceptible to IR-induced cell death. However, IR suppressed proliferation of endothelial cells, as judged by a decreased percentage of cells in S phase and an increased percentage in G0/G1 phase of the cell cycle 6 hours after IR. Genes involved in endothelial cell-specific apoptosis (e.g. ASMase) were not induced at any time point investigated. Inflammation-related genes, on the other hand, were strongly induced, such as CCL2, CCL11 and IL-6. We conclude that endothelial cells do not undergo apoptotic cell death to the same extent as other cell types in the hippocampus and the cerebellum after IR, but that they display reduced cell proliferation. In addition, we demonstrate that endothelial cells play an active, hitherto unknown, role, in the inflammatory response after IR.

Disclosures: **M. Boström:** None. **M. Kalm:** None. **C. Bull:** None. **N. Hellström Erkenstam:** None. **K. Blomgren:** None.

Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 318.06/DP04 (Dynamic Poster)

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Imaging neurodegenerative and glial pathology at single-cell resolution using AAV mediated conditional genetics: A proof of principle in focal cerebral ischemia

Authors: *M. EL-SAADI[1], X. TIAN[1], L. RIVERS[1], H. SUN[2], X. LU[1];
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Abstract: A poor mechanistic understanding of neurodegeneration, secondary to the limitations of available biological tools have hindered advancements in this field of research. Developing

biological tools is paramount in the search for novel therapies to stop the onset or halt the progression of ND. Here we describe a simple method- Mosaicism with AAV mediated Genetic Conditionality (MAGiC) for single-cell analysis of neurodegeneration via retro-orbital introduction of AAV for conditional genetic labelling and perturbation in adult mice. MAGiC represents a fast, flexible, less-invasive and easily reproducible method that can be used to reveal detailed morphology and fine structures of genetically defined cell-type specific neurons and glia, allowing for automatic 3D reconstruction and quantification. As proof of principle, we subjected MAGiC mice to middle cerebral artery occlusion (MCAO). Using multi-photon imaging and 3-D reconstruction, we have illustrated the degenerating cortex pyramidal and striatal medium spiny neurons with characteristic features of neurodegeneration. Moreover, we've documented drastic axon degradation consistent with Wallerian pathology while the cell bodies remain intact ("dying back"), opening the possibility of therapeutic intervention. Most notably, this method reveals elaborate morphology of reactive glia and "caught red-handed" the glial engulfment of degenerating neurons and blood vessels. We are employing such "single-neuron degeneration" approach as an in vivo High Throughput Screening (HTS) platform to facilitate translational efforts by improving predicative validity of preclinical assessment of potential efficacy of neuroprotective therapy.

Disclosures: **M. El-Saadi:** None. **X. Tian:** None. **L. Rivers:** None. **H. Sun:** None. **X. Lu:** None.

Poster

318. Neuroinflammation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: T32AG023477-10

NS047663-01

Title: The role of ER stress in Neuropathy Target Esterase-associated disorders

Authors: *E. SUNDERHAUS[1], D. KRETZSCHMAR[2];
[1]Mol. & Med. Genet., [2]Oregon Inst. of Occup. Hlth. Sci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Mutations in Neuropathy Target Esterase (NTE), a phospholipase and regulator of PKA signaling, have been shown to cause a spectrum of disorders. Loss of NTE's ortholog,

Swiss Cheese (SWS), in *Drosophila melanogaster* has been shown to cause an increase in phosphatidylcholine and age-dependent neurodegeneration. SWS is localized to the Endoplasmic Reticulum (ER), and recently, it was shown that perturbing the membrane composition of the ER led to the activation of the ER stress response through the inhibition of the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA). To investigate whether ER stress could be causing the neurodegeneration and other symptoms of NTE associated disorders, we are using the *sws*^{1} null mutant line. To determine if *sws*^{1} flies show an activated ER stress response, we analyzed the levels of GRP78, a chaperone known to be elevated in cells undergoing ER stress. Using Western blots, we showed that 7-day-old *sws*^{1} flies had elevated levels of GRP78 when compared to age-matched controls. We next addressed whether manipulating ER stress genetically and chemically can prevent *sws*^{1}-related phenotypes. Overexpressing XBP1, an ER transcription factor, indeed suppressed the degeneration and locomotion defects in *sws*^{1} detected by histology and negative geotaxis assays. Similarly, overexpression of SERCA was able to significantly improve the behavioral defects and neurodegeneration seen in *sws*^{1}. Feeding the flies tauroursodeoxycholic acid (TUDCA), a chemical known to reduce ER stress, significantly improved the neurodegeneration, as well as the gliopathy observed in *sws*^{1}, but did not prevent the locomotion deficits. We will now investigate effects on the ER stress pathways in the *sws*^{1} flies in more detail, and we will determine how different disease-related NTE constructs affect the ER response in the hopes of identifying targets for potential therapies.

Disclosures: E. Sunderhaus: None. **D. Kretzschmar:** None.

Poster

318. Neuroinflammation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01DA035714

Title: Mutation of histidine 547 of human dopamine transporter increases dopamine uptake and attenuates HIV-1 Tat-induced inhibition of dopamine transport

Authors: *J. ZHU[1], P. QUIZON[2], W.-L. SUN[3], Y. YUAN[4], N. M. MIDDE[3], C.-G. ZHAN[4]; [1]Drug Discovery and Biomed. Sci., South Carolina Col. of Pharmacy, Univ. of South Carolina, Columbia, SC; [2]Drug Discovery and Biomed. Sci., Univ. of South Carolina, Columbia, SC; [3]Drug Discovery and Biomed. Sci., Univ. o South Carolina, Columbia, SC; [4]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY

Abstract: Abnormal dopaminergic transmission has been implicated as a risk determinant of HIV-1-associated neurocognitive disorders (HAND). HIV-1 transactivator of transcription (Tat) protein and cocaine synergistically increase synaptic dopamine (DA) levels by directly inhibiting DA transporter (DAT) activity, ultimately leading to dopaminergic neuron damage. Therefore, an intervention for HIV infection-induced dysfunction of DA systems has the potential to improve neurocognitive function in patients with the early-stage of HAND. Through integrated computational modeling prediction and experimental validation, we have identified key residues in DAT with which Tat interacts, which are critical for Tat-induced inhibition of DAT and transporter conformational transitions. This study investigated the functional influences of mutations of histidine547 (H547A) and its associated residues tyrosine 548 and tyrosine 551 of human DAT in basal DA transport and Tat-induced inhibition of DA transport. Compared to wild type human DAT, H547A-hDAT displayed a 197% increase in the Vmax with no change in the Km. The increased Vmax in H547A was not accompanied by change in DAT surface expression. Results from other substitutions of His547 show that the Vmax was not altered in H547R but decreased by 99% in H547P and 60% in H547D, respectively. Importantly, Tat-induced inhibition of DA transport observed in wild type hDAT was attenuated in H547A, H547R and H547D. PMA, a PKC activator, produced a 40% inhibition of Vmax in wild type DAT and 61% in H547A, whereas BIM, a PKC inhibitor, increased Vmax in WT and H547A by 198% and 142%, respectively. This indicates that H547A-induced increase in DA uptake is dependent on basal PKC activity. These findings demonstrate that His547 plays a crucial role in enhancing basal DA transport and the Tat-DAT interaction. This study may provide novel insights into identifying targets on DAT for therapeutic interventions, improving DAT-mediated DA transmission of HAND in concurrent cocaine abusers.

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Poster

318. Neuroinflammation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF Grant 2011-0018358

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Title: Genetic ablation of Trpm2 accelerates protein and lipid aggregation in the brain by impaired autophagic clearance

Authors: *B. LEE, J. JUNG, J. LEE, H. KIM, G. HONG, J. WEE, H. LU, U. OH;
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Abstract: TRPM2, a non-selective cation channel implicated in neurodegenerative diseases is known to work as an enzyme that hydrolyzes highly reactive, neurotoxic ADP-ribose (ADPR). Although ADPR is hydrolyzed by NUT-9 in major organs, these are defective in the brain. The present study questions the role of TRPM2 in the catabolism of ADPR in the brain by assessing the phenotypic response to its dysfunction. Genetic ablation of TRPM2 results in the accumulation of ADPR, disruption of ADPR catabolism, and reduction in autophagic activity in the brain. *Trpm2*$^{-/-}$ mice show aggregations of proteins and lipids in the brain, aberrant structural changes and neuronal connections in synapses, and neuronal degeneration. The TRPM2-deficient mice also exhibit learning and memory impairment, enhanced neuronal intrinsic excitability, imbalanced synaptic transmission, and bilateral spontaneous multifocal epileptiform discharges. The accumulation of ADPR and reduction in AMP in the brain of *Trpm2*$^{-/-}$ mice owing to the loss of TRPM2 catabolizing activity may account for the aggregation of neurotoxic proteins in dendritic spines, which ultimately leads to neuronal dysfunction and degeneration. These results respond to long-unanswered questions regarding the potential role of the enzymatic function of TRPM2 in the brain, whose dysfunction evokes protein/lipid aggregation by autophagic disturbance.

Disclosures: B. Lee: None. **J. Jung:** None. **J. Lee:** None. **H. Kim:** None. **G. Hong:** None. **J. Wee:** None. **H. Lu:** None. **U. Oh:** None.

Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 318.10/U5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Modulating the lysosomal gene network to identify new therapeutic opportunities for neurodegenerative disorders.

Authors: *V. BOUCHE[1], A. PEREZ ESPINOSA[1], D. MEDINA[2], L. LEONE[3], M. SARDIELLO[1], A. BALLABIO[2], J. BOTAS[1];
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Abstract: Protein and organelle turnover in neurons and in other cell types is controlled by the lysosome-autophagosome system. Increasing evidence shows that the lysosome is strongly involved in the pathogenesis of several neurodegenerative conditions, in which abnormal protein turnover and degradation contribute to the progression of the disease. The evolutionary conserved CLEAR network regulates the expression of genes involved in lysosome biogenesis, autophagy and lipid metabolism. In mammals, the master gene TFEB and other members of the MiTF-TFE family of transcription factors control this network. The genome of *Drosophila melanogaster* includes only one member of the MiTF-TFE family, namely Mitf, who functions as mammalian TFEB. The genetics of the *Drosophila* model system and the absence of redundant MIT transcription factors can be exploited to identify new interacting modulators of TFEB/Mitf function. An important feature of *Drosophila* is their suitability for large genetic screens, which are now possible thanks to the near saturation of their genome with mutations. Therefore, we are conducting an *in vivo* screening using transgenic *Drosophila* strains in which kinases are genetically downregulated. Several assays are being performed in order to evaluate Mitf nuclear translocation and transcriptional activation of target genes. We hypothesize that the genetic screen will identify candidate kinases and pathways involved in TFEB-mediated activation of lysosomal function. Additionally, we will test candidate hits in the context of several *Drosophila* models of proteinopathies, inducing lysosomal-mediated clearance of accumulated toxic protein. We expect that data from the genetic screen will provide novel insight into pathways not previously known to control the lysosomal network through activation of TFEB. Perhaps more importantly, hits from the screen may identify new potential targets for therapeutic intervention in several neurodegenerative disorders.

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Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 318.11/U6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of nitric oxide synthase inhibitors on methamphetamine-induced hyperthermia and dopaminergic neurotoxicity in mice

Authors: *A. S. DARVESH[1], W. J. GELDENHUYS[2], M. M. HOSSAIN[1], P. SADANA[1], A. J. PRUS[3], S. P. BERGER[4], J. R. RICHARDSON[1];
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WV; [3]Northern Michigan Univ., Marquette, MI; [4]Portland Veterans Affairs Med. Ctr., Portland, OR

Abstract: The psychostimulant methamphetamine (MA) produces hyperthermia and long-term nigrostriatal dopaminergic neurotoxicity in both humans and animals. The MA-induced increase in body temperature has been suggested to contribute to dopaminergic neurotoxicity as depletion of striatal dopamine (DA) and reduction of protein levels of tyrosine hydroxylase (TH) was observed following exposure to MA. Oxidative stress, including production of both reactive oxygen and nitrogen species, has been implicated in the neurotoxic effects of MA. Here, we examined the effect of two nitric oxide synthase (NOS) inhibitors on MA-induced hyperthermia and dopaminergic neurotoxicity in adult male CD1 mice. Treatment with a single dose of MA (40 mg/kg, i.p.) exhibited a significant increase in body temperature (+2.2 ^{o}C) up to 2 hr after MA administration. MA-induced hyperthermia was accompanied by induction of heat shock protein 70 (HSP70) (186%), a marker for cellular stress, in the striatum at 16 hr after MA exposure. Mice euthanized six days after MA administration showed significant depletion of cellular DA (47%) and levels of TH protein (52%) in the striatum. Pre-treatment of mice with the non-specific NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 60 mg/kg, i.p.) or the inducible NOS (iNOS) inhibitor aminoguanidine (AG, 100mg/kg, i.p.), 30 min before MA exposure, significantly suppressed the MA-induced elevations of body temperature, and striatal HSP70 expression. Finally, L-NAME and AG pre-treatment prevented the MA-induced reduction of striatal DA and protein levels of TH. Since pre-treatment with both L-NAME and AG also attenuated MA-induced hyperthermia, it is plausible that this effect may contribute to the NOS inhibitor-mediated neuroprotection. Together, these results suggest that NOS mechanisms may contribute to the dopaminergic neurotoxicity of MA and inhibition of oxidative stress could be a potential therapeutic target for reducing MA neurotoxicity.

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Poster

318. Neuroinflammation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 318.12/U7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Impact of stressors on lambda-cyhalothrin induced brain cholinergic dysfunction in rats: Role of mitochondrial bioenergetics

Authors: *R. K. SHUKLA[1], R. GUPTA[1], M. H. SIDDIQUI[2], A. KUMAR[3], A. B. PANT[1], V. K. KHANNA[1];
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Abstract: Recently, we have found that pre-exposure to immobilization stress (IMS), a psychological stressors and forced swim stress (FSS), a physical stressors exacerbated lambda-cyhalothrin (LCT) induced brain cholinergic dysfunctions in rats. In continuation to this, studies have been carried out to understand the impact of IMS and FSS on LCT induced apoptosis and mitochondrial impairments. No significant change in plasma corticosterone levels, blood brain barrier permeability and expression of anti-apoptotic (Bcl2) and pro-apoptotic (Bax, Caspase-3) proteins was observed in frontal cortex and hippocampus in rats subjected to IMS (one session, placed in plastic restrainer, 15 min/day) or FSS (one session, 3 min/day) for 28 days or exposed to LCT (3.0 mg/kg body weight, p.o.) for 3 days (on days 26, 27 and 28) alone in comparison to controls. Marginal changes in mitochondrial activity, ROS generation and membrane potential both in frontal cortex and hippocampus were evident in rats subjected to IMS or FSS or those exposed to LCT alone as compared to controls. Pre-exposure to IMS or FSS for 28 days followed by LCT treatment for 3 days in rats resulted to increase plasma corticosterone levels, disrupt blood brain barrier permeability and affect the mitochondrial bioenergetics as compared to rats exposed to IMS or FSS or LCT alone. Further, pre-exposure to IMS or FSS caused a marked enhanced ROS generation and decrease in mitochondrial membrane potential associated with alteration in pro and anti-apoptotic proteins in frontal cortex and hippocampus on LCT treatment as compared to rats exposed to IMS or FSS or LCT alone. These changes affect the learning and memory activities on pre exposure to IMS or FSS for 28 days followed by LCT treatment rats. The results clearly exhibit that both psychological and physical stressors contribute in the LCT induced mitochondrial impairments associated with cholinergic dysfunctions. Alterations in behavioural and neurochemical end points were more intense in LCT treated rats pre-exposed to IMS as compared to those pre-exposed to FSS.

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Poster

318. Neuroinflammation

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Program#/Poster#: 318.13/U8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Lipidomic analyses identify mitochondrial lipids and omega-3/omega-6 phospholipid decreases in a mouse model of Gulf War Illness

Authors: *U. JOSHI;
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Abstract: Introduction: Gulf war illness (GWI) is a multi-symptom illness that affects 25% of veterans from the 1991 Gulf War (GW). Using chemicals implicated in the pathogenesis of GWI (a nerve gas antidote pyridostigmine bromide and a pesticide permethrin), we developed a mouse model of GWI, which presents with cognitive impairment and anxiety, features that are similar to symptoms reported by veterans with GWI. Studies suggest that chronic clinical presentation of GWI accompanies impaired energy utilization in the brain and altered inflammatory parameters in the periphery of ill GW veterans. Therefore, we examined brain lipid profiles in this GWI mouse model at a chronic 16-months post-exposure, a timepoint that is relevant to the current clinical condition of veterans with GWI. Methods: Brain cardiolipin (CL) were extracted using a modified Bligh-Dyer method and analyzed using normal phase high pressure liquid chromatography (HPLC) followed by mass spectrometry (MS) in the fourier transform mass spectrometry (FTMS) mode at 100,000 resolution on a LTQ-Orbitrap mass spectrometer. Following acetonitrile-methanol crash, acylcarnitines were analyzed using hydrophilic interaction liquid chromatography (HILIC) and MS analysis in the FTMS mode at 30,000 resolution on the LTQ-Orbitrap. Lipid extracts obtained using the Folch method were subjected to normal phase LC/MS to separate phospholipid (PL) classes and individual species were identified and quantified with the LipidomeDB software. Lipid extracts were saponified for gas chromatography/mass spectrometry analysis to examine the total fatty acid (FA) content. Results: We observed decreases in acylcarnitine, CL and in PL that contained omega-3 and omega-6 FA in the brains of exposed animals. Total FA analysis also confirmed decreases in omega-3 and omega-6 FA in the brains of GW agent exposed mice. Conclusion: These results indicate that a further examination of mitochondria and biological functions involved in transport and metabolism of omega-3 and omega-6 FA may lead to the development of novel therapies for treating GWI.

Disclosures: **U. Joshi:** A. Employment/Salary (full or part-time): Roskamp Institute.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant T32 GM08716

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Title: Probing the contractility of capillary pericytes *In vivo* with optogenetics

Authors: *D. A. HARTMANN, R. I. GRANT, A. Y. SHIH;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: The cerebrovasculature modulates its resistance to flow in order to match blood supply with the high metabolic demand of the brain. While it is accepted that arterioles ensheathed by vascular smooth muscle cells are capable of modulating blood flow resistance, it is debated whether capillaries lined with pericytes also regulate blood flow. In an attempt to clarify the capacity for pericyte-lined capillaries to regulate blood flow, we examined the contractile ability of cerebral pericytes ~4-9 branch points beyond the penetrating arteriole, which constitutes the middle of the capillary bed. To do this, we generated a transgenic mouse expressing channelrhodopsin-2 in all mural cells (smooth muscle cells and pericytes), and imaged vascular dynamics through an acute skull-removed cranial window using two-photon wavelengths that excite channelrhodopsin-2 primarily in the plane of focus during real-time image collection. In mice lightly anesthetized with isoflurane (0.7% MAC in air), diameters of some capillaries that were 4 or more branch points away from the penetrating arteriole decreased over 20 seconds of excitation, resulting in lumen diameter reductions from baseline averaging ~10%. We only observed diameter changes when using 800 nm two-photon excitation, a stimulation protocol previously used by Hill, et al. (Neuron 87, 2015). The same capillaries exhibited no diameter changes when imaged with 900 nm two-photon excitation, thus making artifacts due to movement of the animal, breathing or heart-beat less likely a contributor to the observed changes. Critically, we did not observe the same extent of capillary constriction using an equivalent stimulation protocol on littermates lacking channelrhodopsin-2 in mural cells. This indicates that channelrhodopsin-2 excitation is the most likely cause of capillary constriction, rather than laser-induced vessel damage. Ongoing studies are being done to ensure that diameter changes are not the result of vessels moving out of the focal plane, and also to examine if these small diameter changes have a meaningful influence on blood flow. Although these are not physiological conditions (i.e. anesthetized with optogenetic stimulation), this work suggests that pericytes deep in the capillary bed are capable of modulating lumen diameter.

Disclosures: D.A. Hartmann: None. **R.I. Grant:** None. **A.Y. Shih:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.02/U10

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: AHA Grant 14GRNT20480366

NIGMS Grant P20GM12345

Title: The cerebrovascular mural cell continuum: A structural and biochemical characterization of smooth muscle cells, pericytes and intermediary hybrids

Authors: *R. I. GRANT, D. A. HARTMANN, R. G. UNDERLY, A. N. WATSON, A. Y. SHIH;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Vascular smooth muscle cells (VSMCs) and pericytes are the two primary mural cell types that reside on blood vessels of the brain. VSMCs canonically reside on arteries and arterioles, exhibiting ring-like morphology that completely encircles the arteriolar endothelium without any obvious central cell body. In contrast, capillary pericytes have a prominent ovoid cell body and elongated cellular processes that partially cover the capillary endothelium. It is widely accepted that VSMCs regulate blood flow, but whether capillary pericytes possess this ability is a topic of debate. One reason for this debate is a lack of information on how to define mural cells as the cerebral vasculature transitions from arterioles to capillaries. To address this issue, we imaged mural cells in optically-cleared and immunostained mouse cortical tissues with two-photon microscopy. Mural cells were labeled in transgenic mice that expressed tdTomato under control of the PDGFRβ or NG2 promoters. We classified mural cells based on their morphology and expression of the contractile protein, α-smooth muscle actin (α-SMA). These characteristics were then related to subsurface microvessel branch order, defined as the number of branch points between a microvessel of interest and the nearest penetrating arteriole. As arterioles transitioned to capillaries, we found 'hybrid' mural cells with characteristics of both VSMCs and pericytes. These cells possessed protruding, ovoid cell bodies and were oriented longitudinally along their associated vessel, similar to capillary pericytes. However, they also exhibited strong α-SMA expression and completely encircled the endothelium similar to VSMCs. With respect to branch order, VSMCs resided on the penetrating arteriole defined as zero order, hybrid mural cells were typically on 1st to 4th order branches, and capillary pericytes were found on 5th order vessels and beyond. This cellular layout, however, depended upon the size of the branch emerging from the penetrating arteriole. Our data should facilitate a discussion of how to define mural cell types in relation to vessel branch order, a common vascular metric that can be determined both *in vivo* and *ex vivo*.

Disclosures: **R.I. Grant:** None. **D.A. Hartmann:** None. **R.G. Underly:** None. **A.N. Watson:** None. **A.Y. Shih:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.03/U11

Topic: C.08.Stroke

Support: CIHR Operating Grant

Heart and Stroke Foundation GIA

NSERC Discovery Grant

Title: Chemogenetic modulation of disinhibitory VIP interneuron circuits to enhance recovery from stroke

Authors: ***C. E. BROWN**[1], E. WHITE[2], N. LIANG[2], K. GERROW[2];
[1]Island Med. Program, [2]Univ. of Victoria, Victoria, BC, Canada

Abstract: Functions lost due to stroke such as movement or sensation of the limbs can be partially restored through the rewiring of surviving brain circuits. However, the extent to which neural circuits can regain lost function is limited by excessive inhibitory signalling in the stroke affected hemisphere. One inhibitory circuit that plays a key role in regulating cortical excitability involves those that express vasoactive intestinal peptide (VIP). These VIP interneurons specialize in inhibiting other classes of inhibitory neurons thereby forming a "disinhibitory" network. As such, they are well positioned to modulate post-stroke cortical excitability and recovery of function. In order to first understand how stroke affects these circuits, we performed longitudinal *in* vivo two photon imaging of VIP interneuron structure before and several weeks after focal ischemic stroke in the forelimb somatosensory cortex. Our results show that VIP neurons in the peri-infarct cortex lose a significant number of dendritic spines and axonal boutons in the first week after stroke. This loss of inputs and outputs was followed by a protracted period of growth where new, but generally unstable dendritic spines and axonal boutons formed in the peri-infarct zone. Given the disruptive impact of stroke on VIP circuit structure and presumably function, we hypothesized that chemogenetic enhancement of VIP interneuron excitability could facilitate its disinhibitory role in the stroke affected hemisphere, and help restore lost functions. Our preliminary data show that chemogenetic activation of VIP neurons (expressing excitatory hM3D(Gq) DREADD) enhances normally dampened cortical

responses to forepaw touch after stroke. Furthermore, we have behavioural evidence that chronic chemo-genetic activation of these neurons (from 5 to 42 days post-stroke) significantly enhances the recovery of sensory and motor functions of the affected forepaw. Collectively, these findings suggest that augmenting disinhibitory circuit activity in the stroke affected hemisphere may represent a promising new experimental approach to restoring sensori-motor function.

Disclosures: C.E. Brown: None. **E. White:** None. **N. Liang:** None. **K. Gerrow:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.04/U12

Topic: C.08.Stroke

Support: NIH R01NS093057

Title: Whole brain activation dynamics after stroke

Authors: *S. LEVY[1], M. ASWENDT[1], B. HSUEH[2], G. SUN[1], S. ISHIZAKA[1], D. SMERIN[1], M. CHENG[1], K. DEISSEROTH[2], G. STEINBERG[1];
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Abstract: Recovery following stroke occurs in both animals and humans, and this is attributed in part to rewiring of neural connections in areas adjacent to or remotely connected to the infarct. As stroke causes brain-wide network changes, it is important to investigate brain activity patterns at the cellular level after stroke. In this study we use a high resolution 3D whole brain imaging technique called CLARITY to visualize whole brain/circuit activation at the cellular level during poststroke recovery. An activity reporter mouse line (FosTRAP) was used in this study to visualize cells activated by the immediate early gene Fos. We hypothesize that the CLARITY procedure will provide a more detailed and complete visualization of brain activation patterns in the whole brain.
We used C57Bl6 WT male mice and the activity reporter mouse line (FosTRAP) which utilizes a tamoxifen-dependent Cre recombinase driven under the immediate early gene Fos. Ischemic stroke was induced by transient middle cerebral artery occlusion with an intraluminal suture (30min). Mice were sacrificed at different time points (Post-stroke day 1, 7, 14 and 28) and brains were processed with the CLARITY protocol. Brains were also immunostained with antibodies for neurons and glia.
Stroke brains collected at different post-stroke timepoints became optically transparent after the CLARITY process. Interestingly, the ischemic area turned transparent for all timepoints except

post-stroke day 7 and 28. At these time points the ischemic area remains opaque after the CLARITY process, suggesting that that there may be biphasic physiological processes occurring at these time points that prevented successful clearing. Using Fos-TRAP reporter mice, we observed increased cellular activation in the uncleared infarct and surrounding areas.
Stroke brains can be made optically transparent after the CLARITY process. Using the FosTRAP activity reporter mice, CLARITY provided high resolution visualization of cellular activation patterns during stroke recovery. Ongoing studies include brain maps of activated cells with colocalizations of activated cells using various cellular markers, which may reveal important physiological mechanisms underlying post-stroke recovery.

Disclosures: **S. Levy:** None. **M. Aswendt:** None. **B. Hsueh:** None. **G. Sun:** None. **S. Ishizaka:** None. **D. Smerin:** None. **M. Cheng:** None. **K. Deisseroth:** None. **G. Steinberg:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.05/U13

Topic: C.08.Stroke

Title: Investigating neural and molecular mechanisms of spontaneous recovery in experimental stroke

Authors: *M. ITO[1], M. ASWENDT[1,3], M. CHENG[1], S. ISHIZAKA[1,4], A. LEE[2], S. LEVY[1], D. SMERIN[1], E. WANG[1,5], G. K. STEINBERG[1];
[1]Neurosurg., [2]Pediatrics, Stanford Univ., Stanford, CA; [3]Max Planck Inst. for Metabolism Res., Cologne, Germany; [4]Natl. Hosp. Organization Nagasaki Kawatana Med. Ctr., Kawatana-cho, Japan; [5]Univ. of San Diego, San Diego, CA

Abstract: Spontaneous recovery after stroke occurs in both human and animals. A number of events have been described, including changes in extracellular matrix, glia and angiogenesis. However, the underlying neural and molecular mechanisms driving spontaneous recovery are still unclear. We investigated brain/circuit activation patterns in the ipsi- and contralesional hemispheres of spontaneously recovered mice after experimental stroke. In addition, cortical regions from both hemispheres were processed for RNA sequencing to examine the molecular pathways underlying spontaneous recovery. *Method* Ischemic stroke was induced in C57BL/6J adult male mice by transient MCAO for 30 min. Neurological score, vertical pole, and rotating horizontal beam test were performed at baseline and poststroke days 4, 8, and 14 to evaluate recovery. Infarcts were visualized by acquiring T2WI at poststroke day2 using 7-Tesla MR scanner. Mice were sacrificed at poststroke day 15 for immunostaining with antibodies for

astroglial (GFAP), inflammatory (CD68), and chronic neuronal cell activity (FosB). Primary motor cortices were processed for RNAseq. All stroke mice in this study exhibited similar prestroke behavior, poststroke day 4 deficits and cortico-striatal infarct, as verified by MRI and/or histology. These mice were then categorized into spontaneously recovered and non-recovered groups based on their rotating beam performance. *Result* Out of 36 stroke mice with above mentioned similar conditions, 10 (28%) were categorized into spontaneously recovered group and 26 were categorized into non-recovered group. Hierarchical clustering analysis supported this categorization. At poststroke day 14, the recovered group exhibited significantly better beam performance in distance traveled ($p < 0.001$) and speed ($p < 0.001$). FosB immunostaining analysis indicated that higher percentage of the recovered mice exhibited FosB activation in the corresponding contralesional cortex that mirrored the ipsilesional cortical infarct than in non-recovered group (83% and 25%, respectively, $P = 0.05$). *Conclusion* Our study demonstrates that stroke mice with similar cortico-striatal infarct can exhibit significant difference in their behavioral recovery outcome. A higher percentage of spontaneously recovered mice exhibit FosB activation in the contralesional cortex, suggesting that recruitment of contralesional cortex during recovery is beneficial. Current studies include comparison of the RNAseq transcriptome between ipsi- and contra-lesional hemispheres in both groups, which would provide insights into the molecular pathways mediating spontaneous recovery after stroke.

Disclosures: **M. Ito:** 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; SENSHIN Medical Research Foundation, Japan Society for the Promotion of Science. **M. Aswendt:** None. **M. Cheng:** None. **S. Ishizaka:** None. **A. Lee:** None. **S. Levy:** None. **D. Smerin:** None. **E. Wang:** None. **G.K. Steinberg:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.06/U14

Topic: C.08.Stroke

Support: NIH 2015 K08 NS

Hillblom Startup Grant

Title: The dramatic alterations of microvascular complexity and oligovascular unit in mice with metabolic syndrome

Authors: *G. XIAO, S. NUNEZ, J. HINMAN;
Neurol., UCLA, Los Angeles, CA

Abstract: Metabolic syndrome (MetS) and obesity are becoming one of the most significant public health problems all over the world, which produce various vascular risk factors and increase the risk of developing chronic vascular disease, particularly cerebral vascular disease. Studies show that patients with MetS have a six-fold increase in the incidence of silent white matter changes and microvascular infarcts, which may lead to dementia, disability and even death. The underlying mechanisms of these changes in white matter are likely involved the interaction of the dominant cell type, oligodendrocyte lineage within white matter. However, the molecular mechanisms that maintain and potentially perturb the oligovascular unit in white matter disease are still poorly understood. To reveal the oligovasuclar changes and molecular mechanisms that mediate disease-activated disruption of the oligovascular unit within white matter, we used cell-specific reporter and RiboTAG transcriptional profiling to identify the structural alteration and molecular signature of the cerebral endothelia damaged by MetS. Transgenic mice (Tie2-Cre:flox-stop tdtomato plus flox-RiboTAG; PDGFRa-CreERT2:RiboTAG) were fed with 60%kCal from fat diet for 12 weeks at 2 months age to induce MetS and 10%kCal from fat diet was used as control. In our findings, MetS alters the cellular and molecular integrity of white matter, which results in an overall loss of nodal-paranodal complexes as well as a decrease in the length of paranodal segments. Electron microscopic analysis reveals a notable change in the density of axons within the white matter of MetS mice. In addition, the myelin profiles of these axons are more prominent in MetS mice compared to controls. Vascular volumes within white matter are significantly decreased in both large and micro vessels in mice with MetS. Surprisingly, the ratio of oligodendrocyte precursor cell increases in MetS mice. Transcriptional profiling of white matter endothelial cells reveals a difference of expression profile between normal mice and mice with MetS. The significant changes in endothelial cell gene expression are associated with the decreased volume of vessels in white matter with MetS through apoptotic signaling pathway. These findings may indicate the loss of vasculature leads to the structural disruption of myelin, alterations of the oligodendrocyte linage and axonal organization. Taken together, our studies suggest a molecular interaction between cerebral microvasculature and the cellular biology of white matter and in doing so create a potential molecular target for diffuse white matter disease.

Disclosures: G. Xiao: None. **S. Nunez:** None. **J. Hinman:** 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 2015 K08 NS.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.07/U15

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSERC Postdoctoral Fellowship

International Headache Society 2014

TUBITAK 2015

NIH Grant NS057198

NIH Grant EB00790

NIH Grant NS082097

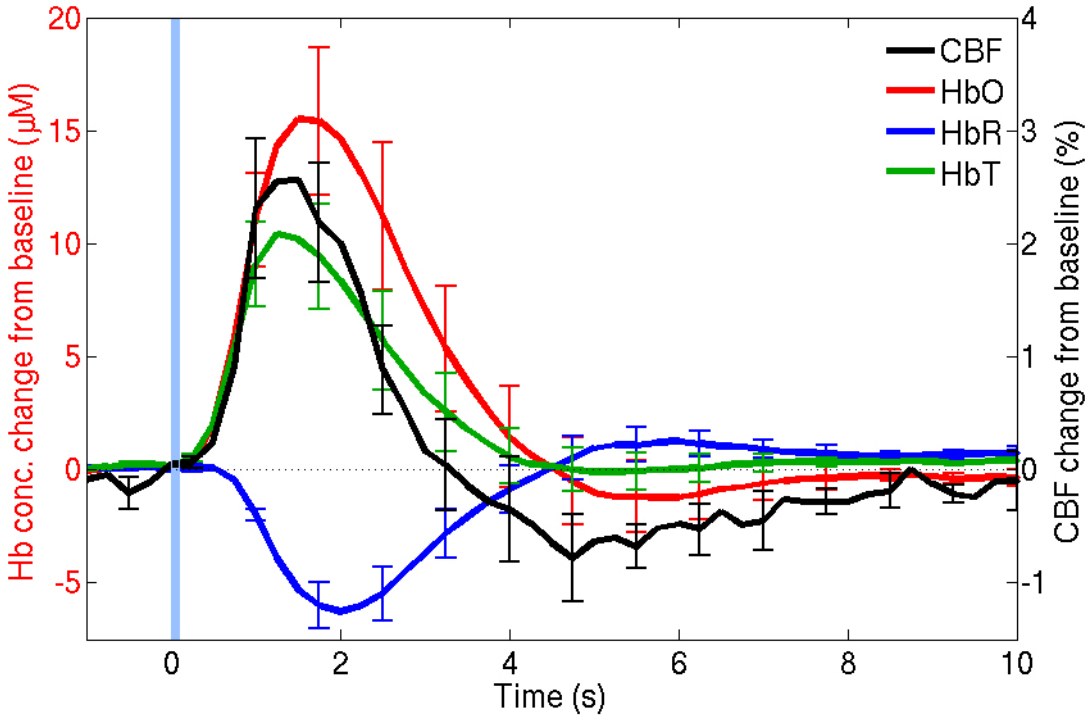
NIH Grant EB003832

Title: Vasoconstriction induced by cortical inhibition reduces cerebral blood flow and hemoglobin oxygenation

Authors: *M. DESJARDINS[1,2], K. KILIÇ[3], C. MATEO[4], P. SAISAN[3], C. L. G. FERRI[3], Q. CHENG[3], K. WELDY[3], D. KLEINFELD[4,5,6], A. DALE[2,3], A. DEVOR[3,2,7];
[2]Radiology, [3]Neurosciences, [4]Physics, [5]Section of Neurobio., [6]Electrical and Computer Engin., [1]UCSD, La Jolla, CA; [7]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp. / Harvard Med. Sch., Charlestown, MA

Abstract: Positive Blood Oxygen Level Dependent (BOLD) functional magnetic resonance imaging (fMRI) signal arises in response to neural activity when an increase in cerebral blood flow (CBF) overcompensates for the competing increase in cerebral metabolic rate of oxygen ($CMRO_2$), causing a decrease in deoxyhemoglobin concentration ([HbR]). Yet, the origin of the negative BOLD (nBOLD) signal, arising under certain conditions, is still debated. Using optogenetics and two-photon microscopy *in vivo*, we have previously shown that inhibitory activity elicits a biphasic arteriolar response with a dilation followed by a constriction phase [1]. Arteriolar dilation and constriction lead to increase and decrease in CBF, respectively. However, accompanying changes in blood oxygenation also depend on $CMRO_2$ and thus remain unknown. To address this question, we performed simultaneous measurements of intrinsic optical signals (IOS) and laser speckle contrast (LSC) in response to optogenetic stimulation of inhibitory neurons through thinned skull over the somatosensory cortex of awake, head-fixed VGAT-ChR2(H134R)-EYFP mice. IOS provide measurements of oxy- ([HbO]), deoxy- and total

([HbT] = [HbO]+[HbR]) hemoglobin concentrations. LSC is inversely proportional to CBF. The measured ROI- and group-averaged timecourses of [HbO], [HbR], [HbT] and CBF responses (Fig. 1) show that activation of inhibitory neurons elicits a biphasic hemodynamic response. During the initial CBF increase, [HbO] increases while [HbR] decreases; during the following CBF decrease, [HbO] decreases and [HbR] increases. Therefore, the vasoconstriction phase elicited by neuronal inhibition causes a decrease in both blood flow and oxygenation, which would be reflected as nBOLD in fMRI. [1] Uhlirova H et al. SfN abstr 2014 #352.10. Fig. 1: Average timecourses +/- s.e.m. (N=3, 720 trials total, each trial was a single 100 ms, ~6 mW pulse of 450 nm).



Disclosures: **M. Desjardins:** None. **K. Kiliç:** None. **C. Mateo:** None. **P. Saisan:** None. **C.L.G. Ferri:** None. **Q. Cheng:** None. **K. Weldy:** None. **D. Kleinfeld:** None. **A. Dale:** None. **A. Devor:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.08/U16

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS057198

NIH Grant EB00790

NIH Grant S10RR029050

International Headache Society

TÜBİTAK

Research Council of Norway

Ministry of Education, Youth and Sports of the Czech Republic, Grant CEITEC 2020 (LQ1601)

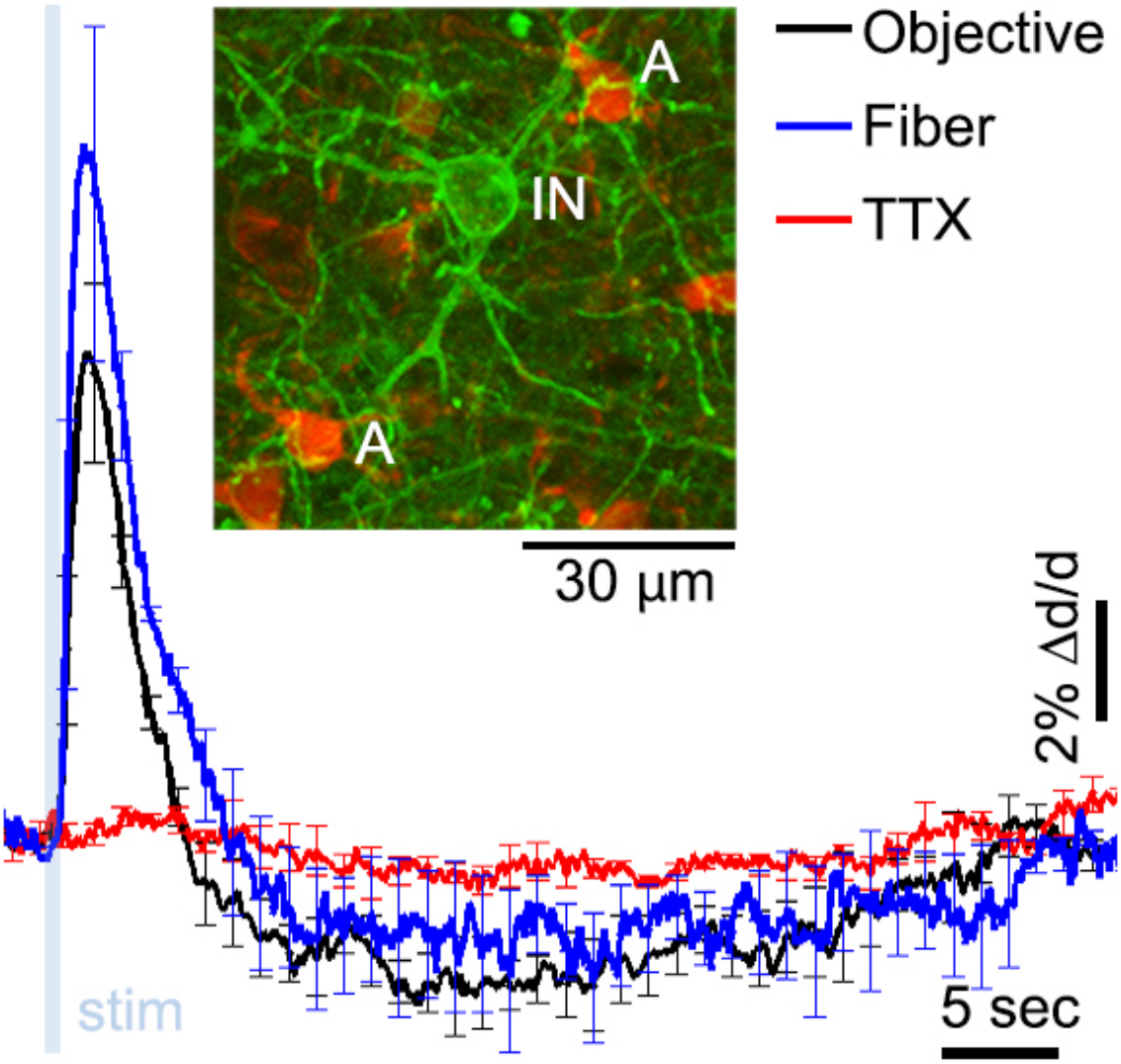
Title: The selective role of cortical inhibitory interneurons in functional hyperemia

Authors: *K. KILIÇ[1], H. UHLIROVA[2,10,11], P. TIAN[1,12], M. THUNEMANN[2], M. DESJARDINS[2], P. SAISAN[1], S. SAKADŽIć[13], T. V. NESS[14], C. MATÉO[3], Q. CHENG[1], K. L. WELDY[1], F. RAZOUX[1], M. VANDENBERGHE[2,15], J. A. CREMONESI[4], C. G. L. FERRI[1], K. NIZAR[5], V. B. SRIDHAR[6], T. C. STEED[5], M. ABASHIN[7], Y. FAINMAN[7], E. MASLIAH[1], S. DJUROVIC[17,18], O. A. ANDREASSEN[15], G. A. SILVA[6,8], D. A. BOAS[13], D. KLEINFELD[3,7,9], R. B. BUXTON[2], G. T. EINEVOLL[14,16], A. M. DALE[1,2], A. DEVOR[1,2,13];
[1]Neurosciences, [2]Radiology, [3]Physics, [4]Biol. Undergraduate Program, [5]Neurosciences Grad. Program, [6]Bioengineering, [7]Electrical and Computer Engin., [8]Ophthalmology, [9]Section of Neurobio., UCSD, La Jolla, CA; [10]Central European Inst. of Technol., [11]Inst. of Physical Engineering, Fac. of Mechanical Engin., Brno Univ. of Technol., Brno, Czech Republic; [12]Physics, John Carroll Univ., University Heights, OH; [13]Martinos Ctr. for Biomed. Imaging, Harvard Med. Sch., Charlestown, MA; [14]Mathematical Sci. and Technol., Norwegian Univ. of Life Sci., Ås, Norway; [15]KG Jebsen Ctr. for Psychosis Res., [16]Physics, Univ. of Oslo, Oslo, Norway; [17]Med. Genet., Oslo Univ. Hosp., Oslo, Norway; [18]KG Jebsen Ctr. for Psychosis Research, Dept. of Clin. Sci., Univ. of Bergen, Bergen, Norway

Abstract: Identification of the cellular players that communicate neuronal activity to the vasculature is important for the basic understanding of cerebrovascular regulation. Previously, we reported that arteriolar dilation in response to optogenetic (OG) activation of all cortical inhibitory neurons (INs) started in deep cortical layers [1]. There, we delivered 473 nm-laser light for OG stimulation to the cortical surface via the objective. Since light at this wavelength does not penetrate deep into the tissue, we reasoned that INs that were driving the early dilation onset in deep layers have superficial axons or dendrites. In principle, OG-induced depolarization of superficial axons may trigger antidromic action potentials (APs) that couple OG stimulation at the surface to the release of vasoactive messengers in deep layers. In this case, the vasoactive effect would be abolished by blocking APs and rescued by delivering OG light directly to layer V. To test this hypothesis, we used a tapered optical fiber positioned with its light-emitting tip in layer V for OG stimulation. Under control conditions, OG stimulation through objective and

fiber induced similar arteriolar responses, but upon AP blockade with TTX, the response was lost in both cases (Figure). These results suggest that spiking is required to enable release of vasoactive agents, and that depolarization along dendrites rather than axons may communicate superficial excitation to vasoactive messenger release in deep layers. Supporting this, computational simulations of INs spanning across cortical layers revealed that depolarization of superficial dendrites could drive the soma, located as deep as in layer V, above its firing threshold. Thus, INs mediating dilation may have dendrites, which extend to the surface, and axons that reach into deep layers. One IN type meeting these morphological criteria are which express vasoactive intestinal peptide (VIP). However, further studies with more selective expression of OG actuators are required to test this prediction.

1. Uhlirova H, Kılıç K, et al. SfN abstr. 2014, No. 352.10



Disclosures: **K. Kiliç:** None. **H. Uhlirova:** None. **P. Tian:** None. **M. Thunemann:** None. **M. Desjardins:** None. **P. Saisan:** None. **S. Sakadžić:** None. **T.V. Ness:** None. **C. Matéo:** None. **Q. Cheng:** None. **K.L. Weldy:** None. **F. Razoux:** None. **M. Vandenberghe:** None. **J.A.**

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Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.09/U17

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Optical control of blood flow in naive animals

Authors: *R. L. RUNGTA[1], B. OSMANSKI[1], D. BOIDO[1], M. TANTER[2], S. CHARPAK[1]; [1]Neurophysiol. and New Microscopies Lab., INSERM U1128 and Univ. Paris Descartes, Paris, France; [2]Wave Physics for Med. Lab., INSERM U979, Paris, France

Abstract: Abstract:
Over the past decade the use of optogenetics to drive genetically distinct populations of brain cells has profoundly increased our understanding of neural circuitry and brain function in health and disease. Optogenetics is now regularly integrated with functional imaging techniques such as BOLD-fMRI or CBV-fMRI, that rely on neurovascular coupling. However, despite the fact that visible light has been shown to dilate peripheral vessels, the effects of light on cerebral blood flow have not been thoroughly investigated. Here we tested whether light stimulation protocols similar to those commonly used in opto-fMRI or to study neurovascular coupling modulate blood flow in mice that do not express any light sensitive proteins.
Combining two-photon laser scanning microscopy and ultrafast functional ultrasound imaging (fUS), approaches that measure blood flow at the microscopic and macroscopic levels, we report that light per se causes a pronounced pseudo functional hyperemia, of similar magnitude to a sensory stimulation, in the neocortex and the olfactory bulb. Pulses or trains of blue or yellow light (471 or 561nm), shone on the mouse brain across a chronic cranial window, triggered a reversible and reproducible dilation of arterioles. The effect was energy-dependent appearing at a threshold of ~0.5mW-1mW. Two-photon imaging of GCaMP6f in transgenic mouse lines revealed that light caused a decrease of vascular smooth muscle cell calcium that preceded the onset of dilation and occurred in the absence of neuronal excitation.
These results impose careful consideration on the use of photo-activation in studies involving blood flow regulation, as well as in studies requiring prolonged and repetitive stimulations to

correct cellular defects in pathological models. They also suggest that light could be used to locally increase blood flow in a controlled fashion.

Disclosures: **R.L. Rungta:** None. **B. Osmanski:** None. **D. Boido:** None. **M. Tanter:** None. **S. Charpak:** None.

Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 319.10/U18

Topic: C.09. Brain Injury and Trauma

Support: Department of Defense within the Center for Neuroscience and Regenerative Medicine

Title: Repetitive model of mild traumatic brain injury produces cortical abnormalities detectable by magnetic resonance diffusion imaging (DTI/DKI), histopathology, and behavior

Authors: K. L. RADOMSKI[1,2], *F. YU[3], D. SHUKLA[1,6], R. C. ARMSTRONG[1,2], C. M. MARION[1,4], R. SELWYN[1,7], B. J. DARDZINSKI[1,5];
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Abstract: Non-invasive detection of mild traumatic brain injury (mTBI) is important for evaluation of acute through chronic effects of head injuries, particularly after repetitive impacts. To better detect abnormalities from mTBI, we performed longitudinal studies of magnetic resonance diffusion tensor imaging (DTI) and diffusion kurtosis imaging (DKI) in adult mice after single mTBI (s-mTBI) and repetitive mTBI (r-mTBI; daily x 5), or respective sham procedures. The s-mTBI impact was attenuated for r-mTBI to differentiate whether repetition lowered the impact threshold for pathology. In the corpus callosum, s-mTBI reduced DTI fractional anisotropy (FA) at all post-injury time points (3, 6, 42 days). Conversely, cortical regions were not altered after s-mTBI but r-mTBI reduced cortical axial diffusivity (AD) at all time points with a corresponding increase in axial kurtosis (Ka) at 6 days post-injury. Post-imaging tissue analysis revealed differential pathology in the corpus callosum versus cortex, without detectable microhemorrhages. The corpus callosum exhibited microglial activation, astrogliosis, and demyelination after s-mTBI, with much less robust changes after r-mTBI. However, microglial activation was increased in the cortex after r-mTBI, with a strong correlation (0.7286) to cortical AD values. Studies in Thy1-YFP-16 mice revealed low levels of

axon damage in the cortex in both models, along with marked axon pathology in the corpus callosum. Finally, r-mTBI, but not s-mTBI, produced social deficits consistent with the function of this anterior cingulate region of cortex. Overall, vulnerability of cortical regions is demonstrated after mild repetitive injury, with underlying differences of DTI and DKI, microglial activation, and behavioral deficits.

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Poster

319. Opto-Chemogenetics and Microvascular Imaging of Stroke and Injury

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Topic: C.08.Stroke

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ISCIII RD12/0014/0001

Title: Neovascularization and functional recovery after intracerebral hemorrhage is conditioned by the Tp53 Arg72Pro single nucleotide polymorphism

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Abstract: Intracerebral hemorrhage (ICH) is a devastating subtype of stroke that lacks effective therapy and reliable prognosis. Neovascularization following ICH is an essential compensatory response that mediates brain repair and modulates the clinical outcome of stroke patients. However, the mechanism that dictates this process is unknown. Bone marrow-derived endothelial progenitor cells (EPC) promote endothelial repair and contribute to ischemia-induced neovascularization. The human *Tp53* gene harbors a common single nucleotide polymorphism (SNP) at codon 72, which yields an arginine to proline aminoacidic substitution *(Arg72Pro*) that modulates the apoptotic activity of the p53 protein. Previously, we found that this SNP controls

neuronal susceptibility to ischemia-induced apoptosis *in vitro*. Here, we evaluated the impact of the *Tp53 Arg72Pro* SNP on vascular repair and functional recovery after ICH. We first analyzed EPC mobilization and functional outcome based on the modified Rankin scale (mRS) scores in a hospital-based cohort of 78 patients with non-traumatic ICH. Patients harboring the *Pro* allele of the *Tp53 Arg72Pro* SNP showed higher levels of circulating EPC-containing $CD34^+$ cells, EPC-mobilizing cytokines -vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1α (SDF-1α)- and good functional outcome following ICH, when compared with the homozygous *Arg* allele patients, which is compatible with increased neovascularization. To directly assess whether *Tp53 Arg72Pro* SNP regulated neovascularization after ICH, we utilized the humanized *Tp53 Arg72Pro* knock-in mice, which were subjected to the collagenase-induced ICH. The brain endothelial cells of the *Pro* allele-carrying mice were highly resistant to ICH-mediated apoptosis, which facilitated cytokine-mediated EPC mobilization and cerebrovascular repair. However, these processes were not observed in the *Arg* allele-carrying mice. These results reveal that the *Tp53 Arg72Pro* SNP determines neovascularization, brain repair and neurological recovery after ICH. This study is the first in which the *Pro* allele of *Tp53* is linked to vascular repair and ability to functionally recover from stroke.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: NIH (NS084398) to JC

Ministerio de Ciencia y Competitividad (SAF2013-48431-R) to RLV

International Foundation for Research in Paraplegia (P148) to RLV

Title: Lysophosphatidic acid receptor 2 contributes to secondary damage after spinal cord injury

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Abstract: Lysophosphatidic acid (LPA) is an extracellular lipid mediator involved in many physiological functions by signalling through six known G-protein-coupled receptors (LPA_1-LPA_6). In the central nervous system (CNS), LPA mediates a wide range of effects, including neural progenitor cell physiology, astrocyte and microglia activation, neuronal cell death, axonal retraction, as well as CNS contributions to pain, schizophrenia and hydrocephalus.
We recently reported that LPA contributes to secondary tissue damage after spinal cord injury (SCI). Interestingly, selective blockade of LPA_1 after spinal cord contusion lesion reduced functional deficits and myelin loss, linking LPA_1 signalling to demyelination, which was in part, mediated by microglial cells.
Here, we provide clear evidence on the deleterious contribution of another LPA receptor, LPA_2 (Lpar2, Edg4), to SCI. We found that LPA_2 is constitutively expressed in the spinal cord parenchyma and its transcripts are up-regulated after contusion injury, in part, by microglial cells. To dissect out the role of LPA_2 in this pathology, we induced contusion injuries in LPA_2 deficient mice. Functional and histopathological analysis showed that motor skills, as well as myelin and neuronal sparing, were significantly enhanced in the absence of LPA_2. To gain insights into the detrimental actions of LPA_2 in spinal cord we performed cell culture studies. These experiments revealed that, similar to LPA_1, activation of microglia LPA_2 led to oligodendrocyte cell death. Moreover, we also found that the cytotoxic effects underlying by microglial cells upon LPA_2 stimulation were mediated, in part, by the release of purines. Overall, this study provides new mechanistic insights into how LPA contributes to SCI physiopathology.

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Poster

320. Injury Responses after Spinal Cord Injury

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Program#/Poster#: 320.02/V3

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS055976

Title: Activity of dorsal raphe serotonergic neurons in a spinal cord injury model of depression

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Abstract: Depression occurs three times more frequently in spinal cord injured (SCI) individuals than in the general population and only a small percentage receives relief with pharmaceutical treatment. The pathology of depression has been attributed to an imbalance in the neurotransmitter serotonin, with the raphe nuclei acting as the primary central source. There also is a suggestion that neuroinflammation may have a prominent role in the development and/or persistence of SCI-depression. In this study we used a battery of behavioral tests (sucrose preference, social exploration, open field, novel object recognition, and forced swim) and hierarchial cluster analysis to identify a depression-like phenotype in approximately 30% of female rats at 4 weeks after a low thoracic, moderate contusion injury. We examined SCI-induced changes in electrophysiological activity of serotonergic dorsal raphe neurons as they relate to long-loop input from pre-frontal cortex glutamatergic neurons to GABAergic interneurons of the dorsal raphe nucleus, using whole cell patch clamp techniques to detect alterations in serotonergic and GABAergic neuron activity in the dorsal raphe at 5 weeks after injury. Further, we used immunocytochemistry to measure changes in levels of GLT1 as an indicator of astrocyte glutamate transport activity. ELISA was used to measure levels of TNFα as evidence of chronic inflammation in higher brain centers (cingulate gyrus, prefrontal cortex and hippocampus) that may contribute to a depressive phenotype and immunocytochemistry identified macrophage-microglial cell activity in the dorsal raphe between non-depressive and depressive phenotypes in SCI rats. Elucidating the role of inflammation in the development of depression after SCI could be instrumental in identifying efficacious treatment strategies for this population.

Disclosures: **K. Farrell:** None. **M.R. Detloff:** None. **J.D. Houle:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Canadian Institute of Health Research

Multiple Sclerosis Society of Canada

Title: Assessment of the contribution of endogenous oligodendrocytic remyelination to locomotor recovery after spinal cord contusion injury in adult mouse

Authors: ***W. TETZLAFF**[1], S. B. MANESH[2], G. J. DUNCAN[2], B. J. HILTON[2], P. ASSINCK[2], J. LIU[2], S. NADERI-AZAD[2], P. CHAU[2], D. E. BERGLES[3], J. R. PLEMEL[4];

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Abstract: The majority of traumatic spinal cord injuries (SCI) are anatomically incomplete and result in some recovery of function. SCI often leads to oligodendrocyte death and the loss of myelin (demyelination) in the weeks post injury; this impairs impulse conduction and leaves axons vulnerable to degeneration. In rodents, there is robust endogenous myelin repair (remyelination) that correlates temporally with a high rate of oligodendrocyte production and functional recovery. However, a causal link between remyelination and spontaneous motor recovery has not been established. Here, we use an inducible conditional knockout (iCKO) of myelin-gene regulatory factor (Myrf) from oligodendrocyte precursor cells using a PDGFRα-CreER^{T2} driver line. Following chemical demyelination we find that iCKO of Myrf greatly impairs the formation of mature oligodendrocytes, process outgrowth and subsequent remyelination, while leaving oligodendrocyte precursor (OPC) recruitment and undamaged myelin intact. Both Myrf inducible conditional knockout mice (iCKO) and littermate controls (n=23) received a 70 Kdyne thoracic contusion at T9/T10. Tamoxifen was administered between 9 and 5 days prior to injury. Motor behaviour was assessed using the Basso Mouse Scale (BMS) for open field locomotion, gait (footprint) analysis and the horizontal ladder test. There were no significant behavioural differences between iCKO and control mice in any of these behavioural assessments in the first 6 weeks after injury. We also crossed Myrf iCKO mice and controls with an inducible membrane-tethered GFP reporter (PDGFRα-CreER^{T2}::ROSA26mGFP(mT/mG) allowing for the visualization of new myelin following injury. De novo oligodendrocyte production was nearly halted, and little new oligodendrocyte myelin was observed in the Myrf iCKO mice. These data show that oligodendrocyte remyelination is not a major contributor to the recovery of overground locomotion following a moderate thoracic spinal cord contusion.

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Poster

320. Injury Responses after Spinal Cord Injury

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Paralyzed Veterans of America Postdoctoral Fellowship (#3080)

Sheldon G. Adelson Medical Foundation

Wings for Life Spinal Cord Research Foundation

Title: Dissecting trauma-reactive astrogliosis *In vivo* by cell-type specific analysis of actively translating mRNA

Authors: *J. E. BURDA[1], Y. AO[1], R. KAWAGUCHI[2,3], S. DEVERASETTY[4,5], G. COPPOLA[2,3,5], M. V. SOFRONIEW[1];
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Abstract: In addition to upholding normal central nervous system (CNS) function, astrocytes respond to diverse forms of CNS injury with dynamic changes in gene expression, metabolism, morphology, proliferative capacity and function collectively referred to as reactive astrogliosis. Many lines of evidence indicate that astrocytes tune their responses to varying degrees of cell death, axonal injury, vascular disruption and inflammation, resulting in distinct and likely function-specific reactivity profiles that may be informed by differences in molecular expression. Using the transgenically targeted (*J Neurosci 28:7231; 2008*) RiboTag procedure (*PNAS 106:13939; 2009*) and RNA deep sequencing, we have obtained gene translation profiles of reactive astrocytes from the mouse spinal cord after traumatic injury (SCI). Bioinformatics analyses of astrocyte ribosome-associated mRNA (ramRNA) are being used to identify gene expression profiles that define trauma-reactive astrogliosis, and the molecular regulators underlying these dynamic changes in cell state. Importantly, many changes in astrocyte-specific gene expression differ from changes observed for non-astrocyte RNA derived from whole spinal cord tissue after SCI, allowing for the elucidation of reactive astrocyte-specific molecular contributions to the post-injury milieu. Further, we developed a user-friendly database of absolute and relative gene expression profiles of murine scar-forming reactive astrocytes and non-astrocyte cells following SCI to enable searching for individual genes of interest (https://astrocyte.rnaseq.sofroniewlab.neurobio.ucla.edu, *Nature*, 532:195-200; 2016). This study provides a qualitative and quantitative measurement of gene expression by reactive astrocytes after traumatic injury *in vivo*. Further investigation into the genomics of reactive astrogliosis will aid our evolving understanding of mechanisms influencing neuroregeneration and repair. Funded by NINDS (FNS096858A, NS084030), Paralyzed Veterans of America (Fellowship 3080), Dr. Miriam and Sheldon G. Adelson Medical Foundation and Wings for Life Spinal Cord Research Foundation.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: NS060801

NS061934

Title: The Aqp4-Trpm4 macromolecular complex mediates astrocyte migration after spinal cord contusion

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Abstract: Glial scar formation, a downstream consequence of spinal cord injury (SCI), maintains BBB integrity after SCI, yet strongly inhibits neuronal regeneration and functional recovery. Astrocytes, which migrate to the site of injury after SCI, contribute to glial scar formation. Recently, it was shown that transmembrane water flux mediated by aquaporin-4 (Aqp4), a passive astrocyte transmembrane water channel, is essential for astrocyte migration and glial scar formation. However, the mediators of ion (osmolyte) flux that drive Aqp4 activity during astrocyte migration are unknown. We hypothesized that Trpm4, a Ca^{2+} activated monovalent cation channel that is de novo upregulated by astrocytes after SCI, drives Aqp4 water flux during astrocyte migration after SCI. We used co-immunoprecipitation (CoIP) and Forester resonance energy transfer (FRET) to determine if Aqp4 and Trpm4 co-associate in astrocytes after SCI. To study the functional interaction of Aqp4 and Trpm4 in astrocyte migration, we used calcein fluorescence imaging, immunocytochemistry, and the xCELLigence migration assay. CoIP and FRET data indicate that Aqp4 and Trpm4 form an integral membrane channel complex in astrocytes following SCI. Calcein imaging data indicates that Trpm4 osmotically drives Aqp4 water flux in response to raised intracellular Ca^{2+}. Immunocytochemistry indicates that Aqp4 and Trpm4 localize to the astrocytic lamellipodia with PIP_2, an enhancer of Trpm4 activity. Migration assays indicate that expression of Aqp4 and Trpm4 are necessary for proper migration of primary astrocytes. In conclusion, transmembrane water transport mediated by the Aqp4-Trpm4 complex is necessary for proper astrocyte migration. Thus, the Aqp4-Trpm4 complex represents a new therapeutic target to modulate glial scar formation after SCI.

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Poster

320. Injury Responses after Spinal Cord Injury

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CHN313739 to JH

Title: The effects of distinct spared nerve injury models on spinal synaptic plasticity following spinal cord injury

Authors: *J. HUIE[1], K. MORIOKA[1], C. OMONDI[1], J. HAEFELI[1], J. SACRAMENTO[1], A. FERGUSON[1,2];
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Abstract: Spinal cord injury (SCI) is often accompanied by concomitant peripheral injuries, yet little is known about the effect of these injuries on central nervous system (CNS) recovery. Pain research suggests that peripheral nociceptive stimulation may induce maladaptive forms of spinal cord plasticity that could undermine recovery of sensory and motor function. We have recently shown that peripheral electro-nociceptive input after SCI induces maladaptive synaptic plasticity in spinal motor neurons that is AMPA receptor-mediated (Huie et al., 2015). Here we investigate the impact of peripheral nerve injury on spinal synaptic plasticity below a complete SCI.The most widely-used model of nerve injury pain is the spared nerve injury (SNI) model which produces both acute and chronic nociception. SNI models involve cutting 2 branches of the sciatic nerve while sparing the third branch. The ‘spared sensory’ model involves cutting a motor nerve (tibial) and sensory/motor nerve (common peroneal), sparing the sensory sural nerve. An alternative ‘spared motor’ model cuts the sural and common peroneal, sparing the motor tibial nerve. Despite both models being referred to in the literature as ‘spared nerve injury’, the differences in modalities that are interrupted and/or spared in these two SNI models may create

divergent responses in the CNS that are relevant to recovery. To assess how peripheral injury affects spinal synaptic plasticity, we used each SNI model separately in conjunction with a complete thoracic spinal transection to model polytraumatic injury. Lumbar spinal cords were harvested 20 minutes after SCI+SNI, and subcellular fractionation of spinal homogenates was performed to produce a synaptoneurosomal fraction. AMPA receptor subunit (GluA1) expression was assessed using linear fluorescent near-infrared western blot. Preliminary findings suggest a divergent effect of SNI models on AMPA receptor subunit expression, with the 'spared motor' model producing a greater increase in GluA1 expression than the 'spared sensory' model. Work is ongoing to further fully characterize the effects of these SNI models on spinal cord plasticity following SCI.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: TWU Research Enhancement Program

TWU Department of Biology

Title: Nano-based drug delivery systems: Targeting to corticospinal tract neurons for controlled release of therapeutics

Authors: *C. MENGJIE[1], R. VEETTIL[1], D. HYNDS[1], S. GHOSH[2], T. MCALLISTER[2]; [1]BIOLOGY, TEXAS WOMAN'S UNIVERSITY, Denton, TX; [2]Southeast Missouri State Univ., Cape Girardeau, MO

Abstract: Damage to axons of corticospinal tract neurons leads to permanent loss of voluntary fine motor control. Nanoparticle-based systems have seen increasing use in therapeutic fields, where they often serve as a vehicle to deliver drugs to the damaged tissues. We have designed a nano-based drug delivery systems that are capable of being remotely actuated to release an imbibed therapeutic on demand. Furthermore, these biocompatible nanomaterials are encased in a hydrogel that can be derivatized to target the drug delivery system to specific cells. Similar systems can cross the blood brain barrier. In this study, we used nanomaterials with polyethylene glycol-based coats derivatized with -COOH and -NH_2 functionl groups on their surface to study

the mechanism of cell targeting in B35, PC12, and corticospinal tract (CST) neurons. Nanomaterials are taken up more efficiently by neurons, including corticospinal tract neurons. Therapeutic drugs that enhance axon extension (e.g. Y27632, C3 exoenzyme) loaded into nanoparticles are used to study the efficiency of drug release and enhancement of neurite outgrowth. These data suggest that our nanoparticle drug delivery systems are able to target specific neurons and provide on-demand release of a specific drug. Therefore, these systems provide potential therapies for encouraging axon regrowth after spinal cord injury.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Alberta Innovates - Health Solutions CRIO Team Program

Title: Age-associated exacerbation of myelin injury is associated with decreases cholesterol synthesis

Authors: *N. MICHAELS, S. K. JENSEN, K. S. RAWJI, M. B. KEOUGH, J. R. PLEMEL, V. W. YONG;
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Abstract: Age is associated with worse clinical outcomes and delayed recovery in many neurological disorders. Animal models suggest age is associated with increased axonal injury, exaggerated micro- and astro-gliosis, and delayed remyelination. However, the impact of age on the early stages of injury, particularly myelin damage, has not been established. In the current study, we investigated age-associated differences in myelin disruption between young and aging animals. We induced a focal injury in the ventrolateral white matter of female young (1.5 month) and aging (8 month) mice via the injection of 0.5% lysolecithin. Mice were then sacrificed 4, 24, and 72 hours afterwards. Analyses of eriochrome cyanine histochemistry and myelin basic protein immunoreactivity revealed a greater amount of myelin disruption in aging animals 24 and 72 hours post lesion. To investigate potential mechanisms underlying age-associated exacerbation of myelin disruption, we have microdissected naïve and 72-hour post lesion ventrolateral white matter of young and aging mice for RNAseq studies and many transcriptomic differences are apparent. The most pronounced differences occurred in the cholesterol

biosynthesis pathway. Cholesterol in the central nervous system (CNS) is primarily synthesized *de novo* in oligodendrocytes. These cells have been reported to continue to produce cholesterol for the maintenance and synthesis of new myelin throughout the life of an organism. Therefore, its decreased synthesis could indicate oligodendrocyte dysfunction, leaving these cells and myelin more susceptible to injury. Indeed, immunohistochemical analysis of oligodendrocyte lineage cells revealed significantly greater loss 4 hours after injury that persisted into the later time points. These novel results emphasize a greater demyelinating injury in aging compared to young mice in response to lysolecithin and they provide a model to identify mechanisms that contribute to exaggerated myelin disruption with aging so neuroprotective therapeutic strategies can be developed.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Buoniconti Fund

Miami Project to Cure Paralysis

Title: Behavioral and histochemical evaluation of phantom limb pain model in rats

Authors: ***S. JERGOVA**, A. LANJEWAR, A. R. NIEDECKEN, C. MARCH, J. SAGEN; Miami Project, Univ. of Miami Sch. of Med., Miami, FL

Abstract: Phantom Limb Pain (PLP) is described as a pain sensation originating from missing body parts. It is very common in amputees (50-70%) and often reported as a severe pain. Therapeutic strategies targeting PLP often provide inadequate pain relief, mostly due to multifactorial features of PLP in terms of origin and mechanism. The goal of this project was to develop a preclinical PLP model in rats to enable evaluation of novel interventive therapies. Animal models of PLP are based on deafferentation injury followed by autotomy behavior. Several clinical studies showed that the presence of pre-amputation pain increase the risk of developing PLP. We used formalin injection prior to axotomy to mimic this scenario and to evaluate development of the behavior in relation to the injection site. Chronic nerve injury prior to axotomy was also evaluated in terms of behavior and expression of various neurotransmitters.

Sprague Dawley males were used in the experiments; their behavior was recorded and scored daily, distinguishing nail biting, distal and proximal digit injury. Animals were perfused within a month and sciatic nerve, dorsal root ganglia (DRGs), spinal cord and brain removed and processed for immunohistochemistry as follows: sciatic nerve was immunostained with NaV 1.7; DRGs with NaV 1.3, 1.7-1.9, CGRP and Substance P; spinal cord with c-Fos, NaV 1.7, CGRP, GAD65/67, NK1 receptor and IBA-1; brain sections were immunostained for c-Fos and GFAP. Results show development of autotomy, as an indicator of disturbed sensory processing, in both models of pre-amputation pain. Animals developed signs of PLP targeting the digits relative to the formalin injection (either lateral or medial side of the hind paw). Control animals (saline injection prior to axotomy) developed delayed and weak or none signs of PLP within the observed period. In the CCI model, the location of the behavior was in correlation with the location of a tactile allodynia at the lateral or medial side of the paw as indicated by Von Frey filaments. The immunohistochemical analysis of the ganglia, spinal cord and brain tissue showed changes in the expression of NaV 1.7 in DRGs and spinal cord related to the development of autotomy. Also, ipsilateral sprouting of CGRP fibers into deeper spinal laminae and enhanced expression of NK1 receptor was observed in animals with PLP behavior compared to animals with the same injury but with mild or no autotomy. The expression pattern of the other neuropeptides was comparable between the different treatment groups. Our results show that the proposed model may be useful in evaluating novel treatment strategies for PLP and suggest possible neurochemical targets for therapy.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Title: Experimental cervical spinal cord injury induces an autoantibody response in the sub-acute phase of the disease

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Abstract: Spinal cord injury (SCI) dysregulates the immune system in an injury level-dependent fashion. The majority of clinical SCI cases occur at the cervical level, which may have a distinct immune signature from thoracic SCI. Autoantibodies against spinal cord and systemic antigens were shown to impair recovery in a mouse model of low thoracic SCI, but whether the development of autoantibodies is induced after cervical SCI remains unknown. Here we explored the development of autoantibody responses in the sub-acute (2 weeks), chronic (10 weeks) and late chronic (20 weeks) phase of cervical SCI in rats. Cervical SCI was induced in female Wistar rats with a 35 g modified aneurysm clip that compressed the spinal cord for 60 sec at the C7-T1 level. Sham animals underwent the same surgical procedure, except for clip compression. Naïve, age-matched rats were also included. We found increased levels of IgG and IgM immunoglobulins in the spinal cord of injured rats at 2 weeks, but not at later time points of this study. Immunohistochemistry and histology revealed increased binding of endogenous IgG and IgM immunoglobulins at the injury epicenter. There, we identified reactive astrocytes surrounding the developing cavity and neurons in the anterior horn as some of the spinal cord cells where endogenous antibodies were primarily located. High antibody levels in the lesioned spinal cord at 2 weeks after cervical SCI were paralleled by a robust autoimmune activation in the spleen. *In vivo*, antibody secreting cells (plasma cells) were significantly expanded after SCI. In an *in vitro* spleen activation paradigm, we found that splenic cells had increased proliferation capacity and produced more immunoglobulins when stimulated with spinal cord homogenate from injured rats, compared to sham controls. This ability was lost after 2 weeks. In conclusion, our results demonstrate that cervical SCI induces an autoantibody response, which is pronounced during the sub-acute phase of injury. This is the first study to characterize the expansion of IgG and IgM immunoglobulin autoimmune responses after cervical SCI and warrants future research that will investigate the mechanisms of autoantibodies and their implication in the progression of cervical SCI.

Disclosures: **A. Ulndreaj:** None. **A. Tzekou:** None. **E.E. Torlakovic:** None. **M.G. Fehlings:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Program#/Poster#: 320.11/V12

Topic: C.09. Brain Injury and Trauma

Support: RO1NS079702

AWIS-PHL Adelaide M. Delluva Student Travel Awar

Dubbs Scholar Fellowship Award

Title: Promoting targeted reinnervation of phrenic motor neurons and restoration of respiratory function using BDNF after spinal cord injury

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Abstract: We are testing a novel approach to promote regrowth of damaged descending bulbospinal respiratory axons and reinnervation of their correct phrenic motor neuron (PhMN) targets after cervical spinal cord injury (SCI). Cervical SCI, which occurs in more than half of all human cases, can be extremely debilitating if the neural circuitry responsible for controlling respiratory function is affected. PhMNs located at cervical levels C3-C5 directly control activation of the diaphragm, which is the major inspiratory muscle. PhMNs are mono-synaptically innervated by bulbospinal projections of respiratory neurons located in a brainstem nucleus called the rostral Ventral Respiratory Group (rVRG). Cervical SCI can result in persistent diaphragm compromise because of damage to these descending rVRG axons, denervation and silencing of spared PhMNs, and consequent paralysis of the hemi-diaphragm. In a rat model of unilateral C2/3 hemisection SCI, we are expressing the axon guidance molecule, brain-derived neurotrophic factor (BDNF), in PhMNs to direct regenerating ipsilateral and/or sprouting contralateral rVRG axons towards PhMNs with the goal of achieving targeted restoration of the critical rVRG-PhMN-diaphragm circuit. Specifically, we are employing anatomically-targeted delivery of adeno-associated virus serotype 2 (AAV2) to the ipsilateral C3-C5 spinal cord to achieve BDNF expression throughout the denervated PhMN pool. Using neuroanatomical tract tracing and *in vivo* electrophysiological approaches, we are exploring the effects of this strategy on rVRG axon regrowth and collateral sprouting, synaptic reconnection with PhMNs, and restoration of ipsilateral hemi-diaphragm activity. Given our findings that rVRG neurons express the BDNF receptor, tropomyosin-related kinase B (TrkB), and that we can efficiently transduce PhMNs using this AAV2-based approach, we hypothesize that BDNF will promote robust PhMN reinnervation by injured rRVG axons and diaphragmatic respiratory recovery.

Disclosures: B. Charsar: None. **M. Urban:** None. **B. Ghosh:** None. **G.M. Smith:** None. **A.C. Lepore:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Craig Neilsen Foundation Grant

Natural Sciences and Engineering Research Council of Canada Discovery Grant

Title: Pharmacological inhibition of CSPGs receptors LAR and PTPσ positively modulates the inflammatory response and promotes oligodendrocyte replacement following spinal cord injury

Authors: *S. M. DYCK[1], H. KATARIA[1], S. THOMAS[1], B. LANG[2], J. SILVER[2], S. KARIMI-ABDOLREZAEE[1];
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Abstract: Traumatic spinal cord injury (SCI) results in significant cell death in oligodendrocytes leading to demyelination and functional deficits. Resident neural precursor cells (NPCs) have a tremendous potential to replace lost oligodendrocytes following SCI. However, the ability of NPCs to proliferate and differentiate into oligodendrocytes is challenged in the post-SCI microenvironment. We previously identified for the first time that injury-induced upregulation of chondroitin sulfate proteoglycans (CSPGs) inhibits several properties of NPCs including their survival, proliferation and differentiation which is meditated through signaling of LAR and PTPσ receptors and activation of downstream Rho/ROCK pathway. In the present study, we evaluated the therapeutic potential of two functionally blocking peptides against LAR (Inhibitory LAR Peptide, ILP) and PTPσ (Inhibitory Sigma Peptide, ISP) in promoting endogenous cell response after SCI. We show that ILP and ISP efficiently block CSPGs inhibitory effects on NPC survival, proliferation, and oligodendrocyte differentiation. In a clinically-relevant model of compressive SCI in rats, we delivered ISP and ILP treatment locally to the injured spinal cord in a sustainable manner for various time-points. Using BrdU incorporation, Western blotting and immunohistochemistry, we identified multiple beneficial effects of ISP and ILP therapy following SCI. Our BrdU studies revealed that ILP and ISP therapy enhances endogenous cell response following SCI by promoting oligodendrocyte preservation and replacement. Conversely, there was a significant decrease in proliferating astrocytes associated with reduced astrocytic scarring. Moreover, ILP and ISP treatment fosters a neuroprotective and anti-inflammatory response in the post-SCI milieu characterized by induced interleukin-10 (IL-10) and arginase-1 expression. Interestingly, ILP/ISP treatment also promoted the phagocytosis ability of microglia which was otherwise reduced upon their activation and by exposure to CSPGs *in vitro*. In conclusion, we demonstrate that CSPGs regulate the response of endogenous precursor cells following SCI by LAR and PTPσ dependent mechanisms. Blocking these receptors by ILP and ISP treatment improves oligodendrocyte differentiation in SCI likely by promoting a pro-regenerative immune response following injury. CSPGs are a key component of the post-SCI milieu with a significant impact on the cell replacement process. Accordingly, our study has identified new clinically relevant therapeutic targets for enhancing cell-based therapies for the treatment of SCI. *Supported by grants from CHN and NSERC.*

Disclosures: **S.M. Dyck:** None. **H. Kataria:** None. **S. Thomas:** None. **B. Lang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

excluding diversified mutual funds); inventors of the intellectual property covering ISP. **J. Silver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); inventors of the intellectual property covering ISP. **S. Karimi-Abdolrezaee:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: SANPORC

NIH RO1OD018272

Title: Induction of immune tolerance by short-course immunosuppresion after spinal grafting of allogeneic neural precursors in pigs with previous chronic spinal cord traumatic injury.

Authors: *M. MARSALA[1], J. D. CIACCI[2], E. I. CURTIS[2], S. MARSALA[1], M. R. NAVARRO[1], P. CHEN[1], S. JUHAS[3], J. JUHASOVA[3], K. YAMADA[4], K. JOHE[5]; [1]Dept. of Anesthesiol., [2]Dept. of Neurosurg., Univ. of California San Diego, La Jolla, CA; [3]Lab. of Cell Regeneration and Plasticity, Inst. of Animal Physiol. and Genet., Libechov, Czech Republic; [4]Columbia Univ. Med. Ctr., New York, NY; [5]Neuralstem, Germantown, MD

Abstract: *Background:* Current clinical protocols use transient or continuous immunosuppression in patients receiving allogeneic neural precursor grafts for treatment of a variety of neurological diseases, including spinal trauma, stroke or ALS. At present there is no solid evidence, however, that would confirm long-term immune tolerance to allogeneic grafts in a large animal model(s) of chronic spinal cord injury. In our current study, we have tested the engraftment of porcine fetal NPCs after transplantation into the lumbar spinal cord of allogeneic transiently-immunosuppressed spinally-injured recipients. *Material and Methods*: Porcine fetal spinal cord-derived NPCs were isolated from 30-day-old fetuses. NPCs were expanded and characterized by immunofluorescence staining after differentiation in vitro. Clones of UBI-GFP expressing NPCs were then prepared and used in vivo for grafting into the lumbar spinal cord of allogeneic pigs with previous spinal cord L3 contusion injury. Animals (n=4) with fully developed paraplegia were grafted at 5.5 months after spinal injury. All animals received between 30-40 injections of NPCs targeted above, at epicenter and below the injury (10 ul/300,000 cells/injection). After cell grafting, animals were immunosuppressed iv with a combination of Prograf and MFF for 4 weeks. At 4 weeks, immunosuppression was terminated

and animals survived for an additional 3 months (n=1), 6 months (n=1) or 12 months (n=2) without immunosuppression. After survival, animals were perfusion fixed and the presence and differentiation of grafted cells analyzed by immunofluorescence. *Results:* i) In vitro induced NPCs showed expression of neural/neuronal markers, including DCX, NeuN, GABA and GFAP. ii) Analysis of previously injured spinal cord tissue grafted with UBI-GFP+ NPCs showed extensive GFP+ grafts occupying previously injured spinal cord regions. iii) Double staining with neuronal markers (NeuN, SYN, NSE) showed the presence of high density grafted neurons throughout the grafts. iv) Staining with excitatory and inhibitory neurotransmitter markers showed a preferential GABA/glycin-ergic phenotype (VGAT and GAD65 +) in grafted neurons. v) Staining with glial markers showed near complete repopulation of individual grafts with GFAP+ astrocytes and regularly distributed Olig2 positive oligodendrocytes at 12 months after grafting. vi) No signs of cell-mediated rejection were seen at any time point after cell grafting. *Conclusion:* These data demonstrate that short course (4 weeks) immunosuppression is effective in inducing immune tolerance to allogeneic NPCs after grafting into chronically-injured spinal cord in pigs.

Disclosures: **M. Marsala:** None. **J.D. Ciacci:** None. **E.I. Curtis:** None. **S. Marsala:** None. **M.R. Navarro:** None. **P. Chen:** None. **S. Juhas:** None. **J. Juhasova:** None. **K. Yamada:** None. **K. Johe:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Program#/Poster#: 320.14/V15

Topic: C.09. Brain Injury and Trauma

Support: Nathalie Rose Barr studentship, International Spinal Research Trust

Title: Astrocytic morphology altered by glycogen synthase kinase 3β (GSK3β) and chondroitinase ABC in the optic nerve and the spinal cord.

Authors: ***A. KALAM**[1], A. D. RIVERA[1], E. J. BRADBURY[2], A. DIDANGELOS[2], A. M. BUTT[1];
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Abstract: Astrocytes constitute a major element of the mature glial scar that forms as a result of spinal cord injury (SCI). The glial scar is a physical and chemical barrier for axonal growth and is a key reason why axons do not regenerate. Our aim is to determine whether combinatorial

treatment with GSK3β inhibitors and ChABC has a synergistic effect on regulating the astroglial scar to a more permissive one for axon growth. To test multiple combinatorial treatments with numerous GSK3β inhibitors that have different mode of action we used *in vitro*, *ex vivo* and *in vivo* models to study glia relevant to SCI.
In vitro astrocyte scratch assay was performed using rat primary astrocytes with different GSK3β inhibitors. Since *in vitro* cultures of astrocytes do not reflect the complex three-dimensional multicellular environment of the spinal cord and it is not feasible to test all the drugs *in vivo*, we developed a 'medium throughput' *ex vivo* slice models, using spinal cord and optic nerves from transgenic mice in which the astroglial promoter glial fibrillary acidic protein (GFAP) drives expression of enhanced green fluorescence protein (eGFP). Thoracic spinal cord slices (P10-15) or adult optic nerves from mice were maintained in culture for 3 to 7 days *in vitro* (DIV) and treated with GSK3β inhibitors (lithium chloride, ARA014418, tideglusib or TWS119) or ChABC. Inhibition of GSK3β and treatment with ChABC induced morphological changes in astrocytes in the spinal cord and optic nerve, with the development of a polarised astrocyte phenotype. An equivalent effect of GSK3β inhibition was demonstrated in cultured optic nerves, with a profound effect on astrocyte morphology. To examine this astrocyte phenotype further, we performed a genome wide microarray analysis on the optic nerve following GSK3β inhibition compared to controls. Pathway analysis (IOA, Ingenuity Systems) indicated Axon Guidance Signalling as one of the major pathways significantly altered by GSK3β inhibition, with prominent effects on sema3, which is known to promote axon growth. The results support the possibility that GSK3β inhibition induces an environment permissive for axon growth and that the polarised astrocyte will provide a scaffold for axon growth.
To examine this, we plan to investigate the best GSK3β inhibitors singly or in combination with ChABC *in vivo* using a contusion model of spinal cord injury in adult rats. These experiments will allow us to determine the optimal combinations of GSK3β inhibition and ChABC for regulating the astroglial scar to one that is more permissive for axon growth, prior to determining whether they promote reformation of connections and recovery of function *in vivo* in SCI.

Disclosures: **A. Kalam:** None. **A.D. Rivera:** None. **E.J. Bradbury:** None. **A. Didangelos:** None. **A.M. Butt:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Title: The combined therapy of estradiol and tamoxifen confers neuroprotection after spinal cord injury

Authors: *J. M. SANTIAGO SANTANA[1], W. I. MALDONADO GEORGE[1], D. N. MILLÁN[2], L. A. RODRÍGUEZ[3], S. M. RIVERA[2], L. M. GARCÍA[3], J. COLÓN[3], A. PÉREZ[3], S. AYUSO[2], J. M. COLÓN[4], A. I. TORRADO[4], I. K. SALGADO[4], Y. ARROYO[3], J. D. MIRANDA[4];
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Abstract: Spinal cord injury (SCI) is a devastating condition that affects 276,000 people in the U.S. SCI triggers a variety of events at the cellular and molecular level, such as apoptosis, demyelination, inflammation and gliosis, generating a non-permissive environment for axonal regeneration and cell survival. A variety of studies show that Estradiol (E2) has neuroprotective effects after SCI. However, the negative effects that estrogen may generate can raise some concern. Fortunately, Tamoxifen (TAM) is a FDA approved drug that can selectively modulate estrogen receptors, selectively allowing for the beneficial effects of E2. This study shows definitive evidence of the effects of the combined therapy of E2 and TAM administration after injury. Adult female Sprague-Dawley rats received a moderate contusion at the thoracic vertebrae (T_{10}) with the NYU impactor device. Sham animals were treated with an E2 implant (placed subcutaneously, in the *mid*-scapular region; 3 mg) and E2 bolus (intravenously; 100 µg), this project consisted of four groups of SCI animals; the groups were depicted as follows, 1) injured with an empty silastic implant (SE) and sesame bolus, 2) injured with a E2 implant and E2 bolus, 3) injured with SE + E2 bolus and TAM (placed subcutaneously, *mid*-scapular region; 15 mg), 4) injured with E2 implant + E2 bolus + TAM. Behavioral studies were performed weekly over a 35 days post-injury (DPI) period and anatomical studies were performed to assess white matter spare tissue with luxol fast blue staining. Results showed that administration of E2 and TAM administration after SCI significantly enhances functional locomotor recovery at 35 DPI (* = $p<0.05$). Luxol fast blue staining showed a significant increase in white matter preservation after SCI in caudal areas in animals treated with E2 (* = $p <0.05$) and in rostral and caudal areas (*** = $p < 0.001$) in animals treated with the combined therapy (E2 + TAM). Together this data suggests that TAM administration may be a suitable approach to develop a possible therapy for SCI in the near future.

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Poster

320. Injury Responses after Spinal Cord Injury

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Program#/Poster#: 320.16/DP05 (Dynamic Poster)

Topic: C.09. Brain Injury and Trauma

Support: NIH NS059622

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VA I01 BX002356

Craig H Neilsen Found 296749

Title: Imaging neural activity in the primary somatosensory cortex using GCaMP transgenic mice

Authors: X. LIN, W. XIONG, W. WU, M. WALKER, X. JIN, *X. M. XU;
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Abstract: The ability to chronically monitor neuronal activity in the living brain is essential for understanding the organization and function of the nervous system. Two-photon laser scanning microscopy (2P) imaging with genetically encoded calcium indicators (GECIs) permit investigating intracellular calcium transients in vivo. In this study, we described an experimental procedure for measuring calcium transients from single neuron in primary somatosensory cortex or S1 from GCaMPs transgenic mouse using 2P. This GCaMP animal model allows for prolonged and stable calcium imaging in intact adult animals in vivo. Key aspects of the protocol, which can be completed in less than 1h, include the use of a variant of high-speed 2P imaging and minimally invasive surgery procedures for an open-skull window on both sides of the skull. Although we only demonstrate the technique in the S1 area, it allows investigation of activity patterns in defined neuronal populations in the living brain and will greatly facilitate dissecting complex structural and functional relationships of neural networks.

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Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: C.09. Brain Injury and Trauma

Title: miR-21 correlates with progression of degenerative cervical myelopathy and is a marker of hypoxia-induced inflammation

Authors: *A. M. LALIBERTE[1], S. KARADIMAS[2], K. SATKUNENDRARAJAH[2], P. VIDAL[2], M. G. FEHLINGS[2];
[1]Genet. and Develop., Univ. of Toronto, Toronto, ON, Canada; [2]Genet. and Develop., Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Degenerative Cervical Myelopathy (DCM), the most common cause of adult spinal cord dysfunction, is a compressive non-traumatic form of spinal cord injury resulting from degeneration of the structural elements of the spine. Although the structural compression can be identified, the progression of DCM pathology is highly variable. This unpredictability is largely due to the scarcity of knowledge of the underlying mechanisms. MicroRNA-21 (miR-21) is up-regulated under hypoxia and is associated with pro-inflammatory macrophage activation, both potentially important mechanisms in DCM pathology. Previously, we have found an increase in mir-21 in the spinal cord and plasma of DCM rodents. Further, preliminary data from the ongoing human DCM microRNA biomarker study have identified a correlation between miR-21 and poor 1-year follow-up outcomes. Given miR-21's relationship to both hypoxia and inflammation, it is possible that hypoxia-induced miR-21 exacerbates inflammation and directly promotes neurological deterioration in DCM. Therefore, we hypothesize that miR-21 is up-regulated by the hypoxic/ischemic conditions arising from cord spinal cord compression, and that this increase contributes to DCM progression. Using a novel mouse model of DCM, we examined wild type and miR-21 knockout mice at 3, 6 and 12 weeks after DCM induction (mild, moderate, and severe deficits, respectively) compared to sham. To determine whether miR-21 levels were associated with hypoxia, we performed qPCR for miR-21 and hif1a and found that expression of both were highest in moderate DCM mice (10 fold, $p<0.001$, and 1.5 fold, $p=0.004$, respectively). The cellular localization of miR-21 expression was determined by in situ hybridization of spinal cord cryosections, where there was colocalization of miR-21 with Iba1+ microglial cells. In order to confirm the relationship between hypoxia, miR-21 and microglia, in vitro hypoxia experiments were performed using the HAPI microglial cell line. HAPI cells increased mir-21 under hypoxia (1% O2) (19 fold, $p<0.001$) and with pro-inflammatory LPS stimulation (7.8 fold, $p<0.001$). With respect to the function of miR-21 in DCM animals, loss of miR-21 in knockout mice improved rotarod performance by 70% compared to wild type ($p=0.004$). Based on these findings, hypoxia is associated with increased microglial expression

of miR-21 and progression of DCM. Furthermore, we demonstrate that miR-21 deletion attenuates this locomotor deterioration in DCM mice. These data support the observed correlation between miR-21 and patient outcome, underscoring the potential utility of miR-21 as both predictive marker and therapeutic target in DCM.

Disclosures: A.M. Laliberte: None. **S. Karadimas:** None. **K. Satkunendrarajah:** None. **P. Vidal:** None. **M.G. Fehlings:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Support: NIH Grant F30NS093811

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Title: Immune modifying microparticles modulate hematogenous monocytes and promote recovery after spinal cord injury

Authors: *J. G. COOPER[1], S. JEONG[1], I. IFERGAN[2], S. B. SHARMA[1], D. XU[2], T. MCGUIRE[1], J. A. KESSLER[1], S. D. MILLER[2];
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Abstract: Spinal cord injury (SCI) disrupts the blood-brain/spinal barrier and causes a neuroinflammatory response at the lesion site that ultimately results in a secondary wave of axonal destruction. Many studies have demonstrated that infiltrating hematogenous monocytes are important contributors to secondary tissue damage and fibrotic scar formation after SCI. This suggests that selectively blocking monocyte infiltration during the acute phase after SCI could help limit tissue damage. However, developing a practical and translatable approach to selectively target hematogenous monocytes has been challenging due to their surface marker similarities with resident microglia and other immune cells. In the present work, we demonstrate that hematogenous monocytes can be safely and selectively depleted through intravenous injection of immune modifying microparticles composed of biodegradable poly(lactic-co-glycolic)acid (PLGA-IMPs) to produce therapeutic benefits after contusion SCI in mice. PLGA-IMPs selectively target circulating monocytes by binding to the macrophage receptor MARCO, which is expressed on the cell surface of monocytes. Monocytes bound to PLGA-IMPs no longer

travel to inflamed sites, but instead, they are sequestered to the spleen and undergo apoptosis. We demonstrate that acute PLGA-IMP treatment after severe contusion SCI in mice markedly improves functional recovery up to 12 weeks post injury as assessed by Basso Mouse Scale (BMS) open field scoring. Animals that receive PLGA-IMP treatment after SCI display decreased intralesional expression of inflammatory cytokines, diminished fibrotic scarring, and reduced levels of inhibitory extracellular matrix molecules, hence making the environment more permissive for repair and recovery. We demonstrate that acute IMP treatment results in the preservation of axonal density caudal to the lesion site at chronic time points. These data support that hematogenous monocytes contribute to neuroinflammatory damage and fibrotic scar formation after SCI, and more importantly establish PLGA-IMPs as a promising tool for treating SCI and encourage further optimization of the technology to move it forward into clinical development.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Scoliosis Research Society

Title: Riluzole prevents functional deficits during mild distraction spinal cord injury

Authors: *E. N. SHIMIZU, K. J. JOHNSON, M. I. ROMERO-ORTEGA;
Bioengineering, Univ. of Texas At Dallas, Dallas, TX

Abstract: Scoliosis, an abnormal curvature of the spine, affects an estimated 6 to 9 million people in the United States. The first treatment involves bracing to mitigate further curvature, however the effectiveness of this approach is limited, leading to 38,000 spinal fusion surgeries annually. During scoliosis corrective surgery significant distraction forces are imparted on the vertebral column, causing spinal cord injury (SCI) in approximately 1-3% of surgeries, reaching as high as 27% in severe deformities where more complex surgical procedures must be used. We previously developed a new SCI model that utilizes linear actuators to apply bilateral distraction forces on the rat spinal cord, resembling those during scoliosis corrective surgery. Characterization of the injury allowed us to define optimal parameters (i.e. distraction length,

and speed). We use a total distraction length of 5mm at 0.5 mm/s and hold distracted for 15 minutes, which does not cause tissue damage as determined by immunocytochemistry. Instead it induced hypoxic damage, possibly by oxidative stress, indicated by significant increase in protein carbonyls 30 minutes post injury. These changes correlate with a mild locomotor deficit, which persisted for the 7 day assessment period. In this study, we investigated the neuroprotective effect of Riluzole, a sodium channel blocker, to mitigate the deficits following a mild distraction SCI. Thirty-two adult female Long Evans rats were randomly divided into four experimental groups in which either Riluzole or vehicle was administered intraperitoneally to each animal prior to undergoing a sham operation or a distraction injury. Behavioral assessment was evaluated using the CleverSys TreadScan kinematic system on post- operative days 1,3,5, and 7. Statistical analysis showed significant deficits ($p<0.05$) in the stride length and stance time in vehicle treated animals after distraction during the 7 day assessment period. This deficit was prevented by Riluzole pre-treatment, as these animals showed comparable stride length compared to sham animals. The data suggests a mechanistic explanation to a mild distraction SCI which involves dysfunction in ion channels creating an excitotoxic environment that can be prevented by Riluzole. These results also support the notion that Riluzole can be used as neuroprotective treatment to reduce the risk of SCI during corrective spine surgery.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: CIHR Grant RFN# 133460

NSERC Studentship

Title: Neuroplasticity in the injured spinal cord following Sox9 ablation three weeks post injury

Authors: *N. M. OSSOWSKI, N. M. GEREMIA, T. HRYCIW, K. XU, A. BROWN; Robarts Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Recovery after spinal cord injury is limited, as neuronal connections lost due to trauma are never fully restored. This poor recovery is due to the molecular environment of the spinal cord that limits neuroplasticity. Perineuronal nets are matrix structures that pose a major impediment to reactive sprouting owing to their high content of chondroitin sulfate

proteoglycans (CSPGs). Our laboratory has shown that conditional *Sox9* ablation prior to spinal cord injury, effectively decreases CSPG levels in the perineuronal nets distal to the lesion. We have also demonstrated that the decrease in perineuronal net CSPG levels improves locomotor recovery after spinal cord injury. In the present study we have set out to determine whether conditional ablation of *Sox9* in the subacute period after spinal cord injury also yields increased neuroplasticity compared to controls. In this study, *Sox9* was ablated in mice 3 weeks after spinal cord injury. Immunohistochemistry and neuronal tracing to assess neuroplasticity and levels of perineuronal nets will be presented.

Disclosures: **N.M. Ossowski:** None. **N.M. Geremia:** None. **T. Hryciw:** None. **K. Xu:** None. **A. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Names on patents related to Sox9 inhibition.

Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 320.21/W3

Topic: C.09. Brain Injury and Trauma

Support: panish Ministry of Economy and Competitiveness SAF2013-48431-R

International Foundation for Research in Paraplegia Grant P148

Title: IL-13 administration favors microglia and macrophages to adopt an M2-like phenotype after spinal cord injury

Authors: *J. AMO-APARICIO, R. LOPEZ-VALES;
Univ. Autonoma De Barcelona, Bellaterra, Spain

Abstract: Spinal cord injury (SCI) elicits an inflammatory response that comprises mainly microglia and peripheral blood-derived macrophages. These cells contribute directly or indirectly to tissue damage and functional loss; however, they can also promote repair. These paradoxically conflicting roles of microglia and macrophages depend on their polarization state: In response to interferon gamma or lipopolysaccharide, macrophages and microglia undergo “classical” M1 polarization. Contrary, upon interleukin 4 (IL-4) or interleukin 13 (IL-13) stimulation, macrophages and microglia acquire “alternative” M2 polarization. M1 macrophages and microglia release high levels of pro-inflammatory cytokines and free radicals. These compounds are crucial for killing microbes and tumor cells, but they also induce damage in healthy

neighboring cells and are associated with cell loss and secondary damage after SCI. Contrary, M2 macrophages release anti-inflammatory cytokines and are involved in parasite containment, tissue repair and remodeling events after injury. Importantly, the finding of this macrophage dichotomy was originally described using *in vitro* systems. *In vivo*, macrophages are influenced by multiple additional factors. This leads to a wide spectrum of intermediate phenotypes, where the M1 and M2 archetypal states are located at the ends of this range. Macrophages and microglia display predominantly M1 markers after SCI, whereas the expression of M2 markers is limited. To get insights into the mechanisms that impede microglia and macrophages to acquire an M2-like phenotype after SCI, we found that the expression of IL-13, one of the most important M2 polarizing factors, is detected at very low levels in the contused spinal cord and only for the first 24 hours post-injury. We therefore hypothesized that inefficient induction of IL-13 expression after SCI favors microglia and macrophages to remain in a M1-like state. We first evaluated the expression of IL-13 receptor (IL-13Rα1) and found it is induced in microglia and macrophages following contusion injury. Interestingly, we observed that microglia and macrophages induce the expression of the M2 marker Arg1 upon IL-13 administration into the lesion site. Moreover, IL-13 administration reduced the expression of the M1 markers, iNOS and CD16/32, in these immune cell subsets. These results provide evidence that low levels of IL-13 after SCI hamper microglia and macrophages to acquire an M2-like activation state. Further studies are needed to elucidate whether the redirection of microglia and macrophages polarization by IL-13 minimize tissue damage and functional deficits after SCI.

Disclosures: J. Amo-Aparicio: None. **R. Lopez-Vales:** None.

Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 320.22/W4

Topic: C.09. Brain Injury and Trauma

Support: SANPORC

Title: A potent anti-spastic effect after intrathecal NK1 antisense oligonucleotide or subpial AAV9-NK1-ShRNA delivery in rats with chronic spinal transection-induced muscle spasticity

Authors: *M. BRAVO HERNANDEZ[1], T. YOSHIOZUMI[1], M. R. NAVARRO[1], K. KAMIZATO[1], T. TADOKORO[1], O. PLATOSHYN[1], S. MARSALA[1], J. D. CIACCI[2], C. MAZUR[3], M. MARSALA[1];
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Abstract: BACKGROUND: The spinal NK1 receptor system has been demonstrated to play an important role in the development and maintenance of chronic pain states after peripheral nerve injury. In contrast, its role in the development of spinal hyper-reflexia and muscle spasticity resulting from spinal traumatic injury is not well defined. The goal of the present study was to assess the treatment effect of: i) spinal intrathecal (IT) delivery of NK1 antisense oligonucleotide (NK1-ASO), and ii) subpial (SP) delivery of AAV9-NK1-shRNA in rats with chronic spinal cord transection-induced muscle spasticity. METHODS: Adult Sprague-Dawley (SD) rats (female, 200-300 g) had the Th9 spinal segment transected to induce muscle spasticity. The presence of spasticity was defined as exacerbated EMG response recorded from the gastrocnemius muscle after applying progressively increased paw pressures using von Frey filaments (0.6-26 grams). After baseline spasticity measurement, animals received: i) a single lumbar intrathecal bolus of NK-1-ASO or control antisense oligonucleotide (Cont-ASO), or ii) subpial (SP) AAV9-NK1-shRNA or control AAV9-GFP. Before and after treatment, the presence of spasticity response was measured in 1-week intervals for up to 12 weeks. The effect of each treatment on spinal NK1 expression was evaluated by immunofluorescence and confocal microscopy or by qPCR. RESULTS:In spastic animals receiving IT injection of NK1-ASO, a progressive decrease in measured surface EMG activity after paw tactile stimulation was seen with the maximum effect measured at 2 weeks after injection ($p<0.05$). A significant anti-spastic affect was still present at 10 weeks after treatment. No changes in spasticity response were measured in animals receiving control ASO. In animals injected SP with AAV9-NK1-shRNA, a comparable anti-spasticity effect was seen at 2 weeks after treatment. Histological and qPCR analysis of the lumbar spinal cord showed a 75-85% reduction in NK1 signal with no change in substance P expression. CONCLUSIONS: These data show that NK1-ASO or AAV9-NK1-shRNA-mediated suppression of spinal NK1 receptor expression may represent a novel therapeutic approach for modulation of chronic spinal injury-induced muscle spasticity and hyper-reflexia.

Disclosures: **M. Bravo Hernandez:** None. **T. Yoshiozumi:** None. **M.R. Navarro:** None. **K. Kamizato:** None. **T. Tadokoro:** None. **O. Platoshyn:** None. **S. Marsala:** None. **J.D. Ciacci:** None. **C. Mazur:** None. **M. Marsala:** None.

Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 320.23/W5

Topic: C.09. Brain Injury and Trauma

Title: The effects of 10ms pulsed radiofrequency (PRF) treatment on behavioral measures of chronic pain and gene expression changes along the nociceptive pathway

Authors: *J. M. WILLIAMS[1], D. M. TILLEY[2], C. KELLEY[2], D. L. CEDENO[2], R. VALLEJO[2];
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Abstract: Pulsed radiofrequency (PRF) therapy is a clinical treatment utilizing electromagnetic energy aimed to relieve chronic pain states such as neuropathic pain. A previous study in our laboratory used a spared nerve injury model (SNI) of chronic pain to investigate the behavioral efficacy of PRF therapy and associated molecular changes in the sciatic nerve, ipsilateral L5 dorsal root ganglia (DRG) and spinal cord of rats using clinically-relevant settings (500 Hz, 45 V and a 20ms pulse burst within 500ms pulse intervals). The previous study found significant pain relief following PRF therapy that was associated with gene expression changes in TNF-α, IL-6, GABAB-R1, NA/K ATPase, 5-HT3R and c-fos. These changes varied depending upon the tissue examined. The present study investigated whether a 10ms pulse burst with 500ms pulse intervals would yield similar behavioral efficacy and similar changes in gene expression. Results show that 10ms PRF therapy produced significant pain relief 24 hours following treatment. Unlike the previous 20ms study, no significant changes in gene expression were observed in the sciatic nerve (site of injury) following 10ms treatment. In the DRG, 10ms PRF treatment resulted in gene expression changes in subP, NA/K ATPase, 5-HT3R, Gal, VIP and GFAP levels. In the spinal cord, gene expression changes following 10ms PRF treatment were observed in subP, NA/K ATPase, 5-HT3R, Gal, NPY, IL-6 and SNAP25 levels. Overall, 10ms PRF treatment was equally efficacious as 20 ms PRF treatment in relieving neuropathic pain induced by SNI lesions of the sciatic nerve and resulted in gene expression changes in the DRG and spinal cord regions, but not the sciatic nerve.

Disclosures: J.M. Williams: None. **D.M. Tilley:** None. **C. Kelley:** None. **D.L. Cedeno:** None. **R. Vallejo:** None.

Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: Dept of Neurological Surgery Start-Up Funds to CPH

Title: Treatment of postraumatic intraspinal pressure may limit secondary damage in acute rodent spinal cord injury

Authors: *Z. Z. KHAING, L. N. CATES, D. M. DEWEES, Z. BIRJANDIAN, C. P. HOFSTETTER;
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Abstract: Traumatic spinal cord injury (SCI) leads to devastating neurological impairment. Currently, the only clinically effective intervention following traumatic SCI is surgical decompression to remove the impinging bone fragments within 24 hours after injury. Recent data from our lab and others indicate that intraspinal pressure increases significantly (up to five times the normal intraspinal pressure) after a moderate thoracic contusion SCI. Here we examined the histological and behavioral correlates of increased intraspinal pressure after acute SCI. We found that there was an increase in the local edema (spinal cord water content: intact = 64.95 ± 0.80%, 4 hours after post injury = 74.80 ± 0.18%; 7 days after injury = 75.38 ± 2.46%), along a 10-15 mm segment of the injured spinal cord compared to uninjured cord tissue. Next, we examined myelin sparing using the Iorn-Eriochrome Cyanine R method. Preliminary data suggests a trend towards increased myelin sparing in the injury center and in segments rostral to the injury in animals that received surgical decompression by opening the meningeal linings to alleviate increased intraspinal pressure, compared to control animals (injury alone). Importantly, treatment of elevated intraspinal pressure reduced the lesion length (rostrocaudal extent) significantly ($p < 0.005$). There was no significant difference of the spared neurons between injured animals compared to injured animals that received decompression surgery. Intriguingly, decompression by opening the meningeal linings resulted in significant functional recovery after SCI: evaluated using an open-field locomotor test (Basso, Beattie and Breshnahan test), and foot-fault analysis. Results presented here suggest that raised tissue pressure following acute SCI is an important parameter that significantly affects functional recovery in a clinically relevant rodent model. Moreover, elevated pressures may impact the efficacy of experimental therapeutic interventions that are currently evaluated in rodent contusion models of SCI.

Disclosures: Z.Z. Khaing: None. **L.N. Cates:** None. **D.M. Dewees:** None. **Z. Birjandian:** None. **C.P. Hofstetter:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Program#/Poster#: 320.25/W7

Topic: C.09. Brain Injury and Trauma

Support: SANPORC

Title: Potent & long-lasting suppression of muscle spasticity by spinal subpial AAV9-mediated VGAT and GAD65 gene delivery in a rat thoracic 9 transection model of chronic spasticity.

Authors: *T. YOSHIZUMI[1], K. KAMIZATO[1], A. PLATOSHYN[1], M. R. NAVARRO[1], S. MARSALA[1], J. D. CIACCI[2], M. MARSALA[2];
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Abstract: Loss of spinal segmental GABA-ergic inhibition is believed to play an important role in the development of spinal injury-induced muscle spasticity and spinal hyper-reflexia. In our previous studies, we have characterized the anti-spastic potency of spinal parenchymal GAD65 gene delivery once combined with systemic tiagabine (GABA uptake inhibitor) treatment but the overexpression of GAD65 gene alone showed no measurable treatment effect. In our current study, the anti-spastic potency after spinal subpial dual gene delivery, composed of GAD65 & VGAT (vesicular GABA transporter), in a thoracic 9 (T9) complete transection model of chronic spasticity in rats was studied. Adult Sprague-Dawley (SD) rats were used to perform a T8 laminectomy followed by a complete T9 transection of the spinal cord. To measure muscle spasticity, EMG response recorded by surface EMG electrodes from the gastrocnemius was used and analyzed after applying progressively increased paw pressure using von Frey filaments. At 2-3 months after transection and baseline spasticity measurement, animals received a lumbar subpial injection of AAV9 encoding the GAD65 and VGAT under ubiquitin promoter. AAV9-UBI-GFP was used in controls. After AAV9 delivery, the spasticity response was measured in 7-day intervals for 2 months & animals were then perfused with 4% paraformaldehyde. The expression of GAD65, VGAT, VGLUT1 and VGLUT2 was then analyzed in spinal cord sections with immunofluorescence. A potent and time-dependent antispasticity effect was seen in the treatment group. A significant effect was present at 3 weeks and then continued for the duration of the study (i.e., 2 months). No effect was seen in the control group. Immunofluorescence analysis of lumbar spinal cord sections showed a significant upregulation of GAD65 and VGAT and also the presence of those proteins in excitatory VGLUT1 and VGLUT2 terminals. These data demonstrate that dual AAV9-mediated subpial GAD65/VGAT gene delivery leads to a potent and sustained anti-spasticity effect. The use of this treatment approach may represent a novel treatment for modulation of chronic spinal injury-induced muscle spasticity.

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Poster

320. Injury Responses after Spinal Cord Injury

Location: Halls B-H

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Program#/Poster#: 320.26/W8

Topic: C.09. Brain Injury and Trauma

Support: R01-NS092876

SHC-85400

SHC-85220

SHC-84293

Title: Activation of RhoA is contributed to apoptosis of reticulospinal neurons in lamprey brain after spinal cord injury

Authors: *K. G. ZHANG[1], J. HU[1], W. RODEMER[1], M. E. SELZER[1,2];
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Abstract: Disability following spinal cord injury (SCI) is due to failure of axonal regeneration. It is believed that both extrinsic inhibitory factors, e.g., the chondroitin sulfate proteoglycans (CSPG) and the myelin-associated growth inhibitors (ligands for the Nogo receptor), and a developmental loss of intrinsic growth capacity contribute to regeneration failure in CNS axons. The latter is suggested by findings in the lamprey that the 18 pairs of individually identified reticulospinal neurons (RNs) vary greatly in the abilities of their axons to regenerate through the same spinal cord environment. We also found that the same neurons that are bad regenerators undergo very delayed apoptosis, and show common molecular markers. For example, we showed that after SCI, the CSPG receptors PTPσ and LAR are upregulated selectively in the bad-regenerating/bad-surviving neurons. Thus we hypothesize convergence of signaling pathways for cell survival and axon regeneration. In mammals, many of the postulated extrinsic growth-inhibitory factors, including CSPGs, activate intraneuronal signaling pathways that converge on RhoA. Therefore, we cloned lamprey RhoA and studied its role downstream of the CSPG receptors on SCI-induced retrograde neuronal death. RhoA mRNA was expressed widely in normal lamprey brain, but was upregulated by 2 weeks post-SCI selectively in neurons undergoing apoptosis, indicated by labelling with fluorescently labelled inhibitors of caspase activation (FLICA). Thereafter, RhoA mRNA decreased through 9 weeks, the longest time investigated. RhoA mRNA was found in severed axon tips immediately after spinal cord injury, and this was followed by anterograde translocation from the perikaryon down the axon, beginning at 5 days post-SCI. However, RhoA protein was continuously activated in FLICA-

labeled neurons through 9 weeks post-SCI. RhoA activation was correlated with caspase activation, and inversely correlated with the previously-determined regenerative probability of RNs. These findings are consistent with a role for RhoA in signaling apoptosis and restricting axon regeneration.

Disclosures: K.G. Zhang: None. **J. Hu:** None. **W. Rodemer:** None. **M.E. Selzer:** None.

Poster

320. Injury Responses after Spinal Cord Injury

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: C.09. Brain Injury and Trauma

Support: CSRS-21st Century grant

Title: Surgical decompression for degenerative cervical myelopathy induces activation of the immune system

Authors: *P. VIDAL VERA[1], S. K. KARADIMAS[1], A. ULNDREAJ[3], A. M. LALIBERTE[3], J. WANG[2], M. FEHLINGS[3];
[1]Genet. and Develop., [2]UHN, Toronto, ON, Canada; [3]Univ. of Toronto, UHN, toronto, ON, Canada

Abstract: Degenerative cervical myelopathy (DCM) is the most common spinal cord impairment. It is caused by the age-related degeneration of the cervical spine, leading to chronic compression of the spinal cord. Surgical decompression is the current treatment for DCM patients, in order to halt disease progression and further neurological deterioration. However, restoration of the spinal cord blood flow can cause ischemia-reperfusion injury (IRI), contributing to the neurological decline observed within the first months after surgical management.
The primary objective of this study was to characterize the inflammation post-decompression and its contribution to IRI and neurological recovery in an experimental rodent model of decompression. A gradual compression of the spinal cord was induced into two groups by inserting a biomaterial underneath the C5-6 lamina. The first group received an early decompression (6 weeks after DCM), whereas in the second group surgery was delayed (12 weeks after DCM). Locomotor assessment of upper and lower limbs was performed using the Catwalk analysis, whereas manual dexterity was assessed using the Capellini handling test. Our results using injection of fluorescent microspheres show that surgical decompression increases spinal cord blood flow compared to DCM animals in the early decompressed group.

This is accompanied with a concomitant increase in the production of inflammatory cytokines in the spinal cord within 24 hours after decompression. When surgery is delayed there is spinal cord reperfusion followed by astrogliosis and an ongoing release of inflammatory blood monocytes and the production of cytokines, without neurological improvement. Moreover, we found that early decompression attenuates inflammation within 24 hours after surgical decompression, resulting in gait improvement in upper and lower limbs together with a significant recovery in manual dexterity.
Our data show that surgical decompression triggers activation of the immune response. When surgical decompression is delayed, spinal cord reperfusion is followed by ongoing activation of the immune system and glial cells. This is associated with a slow or poor neurological recovery observed in our group with delayed surgery

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: CONACyT by doctoral scholarship number 327854

Project CONACYT - 15523

Title: Assessment of suspensions of the polypyrrole doped with iodine synthesized by plama (PPPy/I) and rat serum albumin (RSA) microinjected as treatment of traumatic injury of the spinal cord (TSCI).

Authors: *O. FABELA[1,4], S. SÁNCHEZ-TORRES[4,5,2], L. ALVAREZ-MEJIA[1,6,4], R. MONDRAGÓN-LOZANO[7,6,4], G. J. CRUZ[8], M. .-G. OLAYO[8], J. MORALES[3], A. DÍAZ-RUÍZ[9], C. RÍOS[9], L. MEDINA-TORRES[10], H. SALGADO-CEBALLOS[6], R. OLAYO[3]; [1]Electric Engin., Univ. Autónoma Metropolitana, CDMX, Mexico; [2]Div. of Biol. Sci. and Hlth., [3]Physic, Univ. Autónoma Metropolitana, Mexico, Mexico; [4]Proyecto Camina A.C., Mexico, Mexico; [5]Med. Res. Unit in Neurolog. Dis., Ctr. Médico Nacional Siglo XXI, México, Mexico; [6]Med. Res. Unit in Neurolog. Dis., Ctr. Médico Nacional Siglo XXI, Mexico, Mexico; [7]CONACyT, Mexico, Mexico; [8]Inst. Nacional de Investigaciones Nucleares, Mexico, Mexico; [9]Neurochemistry, Inst. Nacional de Neurología y Neurocirugía, Mexico, Mexico; [10]Facultad de Química, Univ. Autónoma de Mexico, Mexico, Mexico

Abstract: The spinal cord injury is a social and economic problem which represents a great burden on the patient and their families as well as for public health systems. Differents strategies have been designed for the treatment of this disease, which have focused on two aspects: the first is neuroprotection, i.e., protect the surviving nerve tissue after primary damage, the second is the neuroregeneration, which is to promote the regeneration of nerve tissue post primary and secondary damage; for this, the materials science, has sought to generate biocompatible materials capable of performing the function as a neuroregenerator and neuroprotective agent, at the same time acting as a substrate or cell scaffold. In this regard, biomaterials polypyrrole synthesized by plasma energy and doped with iodine (PPPY / I), have shown the capacity to help in recovery following traumatic spinal cord injury (TSCI) in sub-acute phase [1]. On the other hand, there are reports of biomolecules such as the albumin serum (SA) in which reference to its function as a neuroprotective versus neurotoxic agents such as hydrogen peroxide (H_2O_2) and high concentrations of calcium (Ca^{+2}) under is conditions in vitro [2,3]. Therefore, in this study, we explored the combined application by microinjection of suspensions of two types PPPy/I in the presence or absence of albumin serum rat (RSA) as a treatment in a TSCI in sub-acute phase in a rat model and while it demonstrates the importance of the surface characteristics of those materials in functional recovery response. The results show there is a significant difference between the TSCI + PPPy/I.1 and TSCI + PPPy/I.2 + RSA groups both with respect to TSCI group, and that the surface chemistry of both polymers is involved directly with the biological response obtained. [1] Alvarez-Mejia, L., et al., Functional recovery in spinal cord injured rats using polypyrrole/iodine implants and treadmill training. Journal of Materials Science: Materials in Medicine, 2015. 26(7): p. 1-11. [2] Gum, E.T., et al., Human serum albumin and its N-terminal tetrapeptide (DAHK) block oxidant-induced neuronal death. Stroke, 2004. 35(2): p. 590-5. [3] Fanali, G., et al., Human serum albumin: from bench to bedside. Mol Aspects Med, 2012. 33(3): p. 209 - 290.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: MOST 102-2314-B-075 -031 -MY3

V105E6-004-MY3-1

Title: Neuroprotective strategy at acute phase reduces microglia activation and improves survival of motoneuron after cervical nerve root transection

Authors: *M.-C. HUANG[1,2], C.-T. LIN[3], Y.-L. LIN[5], C.-J. HONG[3], K.-T. CHANG[6], H. CHENG[4,6,3];
[1]Taipei Veterans Gen. Hosp, Taipai, Taiwan; [2]Sch. of Med., Taipei Med. Univ., Taipei, Taiwan; [3]Neural Regeneration Lab., [4]Dept. of Neurosurgery,Neurological Inst., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [5]Grad. Inst. of Vet. Pathobiology, Natl. Chung Hsin Univ., Taichung City, Taiwan; [6]Inst. of Pharmacology,Faculty of Medicine, Sch. of Med., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Cervical root avulsion causes the loss of motor and sensory functions in the upper limb and leads to neuron death in the spinal cord. Avulsion one or multiple nerve roots is commonly observed in severe brachial plexus injuries (BPI), that often causes unbearable neuropathic pain and loss of motor function. Neuroinflammation is involved in development of neuropathic pain. Moreover, inflammation induced oxidative stress is involved in the causes of neuronal death after nerve injury. Since minocycline and acidic fibroblast growth factor (aFGF) has anti-inflammation capability. Herein, we investigated the neuroprotective effects on administration of minocycline and aFGF at acute phase following cervical root injury. After 6th to 8th cervical roots transection (both dorsal and ventral), minocycline was immediately given for one week and aFGF for the following two weeks. Our preliminary data showed that neuroprotective treatment significantly suppressed IBA1 (microglia) and GFAP (astrocyte) expression in the ventral horn in one-week group. Furthermore, the number of survived -motoneurons was significantly elevated after treatment. Our data suggested that neuroprotective treatment at acute phase improved survival of motoneuron and suppressed neuroinflammation in the ventral horn. Since the mechanisms for neuroprotection with cervical root injury model is still unclear, we believe our research will help understand such injuries and provide potential treatment for these patients.

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Poster

320. Injury Responses after Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

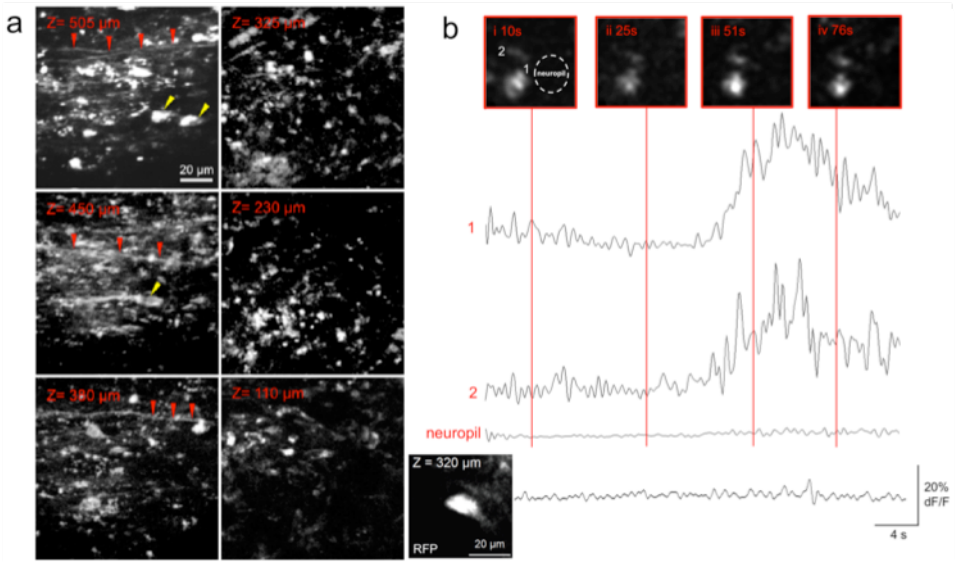
Support: New York State Spinal Cord Research Injury Board C30830GG

Craig H. Neilson Foundation 296332

Title: *In vivo* three-photon excited fluorescence imaging of neural activity in the spinal cord of awake, locomoting mouse

Authors: *Y.-T. CHENG[1,2], S. L. NESS[2,3], S. H. HU[2], J. RAIKIN[2], L. D. PAN[2], D. G. OUZOUNOV[4], T. WANG[4], X. LI[2], J. C. CRUZ HERNANDEZ[2], I. M. BASTILLE[2], N. NISHIMURA[2], J. R. FETCHO[1], C. XU[4], C. B. SCHAFFER[2];
[1]Neurobio. and Behavior, [2]Meinig Sch. of Biomed. Engin., [3]Col. of Vet. Med., [4]Applied and Engin. Physics, Cornell Univ., Ithaca, NY

Abstract: Imaging patterns of neural activity using genetically encoded calcium indicators (GECIs) has enabled studies of brain circuit dynamics, but applying these approaches beyond the cortex is difficult. Spinal cord neurons in central pattern generator (CPG) circuits control rhythmic locomotor behaviors but sit below highly optically scattering white matter, making imaging of these cells challenging, even with two-photon microscopy. With longer excitation wavelength and stronger suppression of background fluorescence, three-photon excited fluorescence (3PEF) microscopy enables deeper penetration into tissue. We developed a long-term implantable spinal imaging chamber that provides optical access to the lumbar spinal cord (L2 - L5), which contains a high density of inter- and moto-neurons that drive hindlimb muscles. We designed a treadmill the animal can run on while held by the implanted chamber under the microscope, optimized such that applied forces and motion of the mouse's back relative to the floor during running are as natural as possible. Once trained, animals exhibited a normal running gait and grooming behaviors while spine fixed atop the treadmill. Running activity was similar in spine-fixed and non spine-fixed sessions: average running speed of 0.36 m/s vs. 0.43 m/s, and fraction of time spent running of 21% vs. 18%. The image motion artifact in running mice was ~3 μm. To image neural activity we expressed the GECI RCaMP1h in spinal cord neurons using an AAV1 viral vector. Using 1.7 μm excitation for 3PEF (Fig. a), we observed spontaneous neural activity (normalized change in fluorescence > 50%) in awake, running mice at a depth of 0.5 mm (Fig. b). In neurons expressing RFP (not calcium sensitive), normalized fluorescence changes were less than 5% (Fig. b, bottom trace). When combined with quantitative tracking of limb kinematics and targeting of GECI expression to genetically defined neural populations, this capability for 3PEF imaging of cell-resolved neural activity could enable detailed studies of how activity patterns in CPG circuits coordinate rhythmic locomotion.



Disclosures: **Y. Cheng:** None. **S.L. Ness:** None. **S.H. Hu:** None. **J. Raikin:** None. **L.D. Pan:** None. **D.G. Ouzounov:** None. **T. Wang:** None. **X. Li:** None. **J.C. Cruz Hernandez:** None. **I.M. Bastille:** None. **N. Nishimura:** None. **J.R. Fetcho:** None. **C. Xu:** None. **C.B. Schaffer:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.01/X1

Topic: C.09. Brain Injury and Trauma

Support: Swedish Armed Forces R&D (AF.9221006)

Swedish Research Council (04X-2887)

Title: Single versus repeated mild blast exposure; the galanin, serotonin and noradrenalin systems

Authors: L. KAWA[1], U. P. ARBORELIUS[1], A. KAMNAKSH[2], T. HOKFELT[1], D. V. AGOSTON[2], *M. G. RISLING[3];
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Abstract: Traumatic brain injury (TBI) as a result of exposure to blast related mechanisms is a spectrum disorder. At the mild end, mild blast TBI (mbTBI), there is significant overlap with mood/anxiety- disorders such as posttraumatic stress disorder. Little is known about the underlying mechanism(s) as the resulting injury is distinct from the TBI literature in the civilian population; not involving rotational acceleration. mbTBI is often missed during diagnosis, in the case of soldiers they may be cleared to return to duty and are at risk of repeated exposures.
We have previously reported changes following exposure to a single blast in the noradrenaline, serotonin, and the neuropeptide galanin systems. These systems have been firmly linked to emotional regulation evident in the pharmaceutical interventions that exploit these pathways and the wealth of published literature.
However, the cumulative effect of multiple exposures has not been clearly ascertained hence we used a rodent model of blast-induced TBI to study the effects in these systems of interest.
Male rats were anaesthetized placed in a rigid metallic holder, which limited acceleration movements of the head and protected the animals' torso, and thereafter exposed to a single or double blast wave.
Samples have been evaluated for white matter injury and nerve cell death. Brain sections in the regions of interest have been used to investigate the transcript levels of the rate limiting enzymes tyrosine hydroxylase and tryptophan hydroxylase two (TPH2), and the neuropeptide galanin. Serum from these animals has also been processed to look at several biomarkers including, S100, GFAP, VEGF, phospho- and native tau levels.
We do not observe evidence of white matter injury or cell death in either the single or double exposed group, thus we can presume we have induced a mild TBI. We find elevated TH, TPH2, and galanin mRNA levels in the exposed groups relative to shams. This is to a similar level in both the single and double exposed groups, therefore we do not see evidence of a cumulative effect of repeated exposure in these stress systems studied. Thus suggesting one exposure alone elicits maximal response in these salient players in emotional regulation and further exposures may just sustain this response.

Disclosures: L. Kawa: None. **U.P. Arborelius:** None. **A. Kamnaksh:** None. **T. Hokfelt:** None. **D.V. Agoston:** None. **M.G. Risling:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.02/X2

Topic: C.09. Brain Injury and Trauma

Support: Department of Veterans Affairs RRD contract #RX001104-01

Title: Astrocyte reactivity following blast exposure involves aberrant histone acetylation

Authors: *Z. S. BAILEY[1], M. B. GRINTER[1], P. J. VANDEVORD[1,2];
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Abstract: Traumatic brain injury has been deemed the signature injury of current wartime efforts and blast exposure is the most common cause. Clinical manifestations of blast induced neurotrauma (BINT) include a diverse array of cognitive and behavioral symptoms driven by persistent inflammation at the cellular level. Epigenetic regulation serves as an important mediator of gene expression and cellular function which may underlie the chronic inflammation likely resulting in neurodegeneration. We hypothesize that altered histone acetylation patterns may be involved in blast induced inflammation and the chronic activation of glial cells. This study aimed to elucidate changes to histone acetylation occurring following injury and the roles these changes may have within the pathology. Sprague Dawley rats were subjected to blast overpressure exposure (10 or 17 psi) within an Advanced Blast Simulator. Sham animals underwent the same procedure without blast exposure. Memory impairments were measured using the Novel Object Recognition (NOR) test at two and seven days post-injury. Tissues were collected at seven days for Western blot and immunohistochemistry analysis. Sham animals showed in-tact memory at each time point. The novel object discrimination decreased significantly between two and seven days for each injury group ($p<0.05$). This is indicative the onset of memory impairment. Western blot analysis showed glial fibrillary acidic protein (GFAP), a known marker of activated astrocytes, was elevated in the prefrontal cortex (PFC) following blast exposure at 10 and 17 psi. No changes were observed for Ionized Calcium-Binding Adapter molecule 1 (IBA-1), a marker for microglia. Analysis of histone protein extract showed no changes in the level of any total histone proteins within the PFC. However, acetylation levels of histone H2b, H3, and H4 were decreased in both groups ($p<0.05$). Colocalization immunofluorescence was used to further investigate any potential correlation between decreased histone acetylation and astrocyte activation. These experiments focused on the anterior cingulate cortex (ACC) and showed a similar decrease in H3 acetylation in astrocytes exposed to a 17 psi blast but not the 10 psi blast. Such changes may alter transcription and play a role in the chronic astrocyte activation associated with BINT. As such, revealing epigenetic alterations following BINT may lead to novel therapeutic targets.

Disclosures: Z.S. Bailey: None. **M.B. Grinter:** None. **P.J. VandeVord:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.03/X3

Topic: C.09. Brain Injury and Trauma

Support: UNT Research Seed Grant

Title: Network Trauma: Electrophysiological and subcellular damage after tangential 300-600g impacts *In vitro*.

Authors: E. A. ROGERS, *G. W. GROSS;
Dept Biolog Sci., Univ. North Texas, Denton, TX

Abstract: A ballistic pendulum impulse generator was used to apply different levels of g-forces to neuronal networks growing on microelectrode arrays (MEAs, 64 electrodes). This approach allowed simultaneous monitoring of neuronal morphology, action potential (AP) production, network activity patterns, and cell electrode coupling of individual units using AP waveshape templates. Quantitative comparisons of activity and morphology were possible before and within 8 min after impact, with continual post-impact monitoring for 48 hrs. The following results can be reported from 31 experiments to date: (1) Network adhesion was maintained after tangential impacts up to 600g. Pre-selected active units were lost in a range from 0 to only 5% (mean 1.5%). Increases and decreases of AP amplitudes (range: 10-40%) were seen indicating effects on cell-electrode coupling or membrane potentials. (2) Time lapse phase contrast microscopy showed nuclear displacement, including nuclear rotation after impact. Pre-impact observations (2-5 hrs) and non-impacted control networks (12-24 hrs) showed stable nuclei. (3) All 31 experiments showed a two-phase response: recovery and subsequent slow activity decay to a stable level plateau approximately 45% below reference. Phase 1 had three recovery modes: full temporary recovery (n=2), partial recovery (n=4), and a more complex two-plateau response (n=25). Pre-impact reference activity (total network spike production) was recorded for an average 4 hours (range 2 to 14 hrs). All changes were quantified relative to this reference. (4) The dominant Phase 1 response (n=25) featured a stepwise recovery over a period of 1 to 2 hours: A rapid activity increase to within 30 to 60% of reference forming a level plateau lasting from 5 to 20 min, followed by a climb to within 10 to 20% of reference where a second plateau was established for 1 to 2 hrs. A full recovery was never attained. (5) Sequential multiple impacts separated by 60-90 min showed compounding increasing damage (n=4).
We hypothesize that the injury profiles reflect two different mechanisms: Direct somal or synaptic damage that is partially repaired within 60min, followed by a long-term deficit in cell-cell communication reflected in altered spike cross correlations. Here, the cytoskeletal damage and nuclear dislocation manifests itself in disrupted protein transport into axons affecting

synaptic functions in a delayed manner. The heavy nucleus plays a major role in the cytoskeletal damage and concomitant transport disruption.

Disclosures: **E.A. Rogers:** None. **G.W. Gross:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

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Program#/Poster#: 321.04/X4

Topic: C.09. Brain Injury and Trauma

Support: VA Merit Award #1I21RX001371

NIH/NIA/IOA Grant #5T32AG00213

Title: Conjugated linoleic acid administration in male rats induces amnesia and exacerbates recovery from functional deficits induced by a penetrating controlled cortical contusion injury

Authors: *C. S. ATWOOD[1], I. M. ANDERSON[1], Q. BONGERS[1], A. JANSEN[1], C. NIER[1], M. WEHBER[1], A. KAPOOR[2], T. E. ZIEGLER[2], K. HAYASHI[1], S. VADAKKADATH MEETHAL[1], R. I. GEDDES[1];
[1]Med., [2]Natl. Primate Res. Ctr., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Conjugated linoleic acids (CLA) possess anti-cancer, anti-atherogenic, anti-adipogenic, anti-diabetogenic, and anti-inflammatory properties. Recently, oral CLA was shown to increase testosterone biosynthesis in exercising, but not sedentary, adult male humans and mice. While similar affects in rats remain controversial, testosterone treatment and/or exercise have been shown to diminish traumatic brain injury (TBI)-induced neuropathology, and reduce deficits induced by stroke in adult rats as well as offset hypogonadism and improve cardiorespiratory fitness in adult men with TBI. To test the impact of CLA on cognitive recovery following a TBI, 5-6 month old male Sprague Dawley rats received a penetrating controlled cortical impact (CCI; n=18) or Sham (n=12) injury and were injected with 50 mg/kg body weight of Clarinol® G-80 (80% CLA in safflower oil; n=16) or saline (n=14) every 48 h for 4 weeks. Plasma testosterone concentrations were significantly reduced post-trauma (from 5.9 ± 1.3 ng/mL to 1.5 ± 0.9 ng/mL; $p < 0.05$). CLA treatment did not restore plasma testosterone (1.7 ± 0.3 ng/mL, $p > 0.05$) to pre-injury concentrations or alter circulating concentrations in Sham animals. In Sham animals, CLA did not alter body weight but did markedly increase the latency to find the hidden MWM platform (40.3 ± 13.0 s) compared to saline treated Sham animals (8.8 ± 1.7 s). CCI injury in saline-treated rats produced a sizable cystic infarct (10.3 ± 1.7% of

cortical surface, $p < 0.05$), reduced mean body weight by ~10.8% ($p < 0.05$) and decreased rotarod performance by 30.2 ± 1.6% of baseline ($p < 0.05$). CLA injections did not alter CCI-induced neuropathology (cortical lesion size was 12.1 ± 1.6% of cortical surface, $p > 0.05$), body weight (~11.4% below baseline weight, $p > 0.05$) or deficits in rotarod performance (latency to fall of rotating rod was 35.7 ± 1.3% of baseline, $p > 0.05$). Like the Sham animals, CLA injections exacerbated the latency of CCI-injured rats to find the hidden MWM platform (66.8 ± 10.6 s) compared to CCI-injured rats treated with saline (30.7 ± 5.5 s, $p < 0.05$). These results indicate that chronic CLA treatment in adult male rats 1) does not alter circulating testosterone concentrations in either uninjured or CCI-injured animals, 2) is detrimental to medium-long-term spatial learning and memory in uninjured animals, and 3) limits cognitive recovery following a moderate-severe penetrating CCI.

Disclosures: **C.S. Atwood:** None. **I.M. Anderson:** None. **Q. Bongers:** None. **A. Jansen:** None. **C. Nier:** None. **M. Wehber:** None. **A. Kapoor:** None. **T.E. Ziegler:** None. **K. Hayashi:** None. **S. Vadakkadath Meethal:** None. **R.I. Geddes:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.05/X5

Topic: C.09. Brain Injury and Trauma

Support: NIAAA RO1AA021121 (AGS, JJC)

Department of Veterans Affairs Office of Research and Development Medical Research Service (DGC)

VA Rehabilitation Research & Development Service (ERP)

University of Washington Friends of Alzheimer's Research (DGC, ERP)

UW Royalty Research Fund (DGC)

Northwest Network Geriatric Research, Education and Clinical Center (DGC, CCW)

Northwest Network Mental Illness Research, Education and Clinical Center (JSM, ERP)

Title: Repetitive blast exposure in mice and combat Veterans promotes a persistent profile of behavioral and dopaminergic dysfunction

Authors: *A. G. SCHINDLER[1], J. S. MEABON[2], K. D. MEEKER[3], G. LI[2], C. W. WILKINSON[3], E. PESKIND[2], D. G. COOK[3], J. J. CLARK[1];
[1]Univ. of Washington, Seattle, WA; [2]Veterans Affairs Puget Sound Mental Illness Res. Educational and Clin. Ctr., Seattle, WA; [3]Veterans Affairs Puget Sound Geriatric Res. Educ. and Clin. Ctr., Seattle, WA

Abstract: Mild traumatic brain injury (mTBI) has been called a "signature injury" of the Operation Iraqi Freedom/Operation Enduring Freedom/Operation New Dawn (OIF/OEF/OND) armed conflicts. Exposure to the primary blast overpressure generated by blast explosions is capable of causing injury; however the underlying pathophysiology is not well understood. Of significant concern is the potential for post-concussive symptoms and psychological dysfunction (e.g. irritability, impulsiveness, risk taking, aggression, substance use/abuse, PTSD, depression, and anxiety), but diagnoses and treatment options are limited. The mesolimbic dopamine system consists of dopamine-containing neurons in the midbrain that project and release dopamine onto their postsynaptic targets, including the nucleus accumbens (NAc). In healthy subjects this system plays a role in reward processing and decision making, but perturbations are implicated in a variety of psychological dysfunctions similar in nature to those seen following blast-related mTBI, raising the possibility that damage to this system might contribute to the underlying neural mechanisms of dysfunction following blast exposure. Indeed, blast-induced damage to the midbrain and NAc has been previously suggested, but a direct investigation of dopamine release post-blast has not been conducted. Likewise, while rodent models of blast-related mTBI have largely focused on subsequent memory-related cognitive effects, no study to date has utilized rodent models to examine the effects of blast exposure on behaviors related to psychological dysfunction and addiction liability such as novelty seeking and irritability. Therefore, in order to study the neurochemical and behavioral consequences of blast-related mTBI in rodents, the present study utilized an established mouse model that effectively recapitulates the blast forces experienced by active military personnel in the open field. Using this model we demonstrate that mild blast exposure(s) resulted in prolonged novelty seeking and irritability. Likewise, blast exposure(s) caused a prolonged potentiation of phasic NAc dopamine release in response to electrical stimulation of the PPT (measured via fast scan cyclic voltammetry; FSCV). Finally, we found an increase in irritability and PTSD symptoms among OIF/OEF combat Veterans with a history of blast-related mTBI that positively correlated with their CSF dopamine levels. Together these data support the hypothesis that dysfunctions following blast-related mTBI may result, at least in part, through perturbation of the mesolimbic dopamine system, highlighting potential new treatment targets and strategies.

Disclosures: A.G. Schindler: None. **J.S. Meabon:** None. **K.D. Meeker:** None. **G. Li:** None. **C.W. Wilkinson:** None. **E. Peskind:** None. **D.G. Cook:** None. **J.J. Clark:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Program#/Poster#: 321.06/X6

Topic: C.09. Brain Injury and Trauma

Support: MOMRP RADIII Task Area I project (Proposal No. 19210)

CDMRP awards W81XWH-08-2-0018 and W81XWH-11-2-0127

Title: Defective methionine metabolism in the brain after repeated blast exposures might contribute to increased oxidative stress

Authors: *P. ARUN, W. B. RITTASE, D. M. WILDER, Y. WANG, I. D. GIST, J. B. LONG; Ctr. for Military Psychiatry and Neurosci., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Blast-induced traumatic brain injury (bTBI) is one of the major disabilities in Service Members returning from recent military operations. The neurobiological underpinnings of bTBI, which are associated with acute and chronic neuropathological and neurobehavioral deficits, are uncertain. Increased oxidative stress in the brain is reported to play a significant role promoting neuronal damage associated with both brain injury and neurodegenerative disorders. In this study, brain regions of rats exposed to repeated blasts in a shock tube underwent untargeted profiling of primary metabolism by automatic linear exchange/cold injection GC-TOF mass spectrometry and revealed acute and chronic disruptions in the metabolism of amino acids and antioxidants. Closely coupled repeated blast exposures (19 psi peak total pressure, 8 msec duration) affected the metabolism of the essential amino acids tryptophan, phenylalanine and methionine. Tryptophan levels decreased on day 1 whereas phenylalanine showed a significant increase in the brain at 28 days after blast exposure. Methionine sulfoxide, the oxidized metabolite of methionine, showed a sustained increase in the brain after blast exposure which was associated with a significant decrease in cysteine, the amino acid derived from methionine. Glutathione, the antioxidant synthesized from cysteine, similarly decreased as also did the antioxidant ascorbic acid. Reductions in ascorbic acid were accompanied by increased levels of its oxidized metabolite, dehydroascorbic acid and other metabolites such as threonic acid, isothreonic acid, glycolic acid and oxalic acid. In view of the fundamental importance of glutathione in the brain as an antioxidant, including its role in the reduction of dehydroascorbic acid to ascorbic acid, the disruptions in methionine metabolism elicited by blast might prominently contribute to neuronal injury by promoting increased and sustained oxidative stress. Increasing the levels of cysteine in the brain by dietary supplementation of cysteine or administration of N-acetyl cysteine could be a potential therapeutic strategy against bTBI.

Disclosures: **P. Arun:** None. **W.B. Rittase:** None. **D.M. Wilder:** None. **Y. Wang:** None. **I.D. Gist:** None. **J.B. Long:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: DARPA (Traumatic Brain Injury (PREVENT) D.O. 0012)

USAMRAA G170217416

USARIEM

Title: A proteomics study of the temporal and spatial changes following blast induced traumatic brain injury

Authors: A. KAMNAKSH[1], R. BEKDASH[1], I.-H. LIN[1], G. MUELLER[1], G. LING[1], A. SCRIMGEOUR[2], L. TONG[3], J. LONG[3], T. WESTMORLAND[4], W. TAYLOR[4], S. PARKS[5], *D. V. AGOSTON[6];
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Abstract: The complexity of traumatic brain injury (TBI) as a disease entity is evident in the clinical spectrum of pathological changes and their neurobehavioral correlates. The affected cognitive and behavioral functions have precise neuroanatomical substrates (e.g., learning and memory are mediated by the hippocampal formation (HPF), anxiety by the amygdala). Apart from individual variability and preexisting conditions, it has been hypothesized that the deficits following TBI are a manifestation of the selective vulnerability of brain regions to blast. In an effort to better understand the pathomechanisms underlying the functional deficits and to identify putative biomarkers for blast-induced TBI (bTBI), we analyzed injury-induced changes in the tissue levels of protein biomarkers associated with inflammatory response, metabolic and vascular changes in functionally relevant brain regions as well as in serum and cerebrospinal fluid (CSF). Using the rodent and porcine models of mild bTBI, we found brain region-specific differences in biomarker levels at various post-injury time points. The inflammatory proteins interleukin 6 (IL-6) and interferon gamma (IFNγ) were significantly higher in the cerebellum (CB) than in the frontal pole (FRP) and HPF. Hydroxynonenal (HNE)-Michael adducts, proteins modified by HNE following lipid peroxidation, were also higher in the CB. Conversely, vascular

endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF1α) were higher in the HPF. In the case of aquaporin-4 (AQP4), a high abundance water channel with distinct localization in the CNS, we detected temporal (as opposed to spatial) differences consistent with the fluid imbalances that are observed clinically (i.e., cerebral edema in the acute phase of TBI and gliosis in the chronic phase). The temporal and spatial differences in protein levels observed here demonstrate the selective vulnerability of brain regions to secondary injury mechanisms (e.g., hypoxia, inflammation, metabolic dysfunction) triggered by the exposure to blast. Therefore, determining injury-induced changes in biomarkers related to different functional classes, in select brain regions as well as in serum and CSF, can provide important and currently missing spatial information about the extent and nature of molecular pathology after TBI.

Disclosures: **A. Kamnaksh:** None. **R. Bekdash:** None. **I. Lin:** None. **G. Mueller:** None. **G. Ling:** None. **A. Scrimgeour:** None. **L. Tong:** None. **J. Long:** None. **T. Westmorland:** None. **W. Taylor:** None. **S. Parks:** None. **D.V. Agoston:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

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Program#/Poster#: 321.08/X8

Topic: C.09. Brain Injury and Trauma

Support: The Miami Project to Cure Paralysis and Florida Department of Health

Title: Durable engraftment, neuronal differentiation of human fetal neural stem cell transplants in penetrating ballistic-like brain injury accompanied by amelioration of cognitive deficits.

Authors: *S. GAJAVELLI[1], M. S. SPURLOCK[2], K. N. RIVERA[2], A. I. AHMED[2], S. YOKOBORI[2], S. W. LEE[2], M. P. HEFFERAN[3], K. JOHE[4], T. G. HAZEL[4], F. C. TORTELLA[5], D. A. SHEAR[5], R. M. BULLOCK[2];
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Abstract: Severe penetrating traumatic brain injury (TBI) is associated with the worst outcomes. No treatments are available, neural stem cell transplantation has emerged as putative therapeutic approach. We hypothesized that transplantation of FDA approved human fetal neural stem cells (hNSC) in a rat model of severe TBI i.e., penetrating ballistic-like brain injury (PBBI) would

uncover their efficacy. Adult Sprague-Dawley rats were subjected to unilateral PBBI and randomized to experimental groups, a week later immunosuppressed and microinjected with vehicle or green fluorescent protein (GFP) expressing hNSCs. Group sample sizes were based on previous literature and pilot data. Animals were tested for spatial learning and memory at eight weeks after transplantation using Morris water maze (MWM) and sacrificed at defined time points. Within the GFP cells expression of nestin was strong at week 1 and diminished by weeks 5 and 8. Immature neuronal marker doublecortin (DCX) was present at weeks 5 and 8. Mature neuronal marker NeuN and synaptophysin could be seen at week8. None of the transplanted expressed glial markers even at week 16. Some cells and processes followed white matter tracts but only GFP processes crossed internal capsule ipsilaterally or wrapped the thalamus bilaterally with processes reaching into the brainstem. MWM testing revealed amelioration of injury induced deficits in animals with transplants. Overall, these results indicate that PBBI is conducive to human fetal neural stem cells engraftment and that hNSC transplants show beneficial effects on cognitive outcome in this model of severe penetrating TBI. Importantly, the transplanted hNSCs appeared to develop normally: downregulating stem cells markers and acquiring neuronal markers. While hNSC cellular migration was more limited, the processes spanned long distances extending primarily through white matter tracts suggesting re-innervation of axonal processes may play a key role in providing therapeutic benefit

Disclosures: **S. Gajavelli:** None. **M.S. Spurlock:** None. **K.N. Rivera:** None. **A.I. Ahmed:** None. **S. Yokobori:** None. **S.W. Lee:** None. **M.P. Hefferan:** A. Employment/Salary (full or part-time): Neuralstem Inc. **K. Johe:** A. Employment/Salary (full or part-time): Neuralstem Inc. **T.G. Hazel:** A. Employment/Salary (full or part-time): Neuralstem Inc. **F.C. Tortella:** None. **D.A. Shear:** None. **R.M. Bullock:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.09/X9

Topic: C.09. Brain Injury and Trauma

Support: CIHR

Title: Inhibition of caspase-3 protects a broad range of developing neurons andneural stem cells from chemotherapeutic-induced cell damage

Authors: *A. J. ELIA[1], J. HENDERSON[2];
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Abstract: Cisplatin represents a widely utilized chemotherapeutic drug which exhibitscommon features in terms of mechanism of action with a variety of otherchemotherapeutic agents. Apoptosis, autophagy and necroptosis have all beenimplicated as mechanisms regulating cisplatin induced cellular injury. Inorder to determine the mechanism whereby agents such as cisplatin act toinduce cellular injury in vivo, CNS injury mapping was performed usinggenetically modified mice in embryos as a model of developing neuroepitheliaand stem cell populations. Cisplatin treatment in wildtype controls resultedin activation of executioner caspase-3 and caspase-7 activity, PARP cleavageand the induction of DNA repair with minimal stabilization of p53 despiteelevation in the expression of cell cycle regulator p21. Analysis ofcellular ultrastructure demonstrated features consistent with apoptosis. Bycontrast caspase-3 null embryos exhibited widespread protection from celldeath resulted in the virtual elimination of cell death for >24 hoursfollowing cisplatin treatment by all measures examined despite demonstrationof caspase-7 activity in these animals. The observed effects are widespreadwithin developing CNS neuroepithelia and extend to non-neural tissues suchas gut, lung, and kidney. Examination of tissues at longer periods followingcisplatin treatment revealed a delayed activation of an necroptotic form ofprogrammed cell death in the absence of caspase-3. This normally quiescentsecondary form of programmed cell death exhibits suggests that coordinatedsuppression of these pathways is critical for long-term inhibition ofp53-mediated cell death in a diverse array of tissues in vivo.

Disclosures: **A.J. Elia:** None. **J. Henderson:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Program#/Poster#: 321.10/X10

Topic: C.09. Brain Injury and Trauma

Title: Effects on the alpha 2 adrenoreceptor antagonist efaroxan on sensorimotor responses and the norepinephrine levels in the dentate gyrus after cortical damage

Authors: *L. RAMOS-LANGUREN[1], S. MONTES[2], G. GARCÍA-DÍAZ[3], N. CHÁVEZ-GARCÍA[2], C. RÍOS[1], R. GONZÁLEZ-PIÑA[3];
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Abstract: Norepinephrine (NE) have shown to play an important role in motor recovery after brain injury. The effects elicited by this monoamine have been reported as distal from the area

directly affected. Remote changes may take place from minutes to weeks and will play an important role in post-stroke recovery. However, the mechanisms involved in spontaneous recovery have not been thoroughly delineated. Previously we reported that 10 and 20 days after cortical iron injection the NE levels in the dentate gyrus (DG, an important structure to plastic processes) increased, which was accompanied by sensorimotor recovery. Here, we analyzed the role of the alpha 2-adrenoreceptor antagonist efaroxan (Efx) as a possible neuroprotector. Therefore, we divided our test subjects (male Wistar rats) in three conditions, a) chronic administration of Efx before cortical injury (n=8), acute administration of Efx immediately (n=7) (b), and 20 days after injury (n=7) (c). Importantly chronic administration of Efx prevents the sensorimotor impair induced by cortical iron injection. Acute administration of Efx after injury did not improve sensorimotor performance. Finally, acute administration of Efx 20 days after injury increased the levels of NE. These results suggest that sensorimotor recovery after brain damage involved changes in NE levels on DG and these changes are modulated by alpha 2-adrenoreceptor. Furthermore, the blocking of these receptors prevents the induction of cortical injury, suggesting that Efx could have a neuroprotector effect enhancing NE release. In brief, these results support evidence to propose NE as a key molecule in the functional recovery of the central nervous system.

Disclosures: **L. Ramos-Languren:** A. Employment/Salary (full or part-time): Maestría en Ciencias Farmacéuticas. **S. Montes:** None. **G. García-Díaz:** None. **N. Chávez-García:** None. **C. Ríos:** None. **R. González-Piña:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.11/X11

Topic: C.09. Brain Injury and Trauma

Support: CONACyT 261323

Title: Immunohistochemical study of nrf2-antioxidant response element as indicator of oxidative stress in the rat brain following by kainic acid and petylenetetrazol treatment

Authors: **A. RUIZ-DÍAZ**[1], J. MANJARREZ[1], C. NAVA-RUIZ[1], A. DÍAZ-RUIZ[1], *M. MENDEZ-ARMENTA[2];
[1]Natl. Inst. of Neurol. and Neurosurg., México City, Mexico; [2]Natl. Inst. Neurol Neurosurg., Mexico City, Mexico

Abstract: Epilepsy is a neurological disorder of the central nervous system characterized by increased and abnormal synchronization of neuronal activity, which is clinically manifested among others by recurrent unpredictable and uncontrollable spontaneous seizures; the temporal lobe epilepsy (TLE) is the most common type of acquired and frequent epilepsy in adults. Epilepsy has been associated with oxidative and nitrosative stress due to prolonged neuronal hyperexcitation and loss neurons during seizures that may result in the mitochondrial dysfunction, increased production of both reactive oxygen species (ROS) and reactive nitrogen species (RNS). It has been clearly demonstrated that oxidative stress interferes with the expression of genes as well as transcriptional factors such asNrf2-dependent Antioxidant Response Element (Nrf2-ARE). In this study we evaluated the morphological changes and the immunohistochemical expression pattern of Nrf2 in the rat brain treated with KA and PTZ considered animal models of epileptic seizures. Nrf2-ARE immunoreactivity was observed mainly in astrocytes of hippocampal brain region in rats exposed at KA; whereas that slight immunoreactivity was observed on rats treated with PTZ. These preliminary results support that fully developed that Nrf2 increased expression is a part of protective mechanism against KA and PTZ toxicity.
Support by CONACyT Grand 261323

Disclosures: **A. Ruiz-Díaz:** None. **J. Manjarrez:** None. **C. Nava-Ruiz:** None. **A. Díaz-Ruiz:** None. **M. Mendez-Armenta:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.12/X12

Topic: C.09. Brain Injury and Trauma

Support: Department of Neurosurgery, Medical College of Wisconsin

VA Research

Title: Evaluation of acute cell death in rat organotypic hippocampal slice cultures exposed to mechanical or blast injury

Authors: *A. GLAVASKI-JOKSIMOVIC[1,2,3], A. S. SHAH[1,3], B. V. APERI[1,3], S. N. KURPAD[1,2,3], B. D. STEMPER[1,3];
[1]Neurosurg., [2]Dept. of Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI; [3]Clement J. Zablocki Veterans Affairs Med. Ctr., Milwaukee, WI

Abstract: Traumatic brain injury (TBI) is a major health problem and its underlying mechanisms remain elusive. In addition, preclinical and clinical studies suggest distinct mechanisms of blunt and blast injuries, although molecular and cellular effects of each are not well characterized. The aim of this study was to elucidate an acute cellular response to the weight-drop and blast overpressure injury in rat organotypic hippocampal slice cultures (OHCs). Hippocampi were dissected from neonatal Sprague Dawley rats (P7-10), cut into 400 μm thick slices, and grown using the membrane interface method. Following recovery period of 7-8 days, OHCs were injured using either blast or blunt mechanisms. For the blunt injury a custom made rod of the NYU weight-drop impactor was placed above the cornu Ammonis 1 (CA1) hippocampal region and dropped from a 6.25 mm height (impact velocity: 0.35 m/s). Alternatively, OHCs were exposed to either 138 ± 22 kPa (low) or 273 ± 23 kPa (high) overpressures using an open-ended helium-driven shock tube. Sham control sections were identically treated, but they were not injured. At 2 h post-injury, cell death in injured and control OHCs was assessed by a propidium iodide (PI) uptake assay. Microglial cells in OHCs were labeled with the isolecitin B4 (IB4) conjugated to FITC for live imaging in the acute phase post injury. Additionally, OHCs were fixed at 2 h post-injury and immunostained against neuronal nuclear antigen (NeuN) and glial fibrillary acidic protein (GFAP) to assess acute effects of injury on neurons and astrocytes, respectively. PI-stained dead cells were observed in traumatized OHCs already at 2 h following mechanical and blast injury. Dead cells were mainly positioned around the site of impact for the blunt injury, although a smaller number of dead cells were observed further away from the site of injury. In the blast-exposed OHCs, dead cells were present throughout the section. However, cells that were co-labeled with PI and IB4, GFAP, or NeuN were detected in both weight-drop- and blast-injured OHCs. Moreover, both injury paradigms evoked the morphological changes of astrocytes, including clasmatodendrosis characterized by cytoplasmic swelling, beading and dissolution of distal processes. Our preliminary data imply that glial cells and neurons in OHCs die in the early phase of mechanical and blast injury, but glial cells appear to be more affected. Future studies will elucidate whether early demise of glial cells is involved in the spread of neurodegeneration following blunt and blast TBI.

Disclosures: A. Glavaski-Joksimovic: None. **A.S. Shah:** None. **B.V. Aperi:** None. **S.N. Kurpad:** None. **B.D. Stemper:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.13/X13

Topic: C.09. Brain Injury and Trauma

Title: Is docosahexaenoic acid neuroprotective after traumatic brain injury in rats?

Authors: ***L. S. BELAYEV**[1], L. KHOUTOROVA[1], A. OBENAUS[2], N. G. BAZAN[1]; [1]Neurosci. Ctr., LSUHSC, New Orleans, LA; [2]Loma Linda Univ., Loma Linda, CA

Abstract: Traumatic brain injury (TBI) remains the most common cause of death in persons under age 45 in the Western world. It is characterized by cerebral damage leading to impairment of neurobehavioral function. Docosahexaenoic acid (DHA) has been shown to possess neuroprotective properties in both *in vitro* and *in vivo* studies. We have previously demonstrated the beneficial effect of DHA in focal cerebral ischemia in rats. The purpose of this study was to determine whether treatment with DHA would be beneficial in a rat model of TBI. Male Sprague Dawley rats were anesthetized with 3% isoflurane, mechanically ventilated, physiologically regulated, and subjected to moderate right parieto-occipital parasagittal fluid-percussion injury. DHA (5 mg/kg) or saline treatment was administered i.v. at 1 h after TBI (n=8-10 per group). Behavior was evaluated on days 1, 2, 3 and 7 after TBI; a grading scale of 0-12 was employed (normal score=0, maximal deficit=12). *Ex vivo* imaging of the brains was conducted on 11.7T MRI on day 7. T2WI and apparent diffusion coefficient (ADC) maps were generated. After completion of MRI study on day 7, histopathology was conducted and the contusion areas and number of normal pyramidal neurons in the CA1 and CA3 regions were quantitated. The physiological variables were entirely comparable among the six groups. Treatment with DHA significantly improved the neurological score compared to saline on day 1 (5.8 ± 0.5 vs. 8.6 ± 0.3), day 2 (5.2 ± 0.6 vs. 9.1 ± 0.3), day 3 (4.6 ± 0.5 vs. 7.5 ± 0.5) and day 7 (3.7 ± 0.5 vs. 7.0 ± 0.6). Lesion areas were significantly smaller in DHA-treated rats compared to the saline group. These results suggest that DHA therapy is neuroprotective in rats following TBI and might provide the basis for future therapeutics in patients suffering from TBI.

Disclosures: L.S. Belayev: None. **L. Khoutorova:** None. **A. Obenaus:** None. **N.G. Bazan:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

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Program#/Poster#: 321.14/X14

Topic: C.09. Brain Injury and Trauma

Support: Veteran's Affairs Office of Research & Development Medical Research Service

NINDS (R01 NS089709)

Title: Polarized aquaporin-4 (AQP4) expression in the cerebellum is disturbed by blast-induced traumatic brain injury (TBI)

Authors: *K. D. MEEKER[1,2], J. ILIFF[5,6], M. SIMON[5,6], J. S. MEABON[2,7], E. R. PESKIND[2,7], D. G. COOK[8,3,4];
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Abstract: Introduction
Modern military conflicts have led to an increasing incidence of mild traumatic brain injury (mTBI) from exposure to explosive blast overpressure (BOP). Recent evidence has identified a novel pathway for clearing extracellular proteins and other substances from brain interstitial fluid via the glymphatic system, which plays an important role in the neuroprotective responses of the brain to TBI. The water channel aquaporin-4 (AQP4) is localized primarily in perivascular astrocytic endfeet and is critical in regulating glymphatic clearance. AQP4 mislocalization is associated with impaired glymphatic function and has been implicated in several neurological disorders. The goal of these experiments was to address in mice the effect of mild blast exposure on AQP4 protein expression levels and localization.
Methods
Using well established procedures, 3-4 month-old male C57BL/6J mice were exposed to primary BOP (19 psi peak intensity) using a helium-driven pneumatic shock tube. All experiments were done following procedures approved by the VA IACUC. Twenty-four hours after a single BOP, brains were analyzed using confocal microscopy. Changes in perivascular AQP4 localization were evaluated as previously described using threshold-based analysis of AQP4 immunoreactivity in different cerebellar cell layers.
Results
We focused on the cerebellum because our recent findings indicate this brain region is especially vulnerable to blast-induced mTBI. Immunofluorescence analysis of the entire molecular layer for all cerebellar lobules revealed no significant changes in perivascular AQP4 localization in blast-exposed versus sham-treated mice (n.s., mean=0.19 and 0.26; n=9 and 14, sham and blast, respectively). However, more fine-grained analysis measuring AQP4 localization in the superficial molecular layer revealed a significant loss of perivascular AQP4 localization in blast compared to shams ($p \leq 0.04$, mean=0.27 and 0.39, n=9 and 14, sham and blast, respectively).
Conclusions
These results indicate that a single blast exposure is sufficient to disturb the normal polarized pattern of AQP4 expression that favors perivascular localization. This suggests that, like other forms of TBI, mild blast exposure may disturb glymphatic function(s) that are subserved by astrocytic AQP4, thereby potentially impairing clearance of metabolic and injury-producing toxic substances from the brain.

Disclosures: K.D. Meeker: None. **J. Iliff:** None. **M. Simon:** None. **J.S. Meabon:** A. Employment/Salary (full or part-time): Neurogenix Pharmaceuticals. **E.R. Peskind:** None. **D.G. Cook:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.15/X15

Topic: C.09. Brain Injury and Trauma

Support: US Army W81XWH-13-1-0016

Title: Mitigation of neuropathology and behavioral deficits in a rat model of brain injury to occupants of vehicles targeted by land mines by an advanced shock absorbing hull design

Authors: *F. TCHANTCHOU[1], W. FOURNEY[3], U. LEISTE[3], A. PUCHE[2], G. FISKUM[1];
[1]Anesthesiol., [2]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD;
[3]Aerospace Engin., Univ. of Maryland Col. Park, College Park, MD

Abstract: Exposure to blasts has resulted in more than 200,000 U.S military victims of TBI in the recent wars in Iraq and Afghanistan. Many were occupants of vehicles targeted by land mines. Using our novel rat model of under vehicle blast induced TBI, we found that underbody blast induced accelerations up to 2400G impaired hippocampus-dependent working memory and long term anxiety-like behavior, but no mortality. Exposure to 2600G resulted in high mortality (67%). These behavioral deficits were associated with several neuropathological markers, suggesting that military vehicle design needs to incorporate acceleration mitigation to reduce brain injury. We hypothesize that blast induced mortality and TBI can be mitigated by two hulls separated by crushable polyurea coated cylinders. Conscious male Sprague Dawley rats were placed in restraints secured on the top of two hulls. An explosive detonated beneath the bottom hull generated an accelerative force of 2600G on the top hull. In some experiments, the two hulls were separated by either uncoated or polyurea-coated aluminum cylinders. Blast survivors and sham rats were tested to assess working memory and anxiety-like behavior over one month. Other animals were euthanized and brain tissue collected for histological or biochemical analyses at 24hr post-blast. Statistical analyses was performed by one way ANOVA with Tukey-Kramer post-test analyses. The presence of polyurea-coated cylinders between the top and bottom hulls reduced blast induced acceleration from 2600G to 550G and completely prevented mortality. The presence of uncoated cylinders only reduced the acceleration to 2300G, with 29% mortality, emphasizing the importance of polyurea coating. The polyurea-cylinder hull design restored working memory to sham levels, while the presence of uncoated cylinders failed to provide protection. Similarly, polyurea coating significantly decreased anxiety-like behavior compared to uncoated cylinders. Histological analysis revealed a >3-fold increase in glial scar/astrocytic gliosis immunoreactivity in the brains of rats after blasts with uncoated cylinders, compared to shams ($p < 0.01$), whereas this form of inflammation was significantly reduced by polyurea-coated cylinders. In conclusion, exposure of rats to underbody blast-induced acceleration results

in brain inflammation within one day and behavioral deficits for at least one month. Mitigation of acceleration by hull designs that absorb the blast force protects against both mortality and neurologic impairment. Grant support: US Army W81XWH-13-1-0016

Disclosures: F. Tchantchou: None. **W. Fourney:** None. **U. Leiste:** None. **A. Puche:** None. **G. Fiskum:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.16/X16

Topic: C.09. Brain Injury and Trauma

Title: Assessing glycomics and neuroproteomic changes in experimental tbi: comparative analysis of aspirin and clopidogrel

Authors: *F. H. KOBEISSY[1], H. BAHMAD[2], N. RAMADAN[2], R. ZHU[3], Y. MECHREF[3]; [1]Dept of Psychiatry, Univ. of Florida, Gainesville, FL; [2]American Univ. of Beirut, Beirut, Lebanon; [3]Texas Tech. University,, Lubbock, Texas, TX

Abstract: As populations age, the number of patients sustaining traumatic brain injury (TBI) and concomitantly receiving pre-injury antiplatelet therapy such as Aspirin (ASA) and Clopidogrel (CLOP) is rising. These drugs have been linked with unfavorable clinical outcomes following TBI, where the exact mechanism(s) involved are still unknown. In this novel work, we aimed to identify and compare the altered proteome profile imposed by ASA and CLOP when administered alone or in combination, prior to experimental TBI. Furthermore, we assessed differential glycosylation post-translational modification (PTM) patterns following experimental controlled cortical impact (CCI) model of TBI, ASA, CLOP and ASA+CLOP. Ipsilateral cortical brain tissues were harvested 48 hours post injury and were analyzed using an advanced neuroproteomics Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) platform to assess proteomic and glycoproteins alterations. Of interest, differential proteins pertaining to each group (22 in TBI, 41 in TBI+ASA, 44 in TBI+CLOP, and 34 in TBI+ASA+CLOP) were revealed. Advanced bioinformatics/ systems biology and clustering analyses were performed to evaluate biological networks and protein interaction maps illustrating molecular pathways involved in the experimental conditions. Results have indicated that proteins involved in neuroprotective cellular pathways were upregulated in the ASA and CLOP groups when given separately. However, ASA+CLOP administration revealed enrichment in biological pathways relevant to inflammation and pro-injury mechanisms. Moreover, results showed differential upregulation of glycoproteins levels in the sialylated N-glycans PTMs which can be implicated

in pathological changes. Omics data obtained have provided molecular insights of the underlying mechanisms that can be translated into clinical bedside settings.

Disclosures: F.H. Kobeissy: None. **H. Bahmad:** None. **N. Ramadan:** None. **R. Zhu:** None. **Y. Mechref:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: DoD GRANT 11162432

NICHD intramural research fund

Title: Role of glia in the pathophysiology of Gulf War Illness

Authors: *D. J. DUTTA[1], D. H. WOO[2], M. ROBNETT[1], W. HUFFMAN[1], P. LEE[1], K. SULLIVAN[3], R. KILLIANY[4], J. O'CALLAGHAN[5], R. D. FIELDS[1];
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Abstract: Gulf War Illness (GWI) is a chronic multi-symptom disorder affecting returning military veterans and civilian workers of the 1990-91 Gulf War, where they were exposed to various chemical agents that disrupt cholinergic signaling. White matter integrity is compromised in these veterans, which led us to hypothesize that cholinergic signaling plays an important role in oligodendrocyte development and myelin physiology. Acetylcholine (Ach) receptors are widely expressed in oligodendrocyte lineage cells, both in-vitro and in-vivo. These receptors are functional and respond to Ach and other cholinergic agonists with increase in intracellular Calcium. Both expression and activity of these receptors, vary spatially and temporally, with development of the oligodendrocyte lineage. Stimulation of these receptors on oligodendrocyte lineage cells, both in isolated cultures and in co-cultures with neurons, have distinct effects on the size of the oligodendrocyte population and the extent of myelination. To assess the role of cholinergic signaling in-vivo and mimic the theater of war, we developed an animal model of GWI. These GWI mice were administered Corticosterone (Cort), a stress hormone, and Diisopropyl Fluorophosphate (DFP), an analogue of Sarin gas and an irreversible inhibitor of Acetylcholinesterase. Consistent with our in-vitro observations, white matter of these

GWI mice, changed in response to the strength and duration of these modulators of cholinergic signaling. Moreover, white matter integrity in these mice, as assessed by Diffusion Tensor Imaging, correlated with such changes.

Disclosures: **D.J. Dutta:** None. **D.H. Woo:** None. **M. Robnett:** None. **W. Huffman:** None. **P. Lee:** None. **K. Sullivan:** None. **R. Killiany:** None. **J. O'Callaghan:** None. **R.D. Fields:** None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.18/X18

Topic: C.09. Brain Injury and Trauma

Title: Phenelzine induces restoration of function following a medial frontal cortex contusion in adult male rats housed in standard environments, but not in adult male rats reared in enriched environments

Authors: *M. A. SEARLES;
Saginaw Valley State Univ., Wheeler, MI

Abstract: Every year approximately 1.7 million individuals suffer the consequences of traumatic brain injury (TBI), which is a leading cause of death and disability in the United States. Due to a lack of effective clinical treatment, many of the functional and cognitive deficits sustained from TBI are left untreated. Currently, Phenelzine (PZ) is used clinically for the treatment of anxiety and atypical depression. Furthermore, PZ has been shown to express neuroprotective effects in several models of ischemia- reperfusion and contusion brain injury. Additionally, research from our lab has shown that rats raised in enriched environment (EE) housing perform better during behavioral tasks than rats raised in traditional or standard laboratory environments (SE). The purpose of the current study was to examine the effect PZ administered post-injury may have on recovery of function following a medial frontal cortex (MFC) contusion in rats reared in EE housing. Twenty-seven, twenty-five day old male Long-Evans rat pups were reared in enriched environments (EE) for ninety days. Twenty-seven adult male rats were purchased from the same vendor and placed into SE upon arrival. After fourteen days in SE, eighteen SE-housed rats received MFC contusion injuries. After Ninety days in the EE, MFC contusion injuries were administered to eighteen EE-housed rats. After each injury, at ten minutes post-injury, half of the animals received a subcutaneous (10mg/kg) injection of phenelzine, and the other half received an injection of saline solution. Behavioral analysis was conducted one week post-injury and included the open field task (OFT), Barnes maze (BM), Morris water maze (MWM), rotor-rod (RR), elevated-plus maze (EPM), and forced-swim task (FST). Upon completion of behavioral

testing, the animals were euthanized and perfused, and their brains extracted. Tissue from the left hemisphere was embedded in paraffin, sectioned, and underwent hematoxylin and eosin staining. The right hemispheres were reserved for thick-sectioning analysis. Stereological analysis was performed to quantify total cortical volume as well as number of surviving cells in the hippocampus.

Disclosures: M.A. Searles: None.

Poster

321. Trauma and Blast-Induced Neurochemical and Cellular Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 321.19/Y1

Topic: C.09. Brain Injury and Trauma

Title: The role of pontine alpha-2 receptors in the reinstatement of the motor deficit after cortical damage

Authors: G. GARCÍA-DÍAZ[1], L. E. RAMOS-LANGUREN[2], J. LOMELÍ-GONZÁLEZ[1], F. AYALA-GUERRERO[3], N. CHÁVEZ-GARCÍA[2], R. GONZÁLEZ-PIÑA[4], *S. MONTES[2]; [1]Escuela Superior de Medicina. Inst. Politécnico Nacional, México, Mexico; [2]Natl. Inst. Neurol. Neurosurg, Mexico City, Mexico; [3]Facultad de Psicología. Univ. Nacional Autónoma de México, México, Mexico; [4]Torre de Investigación. Inst. Nacional de Rehabilitación, México, Mexico

Abstract: Norepinephrine (NE) plays an important role in the motor functional recovery after cortical damage. In addition, it has been suggested that there does exist distal effects from the area directly affected. The remote effects may take place in a period of time from minutes to weeks. However, these functional recovery mechanisms are not entirely clear. Recently, it has been found that NE inhibition in the pons is correlated with motor deficit after injury in the motor cortex. Within the pons, the locus coeruleus (LC) is the major noradrenergic nucleus in the brain and is the sole source of NE in many parts of the central nervous system. The LC area is rich in α2 adrenoceptors. Therefore, the modulation of this kind of receptors could delay or accelerate the time of functional recovery. The aim of this study was to analyze the role of α2 adrenoreceptor agonist clonidine (CL) in the NE content in the pons, in the motor deficit, and in the spontaneous activity after 20 days of cortical damage when the animals show signs of recovery. We divided the subjects into four following conditions: a) administration of clonidine 12μg/kg (n=6), b) administration of clonidine 25μg/kg (n=6), c) administration of clonidine 50μg/kg (n=6) and d) a sham group (n=6). Twenty days after the injury, NE levels in both hemispheres on the pons were similar to the Sham group. However, NE decreased significantly

in the clonidine-treated groups, suggesting that clonidine-treated recovered animals presented a reinstatement of the motor and sensorial deficit such as those which had occurred immediately after cortical damage. These results support the idea that NE is a key neurotransmitter for the recovery as well as the reinstatement of motor deficit.

Disclosures: G. García-Díaz: None. **L.E. Ramos-Languren:** None. **J. Lomelí-González:** None. **F. Ayala-Guerrero:** None. **N. Chávez-García:** None. **R. González-Piña:** None. **S. Montes:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.01/Y2

Topic: C.09. Brain Injury and Trauma

Support: KAKENHI

Title: Effects of P2 receptor blocker MRS2179 against experimental cerebral contusion injury in rat

Authors: *T. KUMAGAWA, K. SHIJO, N. MORO, M. FUKUSHIMA, T. MAEDA, A. YOSHINO;
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Abstract: Previously we have reported the massive efflux of gliotransmitter ATP into the extracellular space immediately after experimental cerebral contusion injury. Released ATP from the injury stimulated astrocyte will generate calcium wave and activate neighbor microglia via P2 receptor. Subsequently activated microglia will initiate immune response including release of several cytokines, chemotaxis and phagocytosis to remove debris from the brain. Here we assessed the effect of MRS2179, a selective P2Y1 receptor blocker, if it will restrict inflammatory response and can be beneficial in a rat cerebral contusion model. Cerebral contusion injury model was made in SD rat. MRS2179 was in situ administrated into the contused brain by osmotic pump. Artificial cerebral spinal fluid was used as a control. Galectin-3, a marker of activated microglia was measured by western blotting and cytokines such as IL-1 beta, IL-4, IL-6, TNF alpha and NF-kB were measured by reverse transcription PCR, 1, 3 and 7 days following injury. Another group of rats were assessed for behavioral performance up to 28 days, including beam walk test, neurological response and plus maze test. Histological analysis revealed that Galectinn-3 was expressed only on the activated microglial cell. Expression of Galectin-3 and some cytokines were significantly suppressed by MRS2179 administration.

Although, MRS2179 did not improve the behavioral outcome. MRS2179 admitted to the contused brain might have some therapeutic effect, however further study is required to make a conclusion.

Disclosures: **T. Kumagawa:** None. **K. Shijo:** None. **N. Moro:** None. **M. Fukushima:** None. **T. Maeda:** None. **A. Yoshino:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: NIH/NINDS R01 NS086570-01

Shriner's Hospital for Children 85110-PHI-14

Title: Traumatic brain injury during adolescence enhances rewarding effects of a subthreshold dose of cocaine in mice

Authors: *L. CANNELLA[1], S. F. MERKEL[1], R. RAZMPOUR[1], M. SEASOCK[1], S. M. RAWLS[2], S. H. RAMIREZ[1];
[1]Pathology and Lab. Med., [2]Ctr. for Substance Abuse Res., Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA

Abstract: Traumatic brain injury (TBI) is an important public health problem as 1.7 million occur annually in the United States. One of the most common, chronic comorbidities seen in TBI patients is the development of a substance use disorder (SUD). Clinical reports suggest that age of injury can affect later drug use. Specifically, among a population of cocaine-dependent patients, 84% reported experiencing their first TBI before the onset of cocaine use. Yet, to date there is limited preclinical data related to the effect of TBI on drug abuse behavior and thus the mechanisms for this phenomenon remain unclear. Previously we investigated the effects of TBI on susceptibility to the rewarding effects of cocaine. Using conditioned place preference (CPP) as a measure of cocaine reward in male mice, we found that moderate TBI inflicted by Controlled Cortical Impact (CCI) during adolescence increased susceptibility to the rewarding effects of 10 mg/kg cocaine during adulthood. These results support clinical evidence that experiencing neurotrauma early in life can facilitate later susceptibility to psychostimulant addiction. The aim of the current study is to further investigate whether TBI during adolescence enhances the effects of a subthreshold dose of cocaine that typically does not produce a CPP

shift. A single CCI impact with a speed of 4.5 m/s, dwell time of 0.5 sec, and compression depth of 2 mm produced a moderate TBI in 6 week old, adolescent or 8 week old, young adult male C57BL/6 mice. Drug seeking behavior was assessed using the CPP assay two weeks after injury. Expression of immune response-associated genes was measured using qRT-PCR. We found that moderate TBI during adolescence, but not during young adulthood, augmented preference to the environment paired with 2.5 mg/kg cocaine, indicative of enhanced sensitivity. Additionally we detected increased expression of immune response-associated genes in the prefrontal cortex. These results further indicate that exposure to TBI during adolescence may enhance the abuse liability of cocaine in adulthood, suggesting that the threshold for the rewarding effects of cocaine could be lower in TBI patients. Moreover, future studies will focus on testing whether dampening of key neuroinflammatory responses post-trauma leads to the restoration of homeostasis in the reward circuitry.

Disclosures: L. Cannella: None. **S.F. Merkel:** None. **R. Razmpour:** None. **M. Seasock:** None. **S.M. Rawls:** None. **S.H. Ramirez:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.03/Y4

Topic: C.09. Brain Injury and Trauma

Support: CDMRP G17017251

Title: Histological evaluation of biomarkers in a longitudinal traumatic brain injury study.

Authors: *S. C. SCHWERIN[1], E. HUTCHINSON[2], K. RADOMSKI[1], A. IMAM-FULANI[1], M. CHATTERJEE[1], C. PIERPAOLI[2], S. L. JULIANO[1];
[1]Anatomy, Physiol. and Genet., Uniformed Services Univ., Bethesda, MD; [2]Natl. Inst. of Child Hlth. and Develop., NIH, Bethesda, MD

Abstract: In this study we used the ferret to study TBI and correlate MRI markers with brain histology and behavioral assessment. The ferret is the smallest mammal with a gyrencephalic brain. We studied 15 adult male ferrets, 3 naïve and 12 receiving a mild controlled cortical impact on the somatosensory cortex (velocity = 5 m/s, depth of impact = 2 mm, dwell time = 100 ms, impactor tip diameter =3 mm). Brains were collected for ex vivo scanning and histology at 1 day post injury (1DPI), 4 weeks post injury (WPI) and 16WPI. The location of MRI abnormalities guided our choice of slices for immunohistochemical analysis. We used antibodies directed against astrocytes (glial fibrillary acid protein), microglia (Iba1), dendrites (microtubule

associated protein; MAP2), myelin (myelin oligodendrocyte glycoprotein), microtubules (Tuj1), phosphorylated tau, and inhibitory interneurons (parvalbumin). At 1DPI, we saw substantial changes in immunoreactivity for microglia that extended away from the injury, but very few changes in astrogliosis. At this time point, most of the histological as well as imaging abnormalities were contained within the gray matter. We also observed more subtle changes in myelin and neuronal markers including beta tubulin (Tuj1) and MAP2 that occurred mostly in the upper layers of the cerebral cortex. At 4WPI chronic reactive gliosis extended up to 2mm anterior and posterior to the injury epicenter. The density and hypertrophy of the activated microglia were most abundant in the subcortical white matter at 4WPI, reactive astrocytes occurred deep in the underlying white matter and extended into the internal capsule. The density of neuronal markers (MAP2, Tuj1) decreased in the cortical region of injury at 4WPI but returned to near normal values by 16WPI. At 16WPI micro- and astroglial immunoreactivity remained elevated and continued substantially into the white matter. Our behavioral analysis found that the ferrets initially showed impairments in mobility, but that these mostly recovered by 16WPI. At this time point, they demonstrated aspects of anxiety, however, by avoiding traveling in the center zone of an open field test. The animals had cognitive difficulties that continued up to 16WPI as seen in a T maze task and novel object recognition. Correlating the persistent behavioral alterations with histologic changes at the same time point suggests that markers representing inflammation may be more relevant to function than neuronal changes. These findings show that the gyrencephalic ferret is an important translatable model for TBI research; our ability to correlate histology with MRI also provides an important noninvasive tool to guide analysis.

Disclosures: **S.C. Schwerin:** None. **E. Hutchinson:** None. **K. Radomski:** None. **A. Imam-Fulani:** None. **M. Chatterjee:** None. **C. Pierpaoli:** None. **S.L. Juliano:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.04/Y5

Topic: C.09. Brain Injury and Trauma

Support: CIHR Grant 125888

CIHR CGS-M

CIHR CGS-D

NSERC Grant 249853

Title: A new model for un-anaesthetised repeat closed head injury produces acute neurological deficits in the juvenile rat

Authors: *A. L. MECONI, R. C. WORTMAN, B. R. CHRISTIE;
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Abstract: Pediatric concussion accounts for 144,000 emergency room visits annually in the United States. The prognosis for this type of injury varies greatly. Most subjects recover within a week, but some suffer motor and cognitive symptoms that can persist for months. The reason for these individual differences in recovery remains under investigation, but epidemiological evidence suggests that metrics like age and sex strongly influence outcomes. Children are more likely to sustain a concussion than adults, males experience a higher incidence than females, and females are more likely to have persistent symptoms. Experimental animal models of concussion have been crucial in identifying the physiological basis of variability in concussion symptoms, but they have disproportionately focused on adult male subjects. We have developed a new model to reliably induce concussion in young rats. This model is unique from previous juvenile concussion models because it does not require anaesthesia - which has neuroprotective properties that may confound experimental outcomes. Omitting anesthesia allows for more rapid and reliable symptom assessment immediately after a concussion. We have used a modified neurological severity score (NSS) to rapidly assess motor and cognitive deficits that occur during this previously inaccessible acute time window. Preliminary characterization in juvenile male and female rats suggests that single and repeat awake closed head injury (ACHI) produced by this new model acutely impairs NSS performance, with recovery after 7 days. Interestingly, similar to clinical evidence, there appears to be a subset of the population that is more severely affected by mild closed head injury despite having similar genetic and environmental histories. Continued efforts to characterize this model will allow us to investigate the physiological basis of concussion symptoms in these special populations.

Disclosures: **A.L. Meconi:** None. **R.C. Wortman:** None. **B.R. Christie:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Program#/Poster#: 322.05/Y6

Topic: C.09. Brain Injury and Trauma

Support: CIHR Grant 125888

NSERC Grant 249853

Title: The effects of mild traumatic brain injury on hippocampal neuroinflammation in female juvenile rats

Authors: *M. A. CLARKSON[1], B. R. CHRISTIE[2], P. C. NAHIRNEY[2], A. L. MECONI[2], A. COLLINS[2], E. TRUESDELL[2];
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Abstract: Mild Traumatic Brain Injury (mTBI) is being increasingly recognized as a serious health issue. Also commonly known as concussion, mTBI accounts for more than 80% of brain injuries. The prevalence of mTBI in athletes, particularly young athletes is of great concern as injury to the young brain can lead to long-term detrimental effects. This injury is known to have two phases; the primary phase, being the initial impact, followed by a secondary phase which constitutes a series of pathophysiological changes within the brain. To examine the role of inflammation in the secondary phase of injury, we performed immunostaining for immune cells, microglia and astrocytes in the hippocampus and cortex 7 days after mTBI. To replicate this common human injury, we used an awake closed head injury (ACHI) model to induce mTBI in non-anaesthetized juvenile female rats. The model allows us to produce acute neurological deficits similar to those produced by a concussion. In this study , we used confocal microscopy to quantify glial fibrillary acidic protein (GFAP) expression by astrocytes and ionized calcium binding adaptor molecule 1 (IBA1) expression by microglia. In both the CA1 region and dentate gyrus, no significant changes were found in astrocyte or microglial density following mTBI, suggesting that in this model there was not a significant chronic neuroinflammatory response following either single or repeated mTBI.

Disclosures: M.A. Clarkson: None. **B.R. Christie:** None. **P.C. Nahirney:** None. **A.L. Meconi:** None. **A. Collins:** None. **E. Truesdell:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: intramural Grant NIAAA/NIH

Title: Repetitive Closed-Head Impact Model of Engineered Rotational Acceleration (rCHIMERA) induced long-term cognitive impairment and persistent astrogliosis and microgliosis in mouse

Authors: *H. CHEN;
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Abstract: Epidemiological and retrospective autopsy data suggested that repeated mild traumatic brain injury (rmTBI) or single severe brain injury is a high risk factor to develop of chronic traumatic encephalopathy (CTE), posttraumatic stress disorder (PTSD), Parkinson's and Alzheimer's diseases many years after the injury. Lack of proper animal models reflecting complex features of human TBI with long-term complications has been the major obstacle for translation from preclinical promises to therapeutic success. Recently developed Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) recapitulated in animals many of the functional and pathological features of human TBI. Using this model, we investigate the long-term neuropathological and cognitive functional consequences of rmTBI. C57BL/6 male mice at 12 weeks old were subjected to CHIMERA for three consecutive days (rCHIMERA) at 1day interval. Motor deficit was evaluated by beam walk and rotarod tests within 1 week post injury, and learning and memory was evaluated using Morris water maze test at 1 and 2 month. Microglia and astrocyte activation and axonal injury were detected by immunohistochemistry (IHC), GFAP and Iba-1 protein levels and GFAP, iba-1, APP and TNF-alpha gene expressions were analyzed by Western blotting and qRT-PCR at different time points. We found that learning and memory function was significantly impaired at 1 and 2 months after rCHIMERA. The injured mice also displayed prolonged loss of righting reflex and increased motor deficits. Extensive induction of GFAP and Iba-1 signals in white matter areas was indicated at the acute phase (1 and 7 days) and long-term phase (1, 2 and 3.5 months). GFAP and Iba-1 protein levels were also elevated at 1 month while GFAP gene level was increased at 1-7 days. APP deposition and TNF-alpha gene expression was upregulated only at 1 day post injury. In conclusion, rCHIMERA leads to a long-term cognitive impairment with persistent activation of astrocytes and microglia in the white matter. rCHIMERA may present a useful animal model to study long-term functional and neuropathological outcomes as well as cellular and molecular mechanisms of human rTBI to develop effective treatments for rTBI-induced long-lasting complications in humans. This research program is supported by Intramural program of NIAAA, NIH.

Disclosures: H. Chen: None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Program#/Poster#: 322.07/Y8

Topic: C.09. Brain Injury and Trauma

Title: Acute pathophysiologies associated with mild, “head on” concussion injury in the Sprague-Dawley

Authors: *S. M. VITA, K. R. CLARK, R. J. GRILL;
Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Approximately 2.5 million people per year are reported to have sustained a traumatic brain injury (TBI). However, as many cases of TBI are mild and are thus not reported, this number likely does not accurately represent the problem. Currently there are no effective treatment options available that will preserve or restore function lost to a mild-to-moderate TBI. While there are multiple reasons to explain this dearth of treatment options, one issue may be due a lack of animal models that adequately reproduce the pathophysiological events associated with mild, concussive TBI. We have chosen to employ the Maryland Model of “head on” concussion injury in the adult, male, rat (Kilbourne et al, Novel model of frontal impact closed injury in the rat, J. Neurotrauma, 26:2233-43, 2009) developed in the laboratory of Dr. Simard at the University of Maryland. This device produces a mild, head-on concussion-type injury, replicating both the impact and torque commonly experienced in human concussion events. In this study, we provide an initial characterization of the effects of mild concussion on acute tissue pathologies, such as deficits effecting the Blood Brain Barrier (BBB), activation of microglia (early indicators of immune and inflammatory activation) as well as neuronal pathology. Design: Adult, Sprague-Dawley rats were first anesthetized and then received bilateral surgical exposure of the malar processes of the skull. One cohort received a single, mild TBI insult while the sham group received only placement of the impounder tips against the malar processes, but no lesion. After lesion, subjects were returned to their home cages for recovery. Subjects were euthanized 24 hours post-injury with the brains removed for histopathological assessment. mTBI subjects showed elevated leakage to both endogenous IgG and albumin; serum proteins normally excluded from the brain by the BBB. While overt neuronal damage was not observed, we detected a breakdown in the cortical perineuronal network as evidenced by tissue labeling with the plant lectin, Wistereria floribunda. Preliminary data suggests that the Maryland Model produces subtle alterations in brain structure/integrity in both forebrain (coup) and cerebellar tissues (counter-coup). Subsequent studies will explore the evolution of these pathological events over time and whether they coincide with mTBI associated functional deficits. Our goal is to determine whether the Maryland Model of mTBI-induced concussive injury can serve as an effective means of both studying the long-term consequences of mTBI as well as developing novel, effective therapeutic interventions.

Disclosures: **S.M. Vita:** None. **K.R. Clark:** None. **R.J. Grill:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.08/Y9

Topic: C.09. Brain Injury and Trauma

Title: Increased ethanol intake and progressive reductions in striatal cannabinoid receptor 1 protein levels following mild traumatic brain injury

Authors: B. L. SCHNEIDER[1,2], L. L. SUSICK[1,2], *A. C. CONTI[1,2];
[1]John D. Dingell VA Med. Ctr., Detroit, MI; [2]Neurosurg., Wayne State Univ., Detroit, MI

Abstract: Traumatic brain injury (TBI), of which mild TBI (mTBI) accounts for more than 80%, significantly increases the risk of developing alcohol use disorders (AUDs) over a patient's lifetime. With its known relationship to ethanol consumption and the physiological effects of ethanol, the endocannabinoid system has become of interest in AUDs secondary to TBI. Specifically, ethanol vapor and self-administration decreased striatal expression of cannabinoid receptor 1 (CB1) and altered ethanol preference has been observed following CB1 antagonism in rodent models. In this study, we investigated changes in ethanol consumption and cannabinoid receptor (CB1 and CB2) protein expression in the anterior striatum (ASTR) and nucleus accumbens (NAC) at 8-25 d post-mTBI. Anesthetized male C57BL/6 mice (8-10 wks) were given a mild, midline impact over the intact skull or sham surgery. At 14 d post-TBI, mice were evaluated for binge and chronic ethanol consumption. Injured mice demonstrated a delayed increase in chronic consumption, a consistent increase in ethanol preference, and an increase in binge pattern drinking compared to sham controls. At 8 and 25 d post-injury, brains from separate groups of mice were harvested and frozen. Protein lysates were made from 1.5 mm diameter tissue punches of striatal subregions taken from 2 mm thick coronal slices for immunoblot analysis. At 8 d post-injury, there was a trending decrease in CB1 in ASTR, which became significantly reduced at 25 d post-injury compared to respective sham controls. The NAC showed an initial increase in CB1 at 8 d following mTBI, but was decreased at 25 d compared to respective sham controls. The observed time- and region-dependent alterations in CB1 expression in the ASTR and NAC may underlie the progression of alcohol misuse that develops in the post-TBI period. This work was supported by resources from the John D. Dingell VA Medical Center, Detroit, MI.

Disclosures: B.L. Schneider: None. **L.L. Susick:** None. **A.C. Conti:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Program#/Poster#: 322.09/Y10

Topic: C.09. Brain Injury and Trauma

Title: Pyridoxamine deficiency induces carbonyl stress and schizophrenia-like phenotypes in *Drosophila*

Authors: *K. KORI;
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Abstract: Advanced glycation end products (AGEs) are formed through non-enzymatic reaction, maillard reaction and oxidation stress, between sugars and proteins, lipids or nucleic acids. Pyridoxamine, one of vitamin B_6 is known to scavenge the intermediates of AGEs and also inhibit maillard reaction. The accumulation of AGEs is termed "carbonyl stress" and is kwon to involve in many diseases such as diabetes, kidney disease and aging. Recent studies reported 20 % of schizophrenia patients have mutation in GLO1 gene, which encodes enzyme detoxifies intermediates of AGEs. These patients exhibit high increase in AGEs and decrease in vitamin B_6.To address whether lack of vitamin B_6 causes carbonyl stress underlying schizophrenia, we tried to develop carbonyl stress model using *Drosophila*. To this end, we reared *Drosophila* on the sterilized defined medium lacking vitamin B_6 and found that deprivation of pyridoxamine increases pentosidine level about 5-fold, indicating that vitamin B_6 deficiency causes carbonyl stress. Moreover, this carbonyl stress model *Drosophila* show shorten life span, increased anxiety, fragmentation of nighttime sleep, reducing sugar preference, decreased courtship activity and memory impairment. Since these behavioral phenotypes are related to the symptom of schizophrenia, we suggest the carbonyl stress also impairs brain functions in flies.

Disclosures: K. Kori: None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R21NS081467

NIH Grant F32NS090822

Title: Regrowth of serotonin axons in the neocortex following a stab injury

Authors: *S. E. DOUGHERTY, Y. JIN, D. J. LINDEN;
Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Axon degeneration is a common occurrence in neurological disorders such as toxic chemical insults, stroke, concussion and traumatic brain injury. While most of this axonal loss is as yet irreversible, some neuron populations have shown a propensity to recover their axonal arbors through regeneration (of injured axons) or compensatory sprouting (of spared axons). Serotonergic neurons display an enhanced capacity for axonal regeneration after acute injury. One potential explanation is the ability of serotonergic axons to survive and grow within hostile extracellular conditions. Previous studies have shown that serotonin axons can extend through otherwise nonpermissive environments including glial scars and within the subventricular zone. To assess serotonin axon degeneration, regeneration, and sprouting following acute brain injury, we used a neocortical stab model in a Slc6A4 (serotonin transporter) - soluble EGFP BAC transgenic mouse. Immunohistochemistry on fixed tissue and chronic in vivo two-photon imaging through a cranial window overlying the neocortex have revealed new growth from the severed ends of axons, which extends across and beyond the stab rift. These studies reveal the dynamic nature of serotonin axonal regeneration and sprouting following a glial scar-forming injury. Can other neuromodulatory axons in the neocortex regrow following stab injury? Further investigations will assess dopamine and noradrenaline axons in this model.

Disclosures: **S.E. Dougherty:** None. **Y. Jin:** None. **D.J. Linden:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: DARPA Cooperative Agreement HR0011-13-2-0017

Cortrop Inc.

Title: Diffusion Tensor Imaging detects alterations in the corpus callosum after mild TBI in the mouse

Authors: *P. N. VENKATASUBRAMANIAN[1], M. SMITH[1], D. R. SCHUBERT[2], J. C. PINA-CRESPO[3], K. MATHEWS[4], P. RIGBY[4], A. MANN[3], E. RUOSLAHTI[3], A. M. WYRWICZ[1], J. SPIESS[3,5];
[1]Ctr. for Basic M.R. Res., Northshore Univ. Healthsystem, Evanston, IL; [2]The Salk Inst., La Jolla, CA; [3]Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA; [4]L3 Applied Technologies, Inc., San Diego, CA; [5]Cortrop Inc., Encinitas, CA

Abstract: Purpose
Mild TBI is thought to induce axonal injury which might progress to chronic traumatic encephalopathy, post-traumatic stress syndrome, major depression and other pathological states. Using high resolution diffusion tensor imaging (DTI), we have investigated changes in brain microstructure in a mouse model of mild TBI and found changes in the corpus callosum (CC) that suggest chronic axonal injury.
Experimental
Male 7-8 week-old C57BL/6 mice were fixed in vertical position, heads down, in an aluminum shock tube with their back oriented towards the exit plane of the tube and hit with a supersonic helium wave at a peak reflective pressure of 70psi. On Days 2, 5 and 12 after the blast, the mice were cardiac-perfused and their brains removed for DTI at 14.1T. Multi-slice DTI of fixed brain was acquired using a spin-echo diffusion weighted pulse sequence: 59 μm x 59 μm in-plane, 0.5mm slice thickness, TR/TE 2500ms/16.5ms, duration of diffusion gradients 3ms, delay between diffusion gradients 7ms, 30 gradient directions and one b value = 1000 s/mm^2. Resulting images were processed to generate maps of fractional anisotropy (FA). Diffusion parameters measured from medial and lateral CC in slices containing the genu (CCg), body (CCb) and splenium (CCsp) of CC were compared between controls and mTBI mice on Day 2 (n=3), Day 5 (n=4) and Day 12 (n=3).
Results
High resolution DTI revealed subtle, region-specific changes in the CC at different time points after injury. On Day 2, FA was lower in both medial and lateral CCb (0.41±0.01 and 0.40±0.02, respectively) relative to controls (0.46±0.02 and 0.44±0.01) indicating axonal injury in CC after blast injury. CCg and CCsp showed no changes in FA on Day 2. Reduced FA values indicated axonal injury on Day 2, whereas in preliminary experiments enhanced tau phosphorylation was found only on Day 5. DTI showed that on Day 5, FA was higher in medial and lateral CCg (0.60±0.02 and 0.56±0.01 in mTBI vs 0.55±0.01 and 0.49±0.02 in controls), as well as in medial CCb (0.50±0.02). Increased FA was associated with increase in diffusivity along the fiber direction (axial diffusivity) and no change in radial diffusivity. The observed increase in FA might represent axonal compression. On Day 12, FA in CCg and CCb returned to normal values, whereas it decreased in medial CCsp (0.48±0.03 vs 0.55±0.02 in control) indicating that mTBI likely results in chronic structural changes in the CC.
Conclusion
Our blast injury model appears to be a useful paradigm to investigate mTBI in mice. High

resolution DTI reveals region-specific, chronic mictrostructural changes following mTBI in mice. (Supported by DARPA Cooperative Agreement HR0011-13-2-0017; Cortrop Inc.)

Disclosures: **P.N. Venkatasubramanian:** None. **M. Smith:** None. **D.R. Schubert:** None. **J.C. Pina-Crespo:** None. **K. Mathews:** None. **P. Rigby:** None. **A. Mann:** None. **E. Ruoslahti:** None. **A.M. Wyrwicz:** None. **J. Spiess:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cortrop Ins., Encinitas, CA.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Topic: C.09. Brain Injury and Trauma

Support: Veterans Affairs RR&D Merit Review Grants 1I01RX000502-01A and 1I01RX001005-01A2

Title: Impact of chronic traumatic brain injury on noradrenergic innervation to the major anxiety-related neural pathways in rats

Authors: ***S. TSUDA**[1,2], J. HOU[1,2], R. NELSON[1], G. MUSTAFA[1,2], J. WATTS[1], F. J. THOMPSON[1,2,3], P. BOSE[1,2,4];
[1]Malcom Randal VA Med. Ctr., Gainesville, FL; [2]Physiological Sci., [3]Neurosci., [4]Neurol., Univ. of Florida, Gainesville, FL

Abstract: Every year, approximately 1.7 million new cases of traumatic brain injuries (TBIs) occur in the United States, which often lead to long-term anxiety disorders. However, there has been no uniform consensus for an effective treatment for this disorder, partly due to the lack of comprehensive knowledge in the related neuropathology. Recently, TBI-induced significant injury of norepinephrine production site (i.e., locus coeruleus) has been shown and dysregulation of noradrenergic (NA) system has been implicated in TBI-induced anxiety related pathophysiology. Although the critical roles of the NA system in regulating anxiety have well been known, few studies have reported the impact of TBI on the NA innervation to anxiety-related brain regions following TBI. The purpose of this study was to characterize the changes in NAI to the major anxiety-related neural pathways following chronic closed-head TBI (cTBI; modified Marmarou TBI model, 450g / 1.25 m) in rats. In the current study, coronal brain sections of intact and injured animals which exhibited chronic anxiety-like behaviors in an elevated plus maze were used. Brain sections of the bed nucleus of the stria terminalis (BNST),

central nucleus of amygdala (CeA), dorsomedial hypothalamic nucleus (DMH), ventral subiculum (vSub), periaqueductal gray (PAG), and locus coeruleus (LC) were immnofluorescently labelled after being incubated with antibodies against dopamine beta-hydroxylase, neuronal nuclei, and NA receptors. Stereological analysis of the NA fiber density in these brain regions was performed and the number of NA cells in the LC was counted. The results showed that the number of NA cells in the LC and the NA fiber densities in the LC, PAG, and medial division of CeA were significantly reduced following chronic cTBI. In contrast, the NAI to the BNST, DMH, and vSub was significantly elevated following TBI. These results suggest that prolonged anxiety-like behaviors observed in these animals may partly be related to the dysregulated NA system in the AMG-BNST and the vSub-paraventricular nucleus of hypothalamus anxiety pathways. Current study would provide new information for understanding the anxiety-related cTBI neuropathology, which in turn may provide insights into a novel therapy for cTBI-induced anxiety disorders.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Topic: C.09. Brain Injury and Trauma

Support: Veterans Affairs Rehab R&D Merit Review # 1I01RX000502-01A

Title: Graded Mild traumatic brain injury (mTBI)-induces different trajectories reaching enduring multiple comorbidities

Authors: *F. J. THOMPSON[1,2,3], J. HOU[2], R. NELSON[1], G. MUSTAFA[2], J. JOSEPH[1], Z. WILKIE[1], P. BOSE[1,2,4];
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Abstract: Traumatic brain injury (TBI) can produce life-long disabilities including cognitive, anxiety, balance, and motor deficits. Although newer imaging technologies and protein biomarkers are promising, these often do not provide accurate diagnosis of mTBI, and clinical consequences can be problematic due to inconsistent symptom clusters. Although acute symptoms typically resolve in days to weeks, a prolonged worsening of an array of somatic, emotional and cognitive symptoms may appear months to years following mTBI (Katz et al.,

2015). A recent report emphasized the chronic nature of disability in Veterans with mTBI who had significantly more cognitive, affective, vestibular and somatic symptoms persisting more than 4 years after the mTBI event(s) (Baldassarre et al., 2015). Most closed head TBIs (cTBI) are produced by head impact and may also include acceleration/deceleration with shearing of brain and white matter. The experimental model of cTBI induced by weight drop is known to produce hallmark TBI injuries (concussion, diffuse axonal injury, micro hemorrhages) (Bose et al., 2013; Foda and Marmarou, 1994; Marmarou et al., 1994; Hou et al., 2015; Mustafa et al, 2016). In addition, this injury is not further complicated by the surgery required for cortical exposure (Buki and Povlishock, 2006). However, graded mild intensities using this experimental cTBI model have not been comprehensively characterized for co-morbidities. The present study, used two intensities of weight drop (1.0 m and 1.25 m/450g) to produce closed head mild TBI (mTBI) to investigate the trajectory and magnitude of four comorbidities (cognitive, anxiety, vestibulomotor, and spinal reflex disabilities). Injuries were produced during general anesthesia (isoflurane), and time for righting recoveries were within 2 to 3 minutes longer than observed for non-injured sham animals. MRI images revealed no anatomical changes or hemorrhagic volumes. Collectively, these observations reflected characteristics of mild TBI. Cognitive tests were performed using spatial serial learning in a Morris water maze. Anxiety-like behavior was tested using measures of behavior in an elevated plus maze. Balance was tested using a rotorod. Spinal reflexes were evaluated for rate-dependent depression. For both TBI intensities, significant cognitive, anxiety, balance, and motor reflex changes were observed over the course of 18 weeks post injury. However, the milder TBI revealed considerably slower trajectories for the development of these changes. These studies emphasize the value for multiple time point neurologic assessments following mTBI.

Disclosures: **F.J. Thompson:** None. **J. Hou:** None. **R. Nelson:** None. **G. Mustafa:** None. **J. Joseph:** None. **Z. Wilkie:** None. **P. Bose:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.14/Y15

Topic: C.09. Brain Injury and Trauma

Support: CENC - VA I01 RX001774

DoD W81XWH-13-2-0095

Title: Chronic cerebrovascular abnormalities in a mouse model of repetitive mild traumatic brain injury

Authors: *C. E. LYNCH[1,2,3], G. CRYNEN[1,2,3], S. FERGUSON[1,2,3], B. MOUZON[1,2,3], D. PARIS[1,2,3], J. OJO[1,2,3], P. LEARY[1], F. CRAWFORD[1,2,3], C. BACHMEIER[1,2,3,4];
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Abstract: Repetitive mild traumatic brain injury (r-mTBI) is a risk factor for development of Chronic Traumatic Encephalopathy (CTE), a disease characterized by Tau pathology throughout the cortices, and often co-presenting with conditions such as Alzheimer Disease (AD). It has been well documented that mild to severe TBI can result in transient reductions in Cerebral Blood Flow (CBF), with severe injuries often accompanied by presence of varying degrees of vascular pathology post-mortem. Aberrant CBF readings precede gross Amyloid pathology in AD patients, suggesting that hypo-perfusion is key in the pathogenesis of conditions such as CTE and AD, for which r-mTBI is a pre-disposing factor. We have herein expanded on our previous animal model of r-mTBI, showing robust neuroinflammation and pronounced spatial memory deficit in wild type mice as late as 18 months post-injury. We now show this pathology and concomitant behavioral phenotype to be emulated in a separate animal model of r-mTBI, described herein, and accompanied by chronic impairment of global CBF, and altered expression of cerebrovascular markers. These results are the first to demonstrate chronic cerebrovascular dysfunction in the pathogenesis and evolution of r-mTBI-induced illness, and validate this model for investigation of CTE.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.15/Y16

Topic: C.09. Brain Injury and Trauma

Title: An adapted model of mtbi in adult zebrafish

Authors: *R. SPENCE[1], B. DIX[1], A. YOUNG[1], V. GILL[1], L. STANISLAW[2], J. ELLIS[3], A. MAHERAS[2], B. FORTINI[1];
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Abstract: We have developed an adult zebrafish model of mTBI using a non-puncturing weight drop method. Whole brain mRNA sequencing and CLARITY were performed at 3 and 21 days post injury and compared to sham controls. Our data suggest that 3 days immediately following mTBI a number of genes undergo expression changes, including several involved in circadian rhythms and cell proliferation. CLARITY protein changes include cell proliferation. At 21 days post mTBI induction, more genes show differential gene expression, including several involved in intermediate filament organization and inflammation. CLARITY protein changes include gliogenesis. Further work will focus on examining genetic and protein regulation at further time points to determine possible neural recovery pathways.

Disclosures: R. Spence: None. **B. Dix:** None. **A. Young:** None. **V. Gill:** None. **L. Stanislaw:** None. **J. Ellis:** None. **A. Maheras:** None. **B. Fortini:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Topic: C.09. Brain Injury and Trauma

Support: NIH-NIGMS P20GM109089-01A1

Title: Transcranial direct current stimulation (tDCS) improves neurological outcomes in a mice model of traumatic brain injury

Authors: *O. BRAGINA[1], E. NEMOTO[1], C. SHUTTLEWORTH[2], D. BRAGIN[1];
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Abstract: Objectives: Traumatic brain injury (TBI) causes long-term neurological disabilities in 7% of survivors for which there are no clinically proven neuroprotective therapies. Transcranial direct current stimulation (tDCS) is an electrotherapy currently being clinically tested for TBI. However, due to a lack of animal studies, optimal parameters for application are unknown. Our objective is to examine the most effective therapeutic parameters for tDCS in a mouse TBI model. We hypothesized that repetitive anodal tDCS, applied after TBI during recovery improves cognitive and motor neurologic outcome. Methods: Moderate TBI was induced by controlled cortical impact (CCI) with a Benchmark Controlled Cortical Stereotaxic Impactor using a 3 mm flat-tip impounder deployed at a velocity of 5m/sec and depth of 2.0 mm from the cortical surface. Repetitive anodal tDCS (0.1 mA/15min) or sham stimulation were applied under anesthesia during 2 weeks for 4 consecutive days with 3 days tDCS-free interval starting at 1 week after TBI. The anodal electrode coated with EEG cream was placed over the craniotomy;

the counter electrode on the thorax. Mice were tested for neurological deficits at 3 weeks after trauma using Rotarod testing for coordination and motor deficits, passive avoidance testing for learning and memory and novel object recognition memory testing. Results: CCI-induced moderate TBI caused tissue damage in the cortex and subcortical zones including hippocampus in the ipsilateral hemisphere as shown by T2 MRI and H&E staining. Nissl staining revealed a shrunken hippocampus and obvious shrinkage of parietal somatosensory cortex with 18% counted neuronal loss compared to contralateral hemisphere. TBI impaired motor function as it was shown at one week after injury by three-fold decrease of latency period on Rotarod comparing to uninjured animals. tDCS attenuated TBI-induced neurological impairments. Motor function assessed by Rotarod was better in tDCS mice compared to Sham, with latency to fall of 114.7 ± 17.6 and 79.3 ± 9.9 sec., respectively, $p<0.05$, n=10/group. The step-through retention latency of tDCS mice in Passive Avoidance was significantly longer ($P<0.5$) than that of Sham mice (324.5 ± 68.1 vs. 129.4 ± 59.9 sec., respectively). In the Novel Object Recognition, tDCS mice explored the new object more than the familiar one (58±28% vs. 42±27%) compared to Sham (51±25% vs. 49±26%), however difference was insignificant ($P=0.39$). Conclusions: Our studies show that two weeks of repetitive tDCS improves motor and cognitive deficits in mice with moderate TBI. Support: NIH-NIGMS P20GM109089-01A1

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Topic: C.09. Brain Injury and Trauma

Support: R01NS081189

NS088656

P41 EB002520

Title: Systemic administration of cell-free exosomes generated by human marrow mesenchymal stem cells cultured under 2D and 3D conditions improves functional recovery in rats after traumatic brain injury

Authors: Y. ZHANG[1], M. CHOPP[2,3], Z. G. ZHANG[2], M. KATAKOWSKI[2], H. XIN[2], C. QU[1], E. PIKULA[2], M. ALI[2], A. MAHMOOD[1], *Y. XIONG[1];

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Abstract: Multipotent human mesenchymal stem cells (hMSCs) improve functional outcome after experimental traumatic brain injury (TBI). The present study was designed to investigate whether systemic administration of cell-free exosomes generated from hMSCs cultured in 2-dimensional (2D) conventional conditions or in 3-dimensional (3D) collagen scaffolds promotes functional recovery and neurovascular remodeling in rats after TBI. Wistar rats were subjected to TBI induced by controlled cortical impact; 24 hours later tail vein injection of exosomes derived from hMSCs cultured under 2D or 3D conditions or an equal number of liposomes as a treatment control were performed. The modified Morris water maze, neurological severity score and footfault tests were employed to evaluate cognitive and sensorimotor functional recovery. Animals were sacrificed at 35 days after TBI. Histological and immunohistochemical analyses were performed for measurements of lesion volume, neurovascular remodeling (angiogenesis and neurogenesis), and neuroinflammation. Compared with liposome-treated control, exosome-treatments did not reduce lesion size but significantly improved spatial learning at 33-35 days measured by the Morris water maze test, and sensorimotor functional recovery, i.e., reduced neurological deficits and footfault frequency, observed at 14-35 days post injury ($p < 0.05$). Exosome treatments significantly increased the number of newborn endothelial cells in the lesion boundary zone and dentate gyrus, and significantly increased the number of newborn mature neurons in the dentate gyrus as well as reduced neuroinflammation. Exosomes derived from hMSCs cultured in 3D scaffolds provided better outcome in spatial learning than exosomes from hMSCs cultured in the 2D condition. In conclusion, hMSC-generated exosomes significantly improve functional recovery in rats after TBI, at least in part, by promoting endogenous angiogenesis and neurogenesis and reducing neuroinflammation. Thus, exosomes derived from hMSCs may be a novel cell-free therapy for TBI, and hMSC-scaffold generated exosomes may selectively enhance spatial learning.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: UCLA, Department of Neurosurgery, Brain Injury Research Center

NIH NINDS R01NS091222

Center for Neuroskills, Bakersfield, California

Title: Enduring effects of environmental enrichment on functional network reorganization after experimental TBI in rats

Authors: *A. PAYDAR[1], D. ROBIO[1], S. SRINIVAS[1], Y. CAI[1], A. E. KLINE[2], N. G. HARRIS[1];
[1]UCLA, Dept. of Neurosurgery, BIRC, Los Angeles, CA; [2]Departments of Physical Med. & Rehabilitation, Critical Care Medicine, and Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Current clinical rehabilitation strategies used after brain injury promote beneficial behavioral outcomes. However, therapies might be further optimized by using more detailed feedback from comparison of brain functional connectivity (fc) before and after intervention. We hypothesized that resting state fMRI (rsfMRI) should be an ideal technique to monitor whole brain function in response to rehabilitation. Our recent studies showed that fc is significantly impacted by experimental brain trauma in the rat, and that increased pericontusional and contralateral brain regions fc offsets the loss in connectivity from the injury core. However, it is unknown whether the beneficial effects of rehabilitation can be followed by monitoring brain circuity through changes in fc. We therefore determined fc following controlled-cortical-impact-injured over the forelimb sensorimotor cortex and in sham-injured adult rats that were then housed immediately after injury in either standard (STD) or environmentally enriched (EE) conditions for 4 weeks, and then for an additional 4 wks in STD conditions for all rats (n=5-6/group). We obtained behavioral grid-walk and rsfMRI data at 1, 4 & 8wks post-injury and analyzed data for network-based connectivity over 96 brain regions. The rsfMRI data confirmed our prior published work showing significant injury effects in STD conditions compared to sham-STD at all time-points ($P < 0.05$). There was no global effect of EE vs STD on any network parameter at 1wk post-injury, although there was a trend of reduced modularity (lowered arrangement of circuits into network components), and the number of grid-walk foot-faults was reduced following injury+EE compared to injury+STD. However, 4wks of EE normalized many of the persistent injury+STD effects of reduced shortest path and increased mean local connectivity to sham-STD / sham-EE levels. Low global modularity persisted at 4wks after injury+EE ($P < 0.05$) compared to injury-STD, sham-EE and sham-STD, all of which were not different, indicating a potential biomarker of reorganization due to EE. Importantly, these effects endured when measured 4wks after removal of EE conditions (8wks post-injury). These global effects were underpinned by significant regional effects of normalized connection strength in injured-EE vs injured-STD, bilaterally in S1 cortex, thalamus and caudate, and in contralateral hippocampus and M1 cortex ($P < 0.05$).

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Support: CURE Grant Prevention of Acquired Epilepsies Awards

Title: The impact of MIF binding and CD74 on the activation and expansion of pro inflammatory B cells and gamma delta T cells in a fluid percussion model of traumatic brain injury

Authors: *L. A. SHAPIRO[1], S. K. ROGERS[1], D. NIZAMUTDINOV[1], R. BUCALA[2], M. K. NEWELL0ROGERS[1];
[1]Surgery & Neurosurg., Texas A&M Hlth. Sci. Ctr., Temple, TX; [2]Yale Univ., New Haven, CT

Abstract: The immune system responds to infection or injury, including TBI, via a well-orchestrated series of events that include an innate immune response in which cytokines and chemokines are released that recruit immune cells to the injury. An important pro-inflammatory cytokine released during the early response to injury is macrophage migration inhibitory factor (MIF). This fundamental component of the immune response may be involved in tissue repair, or tissue damage, and is part of the non-specific, innate immune response. If antigen is present following an injury, the innate immune response precedes antigen processing and presentation, and subsequent T cell recognition, the hallmarks of a transition from an innate to a specific, adaptive immune response. Our previous work demonstrated an early expansion and activation of splenocytes within 24 hrs of a traumatic brain injury (TBI), that was highly suggestive of an adaptive immune response. We subsequently demonstrated that the splenocyte expansion is dependent on invariant chain (CD74). The full-length CD74 molecule can exist as a cell surface receptor for macrophage inhibitory factor (MIF). MIF binding to full-length cell surface CD74 results in a signaling cascade that modulates cytokine and chemokine production. However, the proteolytic cleavage product of CD74, CLIP, has an additional function in antigen processing, the first step in the transition from an innate to an adaptive immune response. Depleting CD74 in our experiments resulted in a reduction in lesion size and neuroinflammation following fluid percussion injury (FPI). Several fundamental questions that remain to be answered are whether CD74 contributes to the neurodegenration of TBI via an innate response involving MIF or from its contribution to the transition between innate and adaptive immunity, or both and, importantly, how do these functions influence TBI-induced pathology? To address these questions, we administered ISO1, a MIF antagonist, at 30 minutes after an FPI. We then assessed splenocyte expansion and gamma delta T cell activation at 24 hrs after FPI. The 24 hour time point is when we have previously seen robust expansion of splenocytes, including gamma delta T cells. The

results show that treatment with ISO1 after FPI reduces the expansion of CLIP-bearing B cells, but had little to no effect on the expansion of gamma delta T cells. These results suggest that MIF might have a role in mediating specific, adaptive immune responses, in addition to its well-defined role in contributing to an innate immune response. Follow-up studies are currently underway to determine if ISO1 treatment will also be neuroprotective.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.20/Z3

Topic: C.09. Brain Injury and Trauma

Title: Alteration of cardiac performance after traumatic brain injury through acute signaling mechanisms

Authors: *D. NIZAMUTDINOV[1], J. KAIN[2], L. A. SHAPIRO[3];
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Abstract: Autonomic nervous system innervation of the heart, and neuroimmune responses are possible mechanisms through which the brain can alter myocardial function. Traumatic brain injuries (TBI), are associated with various cardiovascular abnormalities including hyper- and hypotension, electrocardiographic (ECG) abnormalities, release of troponin C, cardiac dysfunction resembling myocardial infarction and significantly increase mortality and morbidity from cardiac pathologies. In animal models, stimulation of the hypothalamus induces cardiac hypertension and/or ECG abnormalities (myocardial necrosis, dysfunction of rhythmic activity-arrhythmias, bradycardia, tachycardia). These symptoms occur with acute stimulation of the hypothalamus, whereas chronic stimulation causes persistent irreversible ECG changes. The focal myo-cytolysis was reported in some patients who suffered from fatal intracranial hemorrhages, known as myofibrillar degeneration. It is now known that any neurological insults, including TBI, stroke, ischemia, and subarachnoid hemorrhage, can lead to adverse cardiovascular events, demonstrating a clear link between the brain and cardiac function. Therefore, the current study sought to examine activation of key cardiac cell signaling molecules responsible for regulation of contractile performance. We examined phosphorylation of protein kinase B (P-AKT) and phosphorylation of c-Jun N-terminal kinase (P- JNK) relative to total forms of both proteins, at 2 and 6 hrs after a fluid percussion injury (FPI), compared to sham FPI

animals. JNK and AKT are known to be involved in the regulation of cardiac calcium mobilization and direct orchestration of contractile elements of the heart.
The results demonstrate a 30% increase of P-JNK at the 2 hrs post-FPI time point and a 21% decrease at 6 hrs after FPI, relative to sham mice. Conversely, the P-AKT exhibited a 7% decrease at 2 hrs after FPI, and a 12% decrease at 6 hrs after FPI, compared to shams. It is possible that the observed 30% increase in phosphorylation of JNK at 2 hrs post-FPI is involved in an acute signaling response to compensate for the decrease of P-AKT at this time point. At 6 hrs post-FPI, the 51% decrease in P-JNK, compared to 2 hrs post-FPI, might be explained as a temporary/partial contractile decompensation. This hypothesis is supported by the observation of greater dephosphorylation of AKT at 6 hrs after FPI.
In conclusion, the presented data are consistent with previously reported clinical findings associated with acute ECG abnormalities in patients with TBI. Follow-up studies are planned to unveil fundamental mechanisms behind cardiac damage / recovery after TBI.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Topic: C.09. Brain Injury and Trauma

Support: NSF grant (1456675)

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Title: Role of oxidation of kcnb1 potassium channels in mouse model of traumatic brain injury

Authors: W. YU[1], R. PARAKRAMA[2], S. TENG[2], M. GOWDA[2], Y. SHARAD[2], S. THAKKER-VARIA[2], J. ALDER[2], *F. SESTI[3];
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Abstract: Keywords: oxidative stress, ROS, potassium channel, K+ channel, Kv2.1, KCNB1, dasatinib, Src kinase, primary neurons, morris water maze, rotating device, transgenic mouse.
Delayed rectifier potassium (K^+) channel KCNB1 (Kv2.1), which conducts a major somatodendritic current in cortex and hippocampus, is known to undergo oxidation in the brain but whether this can cause neurodegeneration and cognitive impairment was not known. Here, we used transgenic mice harboring human KCNB1 wild type (Tg-WT) or non-oxidable C73A mutant (Tg-C73A) in cortex and hippocampus, to determine whether oxidized KCNB1 channels

affect brain's function. Animals were subjected to traumatic brain injury (TBI), a condition characterized by extensive oxidative stress. Dasatinib, a FDA-approved inhibitor of Src tyrosine kinases was used to impinge on the pro-apoptotic signaling pathway activated by oxidized KCNB1 channels. Thus, typical lesions of TBI, namely inflammation (astrocytosis), neurodegeneration and cell death were markedly reduced in Tg-C73A and Dasatinib-treated non-Tg animals. Accordingly, Tg-C73A mice and non-Tg mice treated with Dasatinib exhibited improved behavioral outcome in the rotating test device (rotarod) and in the Morris Water Maze (MWM) compared to control. Moreover, the activity of Src kinases, along with oxidative stress, were significantly diminished in Tg-C73A brains. Together, these data demonstrate that oxidation of KCNB1 channels is a contributing mechanism to cellular and cognitive deficit in TBI and indicate a new therapeutic approach to this devastating condition.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Coalition for Brain Injury Research

CONACYT-COPOCYT

Fundación Marrón Cajiga

Title: Sensor-based quantitation of a closed-skull weight drop model for traumatic brain injury

Authors: *J. ALLENDE LABASTIDA[1], S. ALI[2], J. GAO[1], T. J. DUNN[1], Y. YU[3], D. S. DEWITT[4], D. S. PROUGH[4], P. WU[1];
[1]Neurosci. and Cell Biol., [2]Pharmacol. and Toxicology, [3]Radiation Oncology, [4]Anesthesiol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Traumatic brain injury (TBI) represents a major public health burden, with an estimated 150-300 per 100,000 people suffering from TBI worldwide. The increased awareness of mild TBI such as concussion lead to more research and a growing body of literature being generated in the pre-clinical setting. However, there is a concern in animal models for mTBI and the reproducibility among studies and laboratories. Standardized experimentation will allow for high quality meta-analysis of data and more meaningful translation of preclinical research to the clinical setting. Recently, a closed-skull weight-drop injury model has been developed, including two of the mechanisms (impact and acceleration/deceleration) observed in most cases of TBI, making this model clinically relevant. However, uncertainty of the injury severity and variability are major concerns of this model. To address these problems, we added a set of sensors to the model that allowed us to measure the velocity of the falling weight and calculate its kinetic energy immediately before impact. Male C57BL/6 mice were allocated into 3 groups: sham injury as control and two TBI injury groups (each group injured with either a 95 g or 150 g weight dropped from a height of one meter). Locomotor activity was recorded 30 minutes after injury. We observed a dose-dependent decrease in the exploration activity after injury, and a partial recovery of locomotion at 4 days post-injury. Brains were collected 5 days post-injury for immunohistochemistry and Western blot analysis. Further characterization and optimization will yield a model that generates information of the forces responsible for injury, and ultimately reduces variability of the closed-skull weight drop model to promote translational TBI research.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Program#/Poster#: 322.23/Z6

Topic: C.09. Brain Injury and Trauma

Support: NIH R01 NS037313 15A1

Title: Administration of miR-155 antagomir following experimental traumatic brain injury (TBI) attenuates post-traumatic neuroinflammatory responses and improves neurological recovery

Authors: *R. J. HENRY[1], D. J. LOANE[2], B. E. SABIRZHANOV[2], B. A. STOICA[2], A. I. FADEN[2];
[2]Shock, Trauma & Anesthesiol. Reseach, [1]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a leading cause of mortality and disability worldwide, and is associated with chronic microglial activation that contributes to long-term neurodegeneration and neurological impairments. MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression at a post-translational level. Recent data suggest that miR-155 and miR-124 control microglial activation phenotype balance following CNS injury. This study examined the expression profile of miR-155/miR-124 in mouse brain following TBI. In addition, this study examined the effect of acute intracerebroventricular (i.c.v.) administration of an miR-155 antagomir on post-traumatic neuroinflammation and neurological recovery after TBI.

Adult male C57Bl/6 mice (n = 6/group) underwent moderate controlled cortical impact (CCI) and cortical tissue was isolated from sham, 1h, 6h, 24h, 72h and 7d post-injury for miR-155/miR-124 expression analysis. In a follow up study C57Bl/6 mice (n=8/group) received a single i.c.v. injection of miR-155 or scramble antagomir (0.5nmol) immediately after CCI, and cognitive function was assessed in CCI mice at 7d post-injury using a Y-maze test. At 7d post-injury, mice from the second experiment were anesthetized and trancardially perfused with ice-cold 0.9% saline. Ipsilateral hippocampus was rapidly dissected, snap-frozen and stored at -80°C until assayed for miRs and inflammatory mediator gene expression. Data were analysed using one-way ANOVA followed by Student's Neuman-Keuls *post-hoc* test.

Following CCI, miR-155 expression was significantly increased at 24h post-injury and miR-124 expression significantly decreased at 1h post-injury, when compared to sham-injured controls. This increase and decrease in miR-155 and miR-124 expression, respectively, persisted through 7d post-injury. Administration of a miR-155 antagomir attenuated CCI-induced increases in mRNAs for pro-inflammatory mediator's iNOS and NOX2 at 7 days post-injury. Furthermore, miR-155 antagomir treatment reversed CCI-induced decreases in spontaneous alterations in the Y maze task at 7 days following injury.

In summary, these data demonstrate that experimental TBI is associated with an altered miR-155/miR-124 expression profile up to 7 days post-injury, and pharmacological inhibition of miR-155 following TBI is associated with both anti-inflammatory effects and improvements in functional recovery. Overall, these findings indicate that pharmacological regulation of inflammatory miRs, may offer a novel therapeutic approach for targeting neuroinflammatory responses in experimental TBI.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Congresionaly Dirrected Medical Research Program

Hopkins-LIBRA Fellowship Program

Title: Corticospinal tract pathology with impact acceleration: modeling diffuse axonal injury in the mouse

Authors: N. ZIOGAS[1], J. RYU[1], L. XU[1], P. TSOULFAS[3], *V. E. KOLIATSOS[2]; [1]Neuropathology, [2]Neuropathology, Neurology, Psychiatry, Johns Hopkins Univ., Baltimore, MD; [3]The Miami Project to Cure Paralysis, Maiami, FL

Abstract: Diffuse Axonal Injury (DAI) is a common lesion associated with Traumatic Brain Injury (TBI), from concussion to severe injury. Sequelae of DAI are diverse and include motor impairments, memory loss, loss of coordination and disorders of sleep and mood, suggesting that more than one brain network is affected. The cellular and molecular mechanisms of DAI are probably similar regardless of the network involved, and may be studied using axon bundles accessible to experimental manipulations. Such models can address axonal versus retrograde perikaryal changes, time course of events, and molecular targets for repair. Here, we exposed Thy1-eYFP-H transgenic mice with green labeling of layer V pyramidal neurons and their axons in the corticospinal tract (CST) to impact acceleration TBI of mild severity. Using CLARITY and two-photon microscopy coupled with high-working-distance objective with submicron resolution, we visualized in 3D the CST from cerebral peduncles to pyramidal decussation. Individual axons and classical axonal abnormalities were identified in exceptionally high detail. CLARITY-based immunohistochemistry (IHC) was performed to characterize cellular and molecular pathology including: the precise location of axonal abnormalities, for example nodes of Ranvier identified with Nav1.6 and Caspr IHC; microglial activation and axonal-microglial interactions using IHC for IBA1, CD206 and CD16/32. Using conventional histological methods, we explored molecular hypotheses of axonal demise, including JNK cascade activation ending in phosphorylation of c-Jun or SCG10. Using retrograde tracing, we identified the cell bodies of neurons projecting in the CST. We found that CLARITY is a very sensitive method for axonal lesions that often cluster in sites of crossing of CST with other axon tracts. We also found that pyramidal neurons projecting in the CST lose perikaryal volume by 23% 14 days after injury; perikaryal volume loss is significantly associated with p-c-Jun immunoreactivity. These

findings suggest that impact acceleration reliably causes axonal pathology in the CST that can be characterized with high-resolution neuroanatomy and can divulge important cellular and molecular changes of great significance to DAI and TBI.

Disclosures: N. Ziogas: None. **J. Ryu:** None. **L. Xu:** None. **P. Tsoulfas:** None. **V.E. Koliatsos:** None.

Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 322.25/Z8

Topic: C.09. Brain Injury and Trauma

Title: Chronic retinal injury in a mouse model of blast-induced trauma

Authors: *N. MAMMADOVA[1,2], D. S. SAKAGUCHI[1], S. GHAISAS[2], G. D. ZENITSKY[2], A. G. KANTHASAMY[2], J. J. GREENLEE[3,2], M. H. W. GREENLEE[2];
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Abstract: Traumatic brain injury (TBI) due to blast exposure is currently the most prevalent of war injuries. While secondary ocular blast injuries due to flying debris are more common, primary ocular blast exposure has been reported among survivors of explosions, but with limited understanding of the resulting retinal pathologies. Blast wave pressure of 120 ± 7 kPa causes damage to the brain in a rat model, however, the effects of blast wave pressure on the retina have not been well characterized. Using a compressed air-driven shock tube system, adult male and female C57BL/6 mice were exposed to blast wave pressure of 300 kPa (43.5 psi) per day for three successive days, and euthanized 30 days post injury. We assessed retinal tissues using immunofluorescence for glial fibrillary acidic protein (GFAP), microglia/macrophage specific protein Iba1, and phospho-PHF-tau (AT-270). Primary blast wave pressure resulted in activation of Müller glia, loss of photoreceptor cells, and expression of phospho-tau in retinal neurons and glia. Additionally, we observed activation of microglia based on an increase in Iba1, and CD68 immunoreactivity. These changes were detected 30 days after blast exposure, suggesting the possibility of chronic retinal injury and neuronal inflammation after primary blast exposure.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Program#/Poster#: 322.26/Z9

Topic: C.09. Brain Injury and Trauma

Support: NHMRC

Title: Experimental traumatic brain injury induces changes resembling motor neuron disease that are exacerbated by pathological TDP-43

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Abstract: Motor neuron disease (MND) is characterized by the progressive death of motor neurons, degeneration of the corticospinal tract, and the presence of transactive response DNA binding protein 43 (TDP-43) pathologies. To date the aetiology of MND remains largely unknown. Traumatic brain injury (TBI) has been linked to the later onset of MND, however a causal relationship between these conditions remains controversial. As such, here we administered experimental TBI via the fluid percussion model to rats and assessed for progressive MND-like abnormalities. Volumetric MRI found that TBI resulted in progressive atrophy of the motor cortices, and tensor-based morphometry and diffusion-weighted imaging revealed progressive degeneration within the corticospinal tracts. Rats given a TBI also had a reduction in neurons and an increase in phosphorylated and cytoplasmic TDP-43 in the motor cortex, fewer motor neurons in the spinal cord, muscle atrophy, and motor impairments. To further examine the potential role of pathological TDP-43 in this process we next administered TBI to transgenic mice that overexpress TDP-43 or wild-type mice. All TBI mice had pathological TDP-43 relative to their sham-controls, with TDP-43 + TBI mice having more than all other groups. While all mice given a TBI had significant neuronal death, it was worse in TDP-43 + TBI mice. TDP-43 + TBI mice also had worse cognitive and motor deficits compared to their wild-type counterparts. These findings suggest that TBI can induce a progressive disease process resembling MND and that TDP-43 pathologies may contribute to these effects.

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Poster

322. Traumatic Brain Injury: Models, Mechanisms, and Treatments

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Program#/Poster#: 322.27/Z10

Topic: C.09. Brain Injury and Trauma

Support: Seed Funding from the School of Behavioral Health at Loma Linda University

Title: Pomegranate treatment for repetitive mild brain insults

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Abstract: Humans may be subjected to a number of mild brain insults (e.g., general anesthesia, concussions) over their lifetime. The neuropathological effects of mild brain insults can accumulate, leading to the development of motor and cognitive deficits. Although there are currently no treatments that can restore lost function after repetitive brain injury, our laboratory has shown that dietary supplementation with pomegranate polyphenols can improve Alzheimer's-like neuropathology in transgenic mice, protect mice from the effects of irradiation with protons, and protect humans from post-operative cognitive deficits. Therefore, this study focused on the behavioral and neuropathological effects of repetitive brain insults (concussion/general anesthesia) in mice, and the use of dietary supplementation with pomegranate to ameliorate these effects.
Adult mice were given dilute pomegranate juice or control water for 1 week, followed by anesthesia, repeated anesthesia (3 days apart), or repeated concussive injury (a single closed-head concussion to each hemisphere 3 days apart). They were then maintained on the pomegranate juice or control diet for 2 additional weeks. Behavioral testing was administered 1, 3, 5, and 7-11 days after injury to assess cognitive, motor, and affective function. Repeated concussive injury, but not repeated anesthesia, induced motor and learning deficits, some of which were significantly reduced by pomegranate juice. These data suggest that the model of repeated closed-head concussive injury in mice may be used to test neuroprotective treatments in future translational studies. Furthermore, this study provides further evidence that a phytochemical-rich diet may provide significant neuroprotection from brain injury.

Disclosures: A.M. Briseno: None. **N.M. Bajwa:** None. **A. Obenaus:** None. **R.E. Hartman:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Program#/Poster#: 323.01/Z11

Topic: C.09. Brain Injury and Trauma

Title: Acute vasopressor administration after traumatic SCI: the impact on metabolism, blood flow, oxygenation, pressure and long-term behavioural recovery using a porcine model of SCI.

Authors: *A. GHEORGHE[1], F. STREIJGER[1], **K. SO**[1], E. B. OKON[1], N. MANOUCHEHRI[1], K. SHORTT[1], D. E. GRIESDALE[2], M. S. SEKHON[3], B. K. KWON[4];
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Abstract: Traumatic spinal cord injury (SCI) results in local and systemic vascular changes which make the spinal cord extremely vulnerable to ischemia, hypoxia, and energy dysfunction. Current guidelines recommend maintaining a mean arterial pressure (MAP) of 85-90 mmHg using volume expansion agents and vasopressors in an attempt to support the injured spinal cord with adequate perfusion. While the desire to prevent ischemia is understandable, indiscriminate augmentation of MAP in a traumatically injured cord with impaired vascular autoregulation may have deleterious effects. In this study we sought to determine the impact of MAP augmentation on vascular and metabolic responses as well as long-term behavioural outcomes following a contusion-compression injury of the spinal cord.
Using our porcine model of SCI, female Yucatan miniature pigs received a T10 contusion injury followed by 3-hours of sustained compression. 1-hour post-compression and decompression, norepinephrine (NE) was used to elevate MAP by 20 mmHg for a 1.0-hr period. Laser Doppler/oxygenation and pressure probes were inserted into the spinal cord 0.2 and 2.2 cm from the injury site to monitor the effects of MAP support on spinal cord blood flow (SCBF), $PaPO_2$, pressure over a 7-day period. Microdialysis samples were also collected and subsequently analyzed for lactate, pyruvate, glucose, glutamate and glycerol. Behavioural recovery was scored weekly according to the Porcine Thoracic Injury Behavioural Scale (PTIBS).
Data from the first 6 hours after SCI showed that proximal to the impact (0.2-cm location), NE infusion during the compressed and decompressed state of the spinal cord resulted in only a slight restoration of SCBF and PaO_2, though both still remained well below pre-injury levels. In the decompressed state, a decrease in L/P ratio was observed in the NE group to ~250% of pre-injury levels, while levels remained unaltered in the control group (~800% of pre-injury). Distal to the impact (2.2-cm location), NE infusion increased SCBF and $PaPO_2$ above pre-injury levels

both during the compressed and decompressed state of the spinal cord. Currently we are analyzing the intraparenchymal responses during the subsequent 7 days as well as the long-term behavioural consequences of MAP support.
Combined, our preliminary results suggest that MAP augmentation during compression and following decompression could partially restore post-traumatic ischemia/hypoxia at the injury site, but could potentially lead to hyperemia distal to the injury site. Identifying the functional consequences is currently being carried out.

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Poster

323. Spinal Cord Injury Models and Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 323.02/Z12

Topic: C.09. Brain Injury and Trauma

Title: Trauma-induced alterations of cerebral excitability and cortical reorganization in a porcine model of SCI

Authors: *K. SHORTT[1], C. R. JUTZELER[1,4], F. STREIJGER[1], N. MANOUCHEHRI[1], K. SO[1], J. KRAMER[1,2], B. K. KWON[1,3];
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Abstract: Recent work has shown that deafferentation caused by traumatic spinal cord injury (SCI) can trigger a change in brain state and induce cortical reorganization (Moxon et al. 2014). It is believed that these adaptive changes in response to injury within the motor and sensory systems play an important role in functional recovery after SCI. In an effort to assess time-dependent changes occurring in the forelimb and hind limb sensory-motor cortex, in this study somatosensory (SSEP) and motor evoked potentials (MEP) were used to evaluate immediate (i.e. within 1-3 hours) and long-term changes (12 weeks) using a porcine model of SCI.
Twelve female Yucatan pigs weighing 20-25kg were anesthetised using intravenous Propofol and Fentanyl. All animals received a midline contusion injury at the T10 level using a 50g impactor dropped from a height of 20 cm; this was followed by 5 minutes of sustained compression. Cortical SSEPs were measured using stimulation of the median nerve of the forelimb, and the medial plantar nerve (a branch of the posterior tibial nerve), of the hind limbs.

MEPs were recorded from the extensor carpi radialis muscle in the right forelimb of the animals. SSEPs and MEPs were recorded pre-surgery, post-laminectomy, and 5 minutes, 3 hours and 12 weeks after injury.
Immediately after SCI, upon stimulation of the injured hind limb, cortical SSEPs were completely abolished and did not recover even by week 12. SSEPs recorded upon forelimb stimulation remained unchanged directly after injury, however, a significant amplitude increase was observed at week 12 in comparison to pre-SCI. Moreover, MEPs recorded from the intact forelimb exhibited noticeably greater amplitude at 3 hours and 12 weeks post-SCI.
Taken together, our data demonstrate that besides an obvious loss of cortical responses to hindlimb stimuli, forelimb evoked responses markedly increased following contusion-compression SCI at T10. The increase in forelimb MEP amplitude immediately after SCI may indicate a change in the cerebral state of excitability, while the increase in SSEPs occurring over time (i.e., recovery) may be indicative of cortical reorganization.

REFERENCES:

Moxon, K.A., A. Oliviero, J. Aguilar, G. Foffani. Cortical reorganization after spinal cord injury: always for good? Neuroscience. 2014. 283: p. 78-94.

Disclosures: K. Shortt: None. **C.R. Jutzeler:** None. **F. Streijger:** None. **N. Manouchehri:** None. **K. So:** None. **J. Kramer:** None. **B.K. Kwon:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

Location: Halls B-H

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Topic: C.09. Brain Injury and Trauma

Title: Duraplasty in acute traumatic SCI: the impact on metabolism, blood flow, oxygenation and pressure using a porcine model of SCI.

Authors: *N. MANOUCHEHRI[1], F. STREIJGER[1], K. SHORTT[1], K. SO[1], E. B. OKON[1], B. K. KWON[1,2];
[1]ICORD, UBC, Vancouver, BC, Canada; [2]Vancouver Spine Surgery Inst., Dept. of Orthopaedics, UBC, Vancouver, BC, Canada

Abstract: After traumatic spinal cord injury (SCI), secondary pathophysiologic processes and damage result in worsening the extent of the primary injury and ultimately functional outcome. One commonly observed secondary injury phenomenon in the acute injury period is progressive edema and swelling of the spinal cord. This may increase intraparenchymal spinal cord pressure as the cord swells and is compressed against the dura, resulting in a compromise in perfusion.

One way to potentially alleviate these effects is to expand the subarachnoid space by performing a duraplasty, which could potentially reduce intraparenchymal pressure and improve perfusion. Therefore, in this study, we determine the effect of expansive duraplasty on intraparenchymal pressure, cerebrospinal fluid pressure, blood flow, oxygenation, and metabolic responses in a porcine model of SCI.
Female Yucatan miniature pigs received a T10 contusion-compression SCI either with or without expansion duraplasty using an artificial dural graft. For the SCI-only animals the dura was left intact. Prior to injury, probes for microdialysis, blood flow (SCBF), oxygenation ($PaPo_2$), and hydrostatic pressure measurements were inserted into the spinal cord 0.2 and 2.2 cm from the injury site. Measurements occurred for 4 hours post-injury, after which the animals were recovered for continued monitoring over 7 days.
Contusion-compression SCI resulted in decreased SCBF levels close to the injury site (0.2-cm location) followed by a subsequent increase during the following days. Similarly, $PaPO_2$ plummeted immediately after injury and these levels remained low for the entire 7 day period post-injury. The L/P ratio increased within minutes, with a second continual increase at day 3. A gradual increase in L/P ratio was also observed at 2.2cm. Hydrostatic pressure remained consistently elevated for days and negatively correlated with changes in SCBF. An imbalance between SCBF and tissue metabolism was also observed, resulting in metabolic stress and insufficient oxygen levels.
The next step is to determine if expansion duraplasty can alleviate the observed increase in pressure and ischemia/hypoxia following SCI, a study that is currently being performed. Our preliminary data on 4 pigs shows promise, demonstrating the feasibility of the duraplasty technique and an enlargement of the dorsal subarachnoid space under the duraplasty. Microdialysis, blood flow, oxygenation, and hydrostatic pressure measurements are presently being analyzed.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: CDMRP/USAMRAA W81XWH-13-2-0047

Title: New neurotrauma marker panel of astroglial heterogeneity predicts severity and outcome after recoverable swine spinal cord injury

Authors: *I. B. WANNER[1], J. HALFORD[1], S. SHEN[2], J. A. LOO[2], R. KINSLER[3], P. CRIPTON[4], B. KWON[5], A. MAYER[6];
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Abstract: The broad heterogeneity of spinal cord injury (SCI) complicates patient assessment when determining urgent care, transportation needs and outcome prediction, requiring objective and sensitive techniques for rapid diagnosis. Biofluids offer a protein signature for tracking cellular pathophysiology after neurotrauma. We identified a novel neurotrauma marker panel and associated marker release to cell wounding and cell death in mechanically traumatized astrocytes (Levine et al., 2016). This new panel, measured in cerebrospinal fluid (CSF) and blood by immunoblot densitometry and mass spectrometry, was used to assess severity after contusion SCI in the Yucatan swine, along with quantitative histopathology and magnetic resonance imaging (MRI). In CSF, GFAP, S100β and new astroglial markers aldolase C (ALDOC), brain lipid binding protein (BLBP), and glutamine synthetase (GS) were robustly elevated 15-30 min post-SCI relative to baseline levels. GFAP and S100β decreased over time while ALDOC and GS remained elevated in the CSF 2-7 days post-injury. ALDOC was found in serum by 15-30min and peaked by 2 days post-SCI. Interestingly, acute ALDOC, BLBP, GS and GFAP CSF levels correlated well with cavity size and immunoglobulin extravasation to predict severity of tissue loss and hemorrhage at 7 days postinjury. Acute CSF levels of ALDOC and GFAP associated strongly with recovery of ambulation at 7 days postinjury using the porcine thoracic injury behavioral scale (PTIBS, Lee et al., 2013). Acute astroglial wounding shown by glial fiber fragmentation (clasmatodendrosis) was most abundant 1-2 hours after injury, while tissue loss evolved over 7 days postinjury. Severe, early clasmatodendrosis may contribute to increasing cavitation and acute astroglial biomarker release. At 7 days postinjury, elongated astrocytes formed perilesional bundles located separately from damaged neural tissue with wounded and territorial reactive astrocytes, documenting glial scar formation. Astroglial diversity was further defined by different astroglial marker expression providing molecular and structural heterogeneity in astroglial injury response. In summary, histopathology and cell fate-defined markers with fast release kinetic and prolonged elevation provide new tools for acute diagnosis and prognosis that help elucidate the acute pathophysiology of trauma and support patient risk stratification. Our data indicate that early trauma events predispose the unfolding severity range and recovery potential after contusive SCI. Ongoing investigations will examine acute care conditions like rough ground transportation as well as correlation with quantitative MRI.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Program#/Poster#: 323.05/AA1

Topic: C.09. Brain Injury and Trauma

Title: Relationship between injury severity and miRNA expression in CSF and serum from human spinal cord injury patients

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Abstract: With no real treatment options currently available to clinicians, there is an urgent need for non-invasive biomarkers to aid in the scientific development and clinical validation of novel therapies for acute spinal cord injury (SCI). Micro RNAs (miRNAs) are small regulatory noncoding RNA molecules around 22 nucleotides in length that mediate post-transcriptional silencing of gene expression via the identification of specific sequences in target messenger RNA. Besides their specific spatial, temporal and cellular-level expression, our interest in miRNAs stems from their stability within blood, making it possible for blood samples to be used to measure markers specific to the injured central nervous system. In this study, we compared the miRNA expression profiles of serum and cerebrospinal fluid (CSF) collected from human patients with SCI. Patients classified as AIS A, B, or C were recruited as part of an ongoing, multi-center study (CAMPER; clinicaltrials.gov NCT01279811). In order to collect CSF samples, a lumbar indwelling intrathecal catheter was inserted prior to surgery (within 48 h of injury) and CSF samples were collected every 6-8 h for 5 days. Blood samples were collected simultaneously in order to compare CSF and serum miRNA profiles. Additionally, control ‘‘non-injury’’ CSF samples were collected via a single lumbar puncture from patients undergoing hip or knee surgery during their spinal anesthetics, or intra-operatively from patients undergoing lumbar spine fusions. Next-generation sequencing technology was used to compare effects of injury severity on miRNA levels obtained daily over a period of 5 days post-injury. Extracellular

miRNAs were isolated and sequenced using the Illumina HiSeq 2500 system. Generated data was processed using the Mayo Clinic's Comprehensive Analysis Pipeline for miRNA Sequencing Data (CAP-miRSeq), and aligned miRNA reads were used to compare differential expression. Here, we present miRNA profiles in CSF and serum clinical samples during acute and sub-acute stages after SCI. Our results reveal pronounced trends in miRNA expression and their potential target interactions provide valuable insight into the molecular mechanisms of SCI. This analysis was done in parallel to the investigation of miRNA in the serum of pigs following SCI. This characterization is important to establish whether biomarkers of SCI found in pigs can be transferred to humans and vice-versa.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

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Bryon Riesch Paralysis Foundation

Title: Overexpression of KLF6 in corticospinal tract neurons promotes axon growth after spinal injury

Authors: ***Z. WANG**, I. VENKATESH, N. KRUEGER, D. NOWAK, B. CALLIF, B. MAUNZE, M. G. BLACKMORE;
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Abstract: Axonal regeneration in the central nervous system is limited in part by a developmental decline in the intrinsic regenerative capacity of central nervous system (CNS) neurons. Changes in gene expression are likely involved, and thus transcription factors that orchestrate gene expression are attractive targets to understand and overcome intrinsic limits to axon growth in adult neurons. We have shown previously that forced expression of pro-regenerative transcription factors, including Sox11 and a transcriptionally activated form of

Krüppel-like factor 7 (VP16-KLF7), can enhance the regenerative ability of injured corticospinal tract (CST) neurons. Here we assessed the ability of KLF6, a transcription factor closely related to KLF7, to promote CST regeneration. KLF6 was delivered to cortical neurons by injection of AAV-KLF6 along with AAV-EGFP tracer, and animals were subjected to pyramidotomy or unilateral cervical hemisection. KLF6 expression promoted a robust increase in midline crossing by transduced (EGFP+) CST axons in the pyramidotomy model, and extensive CST growth in the spinal injury model that extended up to 3mm from the injury site. Immunohistochemistry confirmed viral-mediated upregulation of KLF6 protein, but also revealed endogenous expression of KLF6 in cortical neurons that appeared largely unaffected by spinal axotomy. Intriguingly, forced expression of KLF6 had more modest effects in sensory neurons confronted with spinal injury, causing a decrease in net retraction but not sprouting or regeneration beyond the injury site. To identify potential functional interactions with other pro-regenerative transcription factors, either Sox11 or Myc were co-expressed with KLF6 in cortical neurons challenged with spinal injury. Neither combinatorial treatment resulted in significant increases in CST axon growth above the level of KLF6 alone. Ongoing experiments are testing co-expression of KLF6 with additional pro-regenerative factors including Jun and DCLK. In addition, using CRISPR-mediated knockdown in a Cas9-expressing transgenic mouse, we are currently testing combined KLF6 overexpression and knockdown of PTEN. Finally, RNAseq experiments are underway to identify KLF6 target genes. Overall, these data identify KLF6 as a potent transcriptional promoter of axon regeneration in the injured CST.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

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Title: Combined rehabilitation and genetic enhancement of intrinsic regenerative growth ability to improve behavioral outcomes after spinal injury.

Authors: ***A. A. KRAMER**, Z. WANG, E. BALLE, L. K. HOLAN, N. KRUEGER, J. A. EVANS, M. G. BLACKMORE;
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Abstract: Spinal cord injury (SCI) is a debilitating condition as adult CNS neurons are not able to regenerate lost axons, resulting in a loss of function below the injured region. To restore function, axons must be stimulated to regenerate and reconnect with appropriate postsynaptic targets. Recent work from our lab shows that in corticospinal tract neurons, overexpression of pro-regenerative transcription factors; including KLF7, Sox11 and more recently KLF6, leads to a significant increase in axon growth after spinal injury. Moreover, these stimulated CST sprouts show co-localization with the presynaptic marker VGLUT1 and the excitatory postsynaptic marker PSD-95, suggesting synaptic competence. Yet tests of forelimb function including a horizontal ladder task and staircase pellet retrieval showed no improvement in KLF6 or Sox11-treated animals. One possibility is that inappropriate synaptic targeting by newly grown CST axons may limit behavioral recovery. We are therefore exploring wheel running as a form of self-motivated rehabilitation to improve behavioral outcomes. Preliminary data indicate that access to running wheels after SCI improved performance on a horizontal ladder test. In current experiments we are testing the hypothesis that rehabilitation therapy in conjunction with genetic treatment may provide a means to guide newly sprouted axons to appropriate postsynaptic partners. The current study combines wheel running with KLF6 overexpression in the motor cortex, which we have recently shown to produce robust CST axon growth. Pellet retrieval and ladder crossing tasks will assess fine motor skills, and immunohistochemistry will determine the frequency and distribution of synaptic markers associated with sprouting CST axons in injured tissue. This combined genetic and rehabilitation approach may lead to better targeting of the newly sprouted axons to the appropriate postsynaptic targets and ultimately functional synapses and improved behavior.

Disclosures: **A.A. Kramer:** None. **Z. Wang:** None. **E. Balle:** None. **L.K. Holan:** None. **N. Krueger:** None. **J.A. Evans:** None. **M.G. Blackmore:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

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Bryon Riesch Paralysis Foundation

Title: Combined expression of pro-regenerative transcription factors and transplanted stem cells to promote corticospinal tract regeneration.

Authors: *N. JAYAPRAKASH, Z. WANG, N. KRUEGER, A. KRAMER, M. BLACKMORE; Marquette Univ., Milwaukee, WI

Abstract: The failure of axon regeneration in the injured spinal cord results in partial or complete loss of function distal to the injury. To restore the function, severed axons must regrow and functionally reconnect to appropriate targets below the injury site. We have shown previously that forced overexpression of pro-regenerative transcription factors including Sox11 and KLF7 promotes axon growth in corticospinal tracts (CST) neurons. Moreover, using optogenetic stimulation to specifically stimulate CST axon terminals, we have shown that newly sprouted, genetically stimulated axons are able to form functional synaptic connections with spinal neurons. However, these experiments were performed in models of partial spinal injury, and growing CST axons were observed mainly in trajectories that circumvented injuries, taking advantage of spared tissue. Here we tested the ability of Sox11 or KLF7-stimulated axons to regenerate through more complete spinal injuries. Adult mice were subjected to complete thoracic crush injuries or severe cervical injuries in which 1mm of tissue was unilaterally removed. Cortical neurons were treated with AAV-Sox11 or AAV-VP16-KLF7 along with AAV-EGFP tracer. CST axons were not observed to traverse these sites of injury. Combined, these data suggest that KLF7- and Sox11-based interventions enhance innate growth ability while maintaining the capacity for synaptic integration, but do not confer the ability to extend into sites of spinal injury. Recent reports indicate that transplanted stem cells can serve as a substrate for CST growth in the injured spinal cord. Accordingly, in current experiments we are combining transcriptional manipulation of injured cortical neurons with transplantation of embryonic and induced pluripotent stem cells into C5 unilateral injury sites. Preliminary data confirm integration of the transplanted cells and fiber outgrowth into host tissue. Ongoing assessment of CST growth into and beyond the grafts will assess the utility of combined stem cells treatment and gene therapy to re-establish lost synaptic connection following spinal cord injury.

Disclosures: N. Jayaprakash: None. **Z. Wang:** None. **N. Krueger:** None. **A. Kramer:** None. **M. Blackmore:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: This research was funded by the International Spinal Research Trust grant number TRI004

Title: Developing an immune-evading doxycycline-inducible viral vector for gene therapy in the spinal cord

Authors: *F. DE WINTER[1], B. HOBO[1], R. EGGERS[1], S. A. HOYNG[1,2], R. C. HOEBEN[3], R. J. YÁÑEZ-MUÑOZ[4], E. J. BRADBURY[5], E. M. MUIR[6], J. VERHAAGEN[1];
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Abstract: Gene therapy is a powerful strategy to deliver a therapeutic protein to the injured spinal cord. Persistent, uncontrolled expression of a therapeutic gene, however, may have unacceptable side effects. Restricting transgene expression to the appropriate therapeutic time window is therefore essential. The doxycycline (dox)-inducible system is the most commonly used inducible gene expression system, but this system depends on a foreign transactivator (TA) which is immunogenic. This currently limits the in vivo use of this system. The glycine-alanine repeat (GAr) of the Epstein-Barr virus nuclear antigen-1 (EBNA-1) inhibits presentation of EBNA-1 to cytotoxic T cells, allowing virus-infected cells to evade the host immune system. Recently we showed that TA fused to GAr generates a chimeric transactivator (GArTA) which retains its function and has an immune-evading advantage over TA in a bioassay for human antigen presentation (Hoyng 2014).
In this study we compared the performance of the original TA and the new GArTA in the spinal cord. Lentiviral vectors carrying TA or GArTA under a CMV promoter (LV-CMV-TA and LV-CMV-GArTA) were each injected in the spinal cord of adult rats in a 1:1 mixture with LV carrying luciferase (luc) under the control of a doxycycline responsive promoter (LV-TRE-luc). LV-CMV-luc was used as control. The luc reporter allowed longitudinal in vivo monitoring of transgene expression. Control rats injected with LV-CMV-luc show initial high luc expression which significantly declined over a 12 week period. LV-CMV-TA/TRE-luc and LV-CMV-GArTA/TRE-luc injected rats show clear dox-inducible luc expression during the 1st cycle of dox administration, however, a 2nd exposure to dox fails to re-induce expression with both systems.
Methylation is known to silence the CMV promoter. We therefore repeated the experiment with LV vectors in which CMV was replaced by the PGK promoter. Indeed, with 10-fold less viral vector injected, LV-PGK-luc activity was 10-fold higher in the first 3 weeks after injection compared to LV-CMV-luc. After 3 weeks LV-PGK-luc mediated expression also started to decline but stabilizes over time. Both LV-PGK-TA/TRE-luc and LV-GArTA/TRE-luc are inducible 3 times. However, LV-GArTA/TRE-luc continued to be inducible, while the inducibility of PGK-TA/TRE-luc significantly declined during the 4th and 5th dox-cycle, suggesting that the use of GArTA provides an immune-advantage over TA. In future experiments the novel transactivator will be tested in the context of vectors that are gaining

increasing acceptance as clinical gene expression platforms, i.e. integration deficient lentiviral and adeno-associated viral vectors.

Disclosures: **F. De Winter:** None. **B. Hobo:** None. **R. Eggers:** None. **S.A. Hoyng:** None. **R.C. Hoeben:** None. **R.J. Yáñez-Muñoz:** None. **E.J. Bradbury:** None. **E.M. Muir:** None. **J. Verhaagen:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Program#/Poster#: 323.10/AA6

Topic: C.09. Brain Injury and Trauma

Title: Gene therapy using a stealth gene switch for GDNF expression promotes long distance regeneration of motor axons following a spinal ventral root avulsion

Authors: *R. EGGERS[1], F. DE WINTER[1], S. A. HOYNG[1,2], R. C. HOEBEN[3], M. J. A. MALESSY[2,1], M. R. TANNEMAAT[4,1], J. VERHAAGEN[1];
[1]Neth Inst. Neurosci, Amsterdam, Netherlands; [2]Neurosurg., [3]Mol. Cell Biol., [4]Neurol., Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: In patients longitudinal spinal cord lesions of the brachial plexus often cause permanent loss of motor and sensory function. Until recent, such lesions were considered impossible to repair. After experimental lumbar ventral root avulsion, reimplantation promotes motoneuron survival and a limited degree of axon regeneration. Prolonged, uncontrolled viral vector-mediated expression of glial cell-line derived neurotrophic factor (GDNF) in reimplanted ventral roots enhanced motoneuron survival and axonal outgrowth into the root, however, persistent GDNF expression also leads to trapping of regenerating axons and a failure to reinnervate the hind paw (Eggers, MCN 2008). The goal of the current study was to overcome these adverse effects by exerting temporal control over the expression of GDNF using a novel stealth gene switch (Hoyng, Gene Therapy 2014). GDNF expression was regulated in the transduced reimplanted ventral roots (L3-6) of adult Wistar rats using doxycycline-supplemented food for 4 or 12 weeks (wk) post-surgery. This resulted in a 5 fold increased expression of GDNF for either 4 wk followed by a decline to baseline expression levels, or in persistent expression of GDNF for 12 wk. Significantly increased motoneuron survival was observed in all GDNF treated groups irrespective of the doxycycline treatment period. At the reimplantation site, robust regrowth of regenerating motor fibers into the root occurs in both GDNF groups compared to GFP control. However, persistent GDNF expression resulted in coiled fiber growth and disrupted myelination. The diameter of the ventral roots exposed to persistent GDNF

expression is significantly increased in comparison to GFP controls. In contrast, in the 4 wk GDNF treatment group, the large numbers of axons in the ventral root display a longitudinally organized growth pattern and the roots are less enlarged. Biweekly compound muscle action potentials (CMAP) measurements revealed that 4 wk GDNF expression led to an earlier recovery of a CMAP and a significant increase in amplitude compared to animals with persistent GDNF expression and GFP controls. Further histological analysis of axon regeneration and muscle reinnervation is currently ongoing. Taken together, these results show that timed GDNF expression using our novel stealth gene switch in a long distance regeneration model results in enhanced motoneuron survival and prevents local trapping of regenerating motor axons at sites of high GDNF expression. The observed improved CMAP values indicate that timed GDNF expression results in a potentiation of long distance regeneration and muscle innervation.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: The U.K Medical Research Council

International Spinal Research Trust

Title: Regulateable Chondroitinase ABC gene therapy as a treatment for spinal cord injury

Authors: ***E. R. BURNSIDE**[1], F. DE WINTER[2], A. DIDANGELOS[1], N. D. JAMES[1], K. BARTUS[1], E. M. MUIR[3], J. VERHAAGEN[2], E. J. BRADBURY[1];
[1]King's Col. London, London, United Kingdom; [2]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Following spinal cord injury the extracellular matrix undergoes significant remodeling. Scar formation is associated with upregulation of molecules known to be inhibitory to neural plasticity and recovery of function, including chondroitin sulphate proteoglycans (CSPGs). Enzymatic removal of CSPG glycosaminoglycan chains by the bacterial protein Chondroitinase ABC (ChABC) renders the matrix more permissive to recovery, however this is curtailed by rapidly diminishing enzyme activity. We have previously demonstrated that gene therapy using a modified ChABC gene compatible with expression and secretion by mammalian

host cells confers sustained and long-term delivery of ChABC to the injured spinal cord following a single administration. This treatment resulted in dramatic reduction in pathology and significant improvements in functional recovery following clinically relevant spinal contusion injury at both thoracic and cervical levels in adult rats. We now use novel immune-evasive vectors to enable regulatable gene therapy to exert greater control over ChABC expression, where ability to switch off delivery of ChABC greatly improves safety of the treatment. Using this system, doxycycline administration results in high expression of the ChABC gene and extensive functional enzymatic removal of inhibitory components present in the extracellular matrix. We also show this is accompanied by pro-reparative changes in inflammatory markers. We aim to utilise this system to manipulate timing and duration of ChABC delivery to adult rats which have received a clinically-relevant contusion injury to the cervical spinal cord and investigate its efficacy in promoting functional recovery. This represents both an experimental tool to optimise and control ChABC delivery to understand the role of timing in ChABC treatment, and a step towards clinical feasibility of ChABC gene therapy.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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SCIRF Grant 2014 I-02

Title: Rhoa knockdown by pgp/rhoa sirna nanoparticle increases axon growth after spinal cord injury

Authors: S.-J. GWAK[1], C. MACKS[1], K. WEBB[1], M. LYNN[2], *J. LEE[1];
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Abstract: Spinal cord injury results in permanent disruption of axonal pathways that leads to loss of motor and sensory function. The long-term goal of our work is to develop neuron-specific polymeric micelle nanoparticles for combinatorial delivery of bioactive molecules targeting different barriers to neuroplasticity and axonal regeneration. RhoA is a shared target of signaling

pathways activated by diverse extracellular molecules present in the injured spinal cord. In this study, we investigated the ability of amphiphilic copolymers (poly (lactide-co-glycolide)-g-polyethylenimine : PgP) carrying Rho A siRNA to improve axonal regeneration. Compression injuries were created by application of a vascular clip for 10 minutes at the T9 level of the spinal cord in adult male rats and then PgP/RhoA siRNA polyplexes (20 µg siRNA) were injected at the injury site. At 1, 2, and 4 weeks, rats were sacrificed and total RNA isolated for qRT-PCR analysis. For histological evaluation, rats were sacrificed via cardiac perfusion with 4% paraformaldehyde at 4 weeks and the spinal cords retrieved, sectioned longitudinally, stained for neurofilament and GFAP, and digitally imaged. RhoA mRNA expression was significantly reduced in animals receiving PgP/RhoA siRNA nanoparticles compared to the untreated SCI group and were not significantly different from the sham group at all time points. Significant knockdown of RhoA expression in the nanoparticle-treated group was maintained up to 4 weeks. We also observed an extensive necrotic lesion cavity and significant reactive astrogliosis in the untreated SCI animal group, while reduced cavitation/astrogliosis and axonal regeneration into the lesion site in the nanoparticle-treated group was observed at 4 weeks post-injection. These studies demonstrate that PgP is a promising therapeutic siRNA delivery carrier in rat compression spinal cord injury model *in vivo*. Currently, we are evaluating the effect of PgP/RhoA siRNA on functional recovery by Basso-Beattie-Bresnahan (BBB) locomotor rating scale and contact placing test in rat compression SCI model.

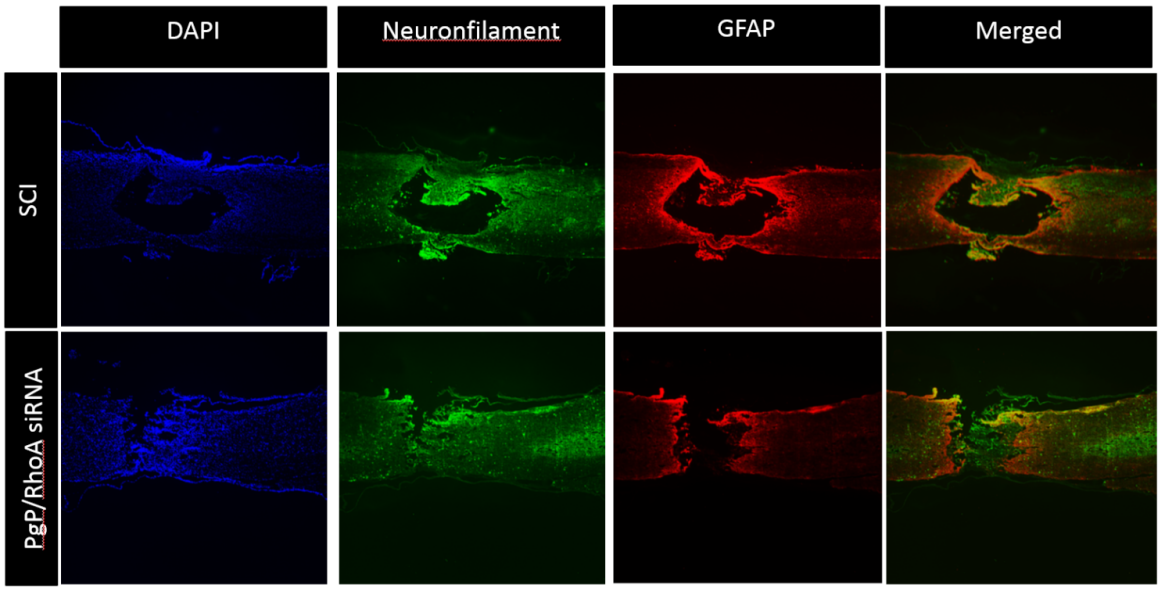

Figure 1.Immunohistochemical staining of neurofilament and GFAP in spinal cord at 4weeks post-injury. Top: Untreated spinal cord, showing an extensive necrotic lesion cavity and significant reactive astrogliosis, Bottom: PgP/RhoA siRNA polyplex-treated spinal cord, showing reduced cavitation/astrogliosis and axonal regeneration into the lesion site. Original magnification: 30X

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: DOD SC110169

Title: Stretching distrupts locomotor function in rats with spinal cord injury: static stretch and hold vs dynamic range of motion patterns

Authors: *A. KELLER[1,2], K. NORD[3], C. HAINLINE[3], D. PRINCE[4], A. SHUM-SIU[4], D. S. K. MAGNUSON[4];
[2]Physiol., [3]Bioengineering, [4]Neurolog. Surgery, [1]Univ. of Louisville, Louisville, KY

Abstract: Stretching therapy remains a leading technique utilized by physical therapists to manage spasticity and contractures in patients with spinal cord injuries (SCI). Preclinical studies in support of current clinical practices are lacking and systematic clinical studies are inconclusive. Using a rat model of incomplete SCI, we showed previously that a daily protocol of hindlimb static stretching (1 min each) had a negative impact on locomotor function of animals with acute or chronic, mild or moderately severe SCI. We found that stretching induced EMG responses in both the stretched and unstretched limbs that were qualitatively similar to clonus and spasms observed in patients in response to stretch. The objective of the current study was to determine if stretching delivered dynamically would also result in locomotor deficits in rats with SCI. Eight female Sprague Dawley (SD) rats were given moderate SCI contusions at T10 and were allowed to recover for 6 weeks to achieve a plateau in locomotor function before the initiation of the stretching protocol. The stretching protocol consisted of our standard 6 stretch positions performed bilaterally in a dynamic (rhythmic) fashion: 2 seconds on, 1 second off for 1 minute, 5 days a week for 4 weeks. Locomotor function was assessed using the BBB Open Field Locomotor Scale, 3D kinematics and gait. Dynamic stretching resulted in significant but temporary disruption to the locomotor function of the animals similar to that observed following static stretching. These results further extend our observation of the stretching phenomenon into a different modality of stretching that can be perceived as less intense yet equally detrimental to the locomotor function. Clinical relevance of the stretching phenomenon is still to be determined. However, based on our previous findings suggesting that rats and humans have similar EMG responses to muscle stretch we hypothesize that stretching may also have a negative impact on the locomotor function of patients with SCI. We believe future clinical studies are warranted in order to determine if stretching is detrimental to patients with SCI.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: DOD Grant SC110169

NIH Grant P30 GM103507

Title: Stretching disrupts locomotor function in rats with spinal cord injury: Role of nociceptive afferents.

Authors: *D. S. MAGNUSON[1], A. KELLER[2], S. KRUPP[3], K. NORD[4], C. HAINLINE[4], D. PRINCE[1], A. SHUM-SIU[1], J. C. PETRUSKA[3];
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Abstract: Stretching remains a front-line approach to deal with the loss of joint range-of-motion and spasticity after spinal cord injuries (SCI). The scientific rationale and evidence-based support for employing a stretching approach is weak, and systematic clinical studies showing efficacy are lacking. Existing studies have focused on understanding the effects of stretch on joint range of motion and have not considered the potential impact on neurological function. We showed recently that a static stretch-and-hold protocol applied to rats with T10 contusive injuries (mild, moderate or moderately-severe) severely but temporarily disrupts locomotor function when applied acutely or chronically. The underlying mechanisms by which stretching is disruptive to locomotion are unknown. The objective of the current study was to determine the role of nociceptive afferents in the mediation of the stretch-induced locomotor deficits by assessing C-fiber depleted animals. Two-day-old female SD rats received intraperitoneal injections of capsaicin (CAP, n=8), vehicle (VEH, n=8) or no injection (CON, n=8). The effectiveness of the capsaicin induced C-fiber depletion was determined functionally when the animals were 3 months old using behavioral pain assessments and by confirming the absence of cutaneous trunci muscle reflexes in response to a noxious heat stimulus. Animals were then given moderately-severe contusive SCI at T9. Our standard static stretching protocol (1 minute stretch-and-hold of each major hindlimb group, repeated twice) was initiated 6 weeks post-SCI and was carried out 5 days a week for 5 weeks. Locomotor function was assessed weekly using BBBs and biweekly using kinematics. Stretching significantly disrupted locomotor function of the VEH animals. In contrast, CAP animals had only minor drops in hindlimb locomotor function which were significantly different from the VEH group at most time points during the stretching protocol. Similar to previous observations locomotor deficits were temporary. Our data suggests that stretch-induced locomotor deficits after incomplete SCI involve the activation

of nociceptive afferents. These findings have significant implications that extend beyond stretching as a therapy due to the multiple sources of nociceptive feedback to the spinal cord after SCI (pressure sores, muscle contractures, etc) that potentially hinder spinal cord motor and locomotor function.

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Poster

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CHN

Wings for Life

Title: Grafts of multipotent neural progenitor cells in models of cervical contusive SCI: engraftment, axonal outgrowth and functional effects.

Authors: *J. H. BROCK[1,2], L. GRAHAM[3], S. IM[3], N. ARMSTRONG[3], M. TUSZYNSKI[3,2]; [1]Dept Neurosci, UCSD, La Jolla, CA; [2]VA, La Jolla, CA; [3]UC San Diego, San Diego, CA

Abstract: We examined whether grafts of rat multipotent neural progenitor cells (NPCs) would support axonal outgrowth and influence functional outcomes in models of cervical contusive injury. Adult Fisher 344 rats underwent unilateral C6 contusive injuries using an Infinite Horizons impactor equipped with a 2 mm impactor tip set at a force of 200 kilodynes and no dwell time. Two weeks later, syngeneic rat E14 spinal cord-derived multipotent NPCs were grafted into the lesion site, and forelimb funtional outcomes were examined using the Montoya staircase. Anatomical analyses demonstrate NPC survival and differentiation in the lesion site, and extensive axonal outgrowth into the host spinal cord. Grafts attenuated host glial responses, assessed by GFAP immunoreactivity, and reduced deposition of inhibitory CSPGs surrounding the lesion site. Grafted animals exhibited superior functional outcomes in forelimb function

compared to lesion-only or fibroblast controls ($P<0.05$). Thus, NPCs survive grafting to sites of contusive SCI, extend axons, alter host injury responses and support partial functional improvement. We are currently assessing NPC grafts in models of chronic contusive injury.

Disclosures: J.H. Brock: None. **L. Graham:** None. **S. Im:** None. **N. Armstrong:** None. **M. Tuszynski:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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NIH R01-NS042291

DOD W81XWH-12-1-0592

CIRM TR3-05628

CH Neilsen Foundation 260965

Bernard and Anne Spitzer Charitable Trust

Title: Human neural stem cell grafts into non-human primate spinal cord contusion or hemisection lesions

Authors: *E. S. ROSENZWEIG[1], J. H. BROCK[1,2], P. LU[1,2], J. L. WEBER[1], R. MOSEANKO[3], S. HAWBECKER[3], E. A. SALEGIO[3], Y. S. NOUT[4], L. A. HAVTON[5], A. R. FERGUSON[6], M. S. BEATTIE[6], J. C. BRESNAHAN[6], M. H. TUSZYNSKI[1,2];
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Abstract: We previously demonstrated that human neural stem cells (hNSCs) and multipotent neural progenitor cells (hNPCs) grafted into sites of rodent spinal cord injury (SCI) survive, extend axons, form synapses, support host axon regeneration, and improve functional recovery (Lu et al., *Cell*, 2012; Lu et al., *Neuron*, 2014; Kadoya et al., *Nat Med*, 2016). We are translating

this approach to non-human primates (Rhesus macaques).
Using the H9 human embryonic stem cell line, we generated neural stem cells using published protocols (Li et al., *PNAS*, 2011). Adult rhesus macaques underwent C7 lateral hemicontusions (N=2; Salegio et al., *J Neurotrauma*, 2016) or lateral hemisection lesions (N= 5; Rosenzweig et al., *Nat Neuro* 2010). H9-derived human NSCs were grafted into the SCI sites between 2 and 12 weeks after injury (2, 4, 6, 6, 8, and 12 wks). Subjects received 20 million GFP-expressing NSCs, suspended in a two-part fibrin matrix and growth factor cocktail (Lu et al., *Cell*, 2012). Subjects were immunosuppressed with prednisone, mycophenolate, and tacrolimus, and were sacrificed 3 - 21 weeks after grafting (3, 8, 16, 16, 18, 18, and 21 wks).
Five of the seven subjects (including both subjects with hemicontusions) had surviving grafts. All surviving grafts differentiated into both neurons and glia, and extended up to hundreds of thousands of new axons; some of these reached very long distances, up to 50 mm, in the host spinal cord. Graft filling of the lesion site varied, indicating the need for further optimization of the grafting method, immunosuppression protocol, or both.
These findings indicate that human neural stem cells can be grafted to sites of subacute to chronic primate SCI, survive, and extend remarkable numbers of axons over long distances. Grafting can be successfully accomplished in sites of contusive SCI, the most common mechanism of human injury. Further optimization of grafting methods is needed prior to potential human translation, highlighting the importance of utilization of larger animal models for methods development and safety assessments.

Disclosures: **E.S. Rosenzweig:** None. **J.H. Brock:** None. **P. Lu:** None. **J.L. Weber:** None. **R. Moseanko:** None. **S. Hawbecker:** None. **E.A. Salegio:** None. **Y.S. Nout:** None. **L.A. Havton:** None. **A.R. Ferguson:** None. **M.S. Beattie:** None. **J.C. Bresnahan:** None. **M.H. Tuszynski:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Title: Distal BDNF delivery to promote axonal regeneration through Schwann cell-seeded alginate hydrogels after spinal cord injury

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Abstract: Grafting of cell-seeded alginate capillary hydorgels into a spinal cord lesion site allows for axonal bridging and physically directs regenerating axonal growth in a linear pattern. However, without an additional growth stimulus, bridging axons fail to extend into the distal host spinal cord. In the present study, we used a combinatorial strategy combining alginate hydrogels seeded with syngeneic Schwann cells (SCs) and a distal gradient of brain-derived neurotrophic factor (BDNF) to explore whether long-descending propriospinal axons can regenerate across an alginate hydrogel to re-enter the host spinal cord. Adult Fischer 344 rats underwent a **l**ateral C5 hemisection immediately followed by 1) implantation of an alginate scaffold pre-seeded with SCs into the lesion site, and 2) injection of adeno-associated virus expressing either tetracycline-regulated (tet-on) BDNF or green fluorescent protein (GFP) as control into the caudal spinal cord ipsilateral to the lesion. After four weeks, ELISAs from spinal cords demonstrated tight BDNF regulation and establishment of a gradient caudal to the lesion. Grafted SCs survived well in the scaffolds, improved the host/graft continuity, and sustained axonal growth along the capillaries. Descending supraspinal and propriospinal axons traced by biotinylated dextran amine (BDA) and immunolabeled for serotonin extended throughout the scaffolds. The number of regenerated axons significantly increased when caudal BDNF was turned on. Ongoing experiments examine longer time points in vivo and more distal virus injections to further extend the gradient of AAV-mediated BDNF expression.

Disclosures: S. Liu: None. **S. Beatrice:** None. **R. Müller:** None. **R. Puttagunta:** None. **N. Weidner:** None. **A. Blesch:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: DFG BL 414/3-1

Title: Surface modification and cell seeding into capillary alginate hydrogels promote axonal regrowth in the acutely injured spinal cord

Authors: *T. SCHACKEL[1], M. GÜNTHER[2], S. LIU[2], B. SANDNER[2], M. MOTSCH[2], R. MÜLLER[3], R. PUTTAGUNTA[2], N. WEIDNER[2], A. BLESCH[4];
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Abstract: Axonal bridging across an extended lesion site remains a major challenge after spinal cord injury (SCI). We have previously shown that axons can be guided in rostrocaudal direction by the implantation of alginate-based hydrogels into a spinal lesion cavity allowing axons to bridge over 2 mm. To further enhance axonal growth into scaffolds, we examined the stability and biological effects of hydrogel surface modification with poly-L-ornithine (PLO) and laminin (Lam) or surface modification combined with cell seeding into hydrogel channels. PLO and Lam peptides electrostatically bound to the hydrogel surface remained stable and biologically active *in vitro* at 37°C for at a minimum of 2 weeks including repeated washes. Surface modification significantly increased cell attachment/survival of bone marrow stromal cells (BMSCs) and postnatal cortical astrocytes, and promoted DRG neurite outgrowth compared to controls. For *in vivo* studies, adult female *Fischer-344* rats underwent lateral C5 hemisection, followed by acute implantation of hydrogels (2 x 2 x 1.3 mm). Four weeks after SCI, uncoated control scaffolds were only partially filled with host cells. In contrast, surface-modified hydrogels showed a significantly higher number of infiltrating cells ($p<0.05$). Host cells colonized the hydrogel channels and covered large portions of capillary walls. Infiltrated host cells were identified primarily as Schwann cells and macrophages, whereas astrocytes were absent in the scaffolds and only found in the periphery of the lesion site. Anterograde tracing of propriospinal as well as other descending tracts with BDA revealed ingrowth of axon bundles into the microchannels. Immunolabeling with β-tubulin showed re-growing axons both at the peripheral and central regions of the scaffold. Surface modification resulted in a 1.5-fold increase of axons at the hydrogel entry sites ($p<0.05$). The effect of additional astrocyte seeding (2 µL; 100,000 cells/µL) into hydrogel microchannels prior to transplantation is subject of ongoing experiments.

Disclosures: T. Schackel: None. **M. Günther:** None. **S. Liu:** None. **B. Sandner:** None. **M. Motsch:** None. **R. Müller:** None. **R. Puttagunta:** None. **N. Weidner:** None. **A. Blesch:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Support: Kentucky Spinal and Head Injury Research Trust (Grant # 14-5)

Paralyzed Veterans of America (Grant #3042)

NIH/NCRR GM103507

Title: Vasopressin and polyuria after acute spinal cord injury

Authors: *L. R. MONTGOMERY, C. HUBSCHER;
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Abstract: Spinal cord injury (SCI) often leads to bladder dysfunction which severely impacts patient morbidity and quality of life. While improvements in bladder function is a top priority for SCI individuals, research in this important area is very limited. Polyuria (the increased production of and/or passage of urine) is often seen following SCI and leads to more frequent bladder catheterizations and disruptions in sleep and daily activities. The mechanisms that underlie the development and continuation of polyuria after SCI are unknown. One of the major hormones responsible for the regulation of water balance is vasopressin. When vasopressin levels are decreased there is a concomitant increase urine production in response. Although vasopressin is a major regulator of urine output and urine output increases after SCI, data is currently scarce with regard to the impact that SCI may have on vasopressin levels. A cohort of adult male Wistar rats was given a moderate-severe T8 spinal contusion (215 kdyn), and found to have a significantly increased urine output over 24 hours at two weeks post injury compared to preinjury baseline levels. Urinalysis revealed that the specific gravity and creatinine levels were significantly decreased at two weeks post-SCI, indicating that the urine was less concentrated. At this same time point, basal levels of plasma vasopressin also decreased significantly. Despite this decrease in vasopressin levels, blood osmolarity (the strongest stimulus that controls circulating vasopressin levels) did not change. To determine the mechanisms by which SCI could influence vasopressin levels, corticosterone levels were also measured at this time point. An increase in corticosterone is known to suppress vasopressin release from the hypothalamus. At two weeks following SCI, corticosterone levels increased significantly. Thus it appears that several factors contribute to the development of polyuria after SCI. Having identified that vasopressin levels decrease after SCI and a mechanism that can lead to this decrease, we can now use these targets to develop interventions that may decrease polyuria within the SCI community and thereby improve the quality of life for SCI individuals.

Disclosures: L.R. Montgomery: None. **C. Hubscher:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Leona M. & Harry B. Helmsley Charitable Trust

Craig H. Nielsen Foundation

Title: Cardiovascular regulation post-epidural stimulation in cervical spinal cord injury

Authors: *B. DITTERLINE[1,2], S. WANG[1], S. ASLAN[1], S. HARKEMA[1];
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Abstract: Background. Persons with SCI often have poor blood pressure (BP) regulation, which decreases quality of life and increases morbidity and mortality when compared to non-injured persons. Increased morbidity results, in part, from impairment to the sympathetic nervous system, resulting in poor reflex response, persistent hypotension and bradycardia, and bouts of orthostatic hypotension. We observed previously that spinal-cord epidural stimulation (SC-ES) for motor rehabilitation resulted in improvements to BP and heart rate (HR). The goal of this study is thus to determine if SC-ES, alone and with activity-based interventions, can re-engage spinal reflexes that would potentially regulate blood pressure, leading to increased resting cardiovascular (CV) outcomes.
Methods. An orthostatic stress test was used to assess hemodynamics, autonomic activity, and baroreceptor sensitivity at rest and during orthostatic stress. Prior to implantation, a 24-year old male, C5 AIS-A, was assessed of CV outcomes and demonstrated an overall impairment to CV regulation, including decreased sympathetic nervous system activity, poor reflex regulation, and orthostatic hypotension. The first intervention, cardiovascular training with epidural stimulation (CV-ES), utilizes SC-ES to increase resting blood pressure to a "normal" range. The second intervention was voluntary-motor training with epidural stimulation (Vol-ES) concurrent with CV-ES. An orthostatic stress test was performed after 80 sessions of CV-ES, and after 80 sessions of combined Vol-ES and CV-ES.
Results. We found improvements to his cardiovascular regulation following CV-ES: orthostatic hypotension that occurred during the pre-intervention assessments (81 ± 6/48 ± 2 mmHg) was ameliorated (120 ± 2/72 ± 5 mmHg); there was increased sympathetic (123±93 ms^2 to 999±918 ms^2) and respiratory-mediated oscillations of HR (56±52 ms^2 to 778±755ms^2) while seated; and an increased baroreceptor effectiveness (up-up: 26 ± 19 % to 60 ± 18%) and sensitivity (up-up: 3±1 to 19±10 ms/mmHg; down-down: 3±1 to 20±12 ms/mmHg) while seated. Additionally, the increases to cardiovascular function were sustained with the addition of Vol-ES: no orthostatic hypotension occurred (120/74 ± 14/16 mmHg), cardiac autonomic activity remained high (LF

power: 1638 ± 2037 ms^2; HF 583 ± 782 ms^2), as did baroreceptor effectiveness (up-up: 42± 14%; down-down: 68±31%).
Conclusions. Epidural stimulation has the potential to improve blood pressure regulation in persons with SCI, which may improve their quality of life and lead to a decrease the morbidity and mortality risk.

Disclosures: B. Ditterline: None. **S. Wang:** None. **S. Aslan:** None. **S. Harkema:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Kentucky Spinal Cord Injury Research Center

University of Louisville Foundation

Title: Activity-based training with spinal cord epidural stimulation promoted the recovery of lower limb motor function independent from spinal stimulation in a chronic motor complete paraplegic

Authors: *E. REJC[1], C. ANGELI[2], D. ATKINSON[1], S. HARKEMA[1];
[1]Neurolog. surgery, Univ. of Louisville, Louisville, KY; [2]Frazier Rehab Institute, Kentucky One Hlth., Louisville, KY

Abstract: In the last lustrum, studies have shown that the combination of spinal cord epidural stimulation (scES) and activity-based training progressively re-enabled standing with balance assistance and volitional control of lower limbs in individuals with chronic motor complete spinal cord injury. However, the presence of scES was always required to perform these motor tasks. In the present study, we show that a chronic (6.8 years post injury) motor complete

individual regained significant lower limb motor function without scES after completing 31 months of activity-based training with epidural stimulation, which included standing, stepping and volitional lower limb movements from supine position. In particular, the participant regained the ability to stand on both legs as well as on one leg without scES, bearing full body weight, while using upper limbs to assist balance. Electromyographic (EMG) activity was properly modulated in a task specific manner. The transition from sitting to standing was accompanied by increased EMG activity of knee extensors and plantar-flexors. Also, during one-leg standing, EMG activity of the unloaded leg muscles was negligible, while the knee extensors of the loaded leg were more active than during bilateral standing. In addition, the research participant recovered the ability to volitionally perform isolated hip flexion and knee extension movements from a supine position without scES. Interestingly, this individual was able to volitionally induce a general co-activation of the lower limb muscles from a supine position without scES as early as 5 months after the beginning of training with scES. However, this achievement did not coincide with any improvement in the generation of motor patterns effective for over-ground standing without scES. In conclusion, we demonstrated that an individual with chronic motor complete spinal cord injury recovered the capability to modulate the excitability of spinal networks to physiological states sufficient for generating: *i)* lower limb antigravity extension patterns allowing independent standing and *ii)* volitional movements performed by proximal lower limb muscles. It can be surmised that prolonged activity-based training with scES promoted the strengthening of supraspinal connectivity to the spinal motor circuitry below the level of injury resulting in descending motor inputs sufficient for enabling lower limb motor control in the absence of scES.

Disclosures: **E. Rejc:** None. **C. Angeli:** None. **D. Atkinson:** None. **S. Harkema:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Craig H. Neilsen Foundation

Title: Targeting improvements in bladder function with epidural stimulation after human spinal cord injury

Authors: *C. HUBSCHER[1], A. HERRITY[2], L. MONTGOMERY[1], A. WILLHITE[2], C. ANGELI[2], S. HARKEMA[2];
[1]Dept Anatom. Sci. & Neuro, Univ. Louisville Sch. Med., Louisville, KY; [2]KSCIRC, Frazier Rehab Inst., Louisville, KY

Abstract: Deficits in urological function after spinal cord injury (SCI) include neurogenic detrusor overactivity and uncoordinated bladder and external urethral sphincter contractions, resulting in inefficient emptying and high intravesical pressure. Urinary retention and an inability of the bladder to store urine under appropriately low pressures can lead to infection and ultimately impact renal health. Current therapeutic approaches aim to manage both the storage and voiding phases of bladder function and include intermittent catheterization, pharmacologic and surgical interventions, as well as urethral stents. While most of these strategies are necessary for urological maintenance post-injury they oftentimes are associated with dose-limiting side effects and therefore remain inadequate. Neuromodulation has also been implemented in various formats as a promising alternative treatment for neurogenic bladder in an effort to regain control of function after SCI. Thus, this study investigated bladder outcomes in AIS grade A and B subjects receiving spinal cord epidural stimulation (scES) at L1-S1 spinal levels in combination with locomotor and/or stand training by our research team at the University of Louisville Human Locomotion Research Center. Urodynamic assessments were performed at pre- and post-training time-points and cystometrograms were captured with and without the use of scES. In addition, specific configurations and parameters optimal for continence and micturition were identified in several subjects during filling cystometry. We found that while locomotor training resulted in improvements in bladder capacity and voiding efficiency, the use of scES further enhanced these parameters and in a frequency-dependent manner. Importantly, as capacity increased, bladder pressures continued to remain low, indicating better compliance. Overall, scES may help contribute to an improvement in quality of life by providing a means of extending the time to catheterization under safe pressures and restoring efficient bladder emptying, ultimately preserving lower and upper urinary tract health.

Disclosures: C. Hubscher: None. **A. Herrity:** None. **L. Montgomery:** None. **A. Willhite:** None. **C. Angeli:** None. **S. Harkema:** None.

Poster

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Leona M. and Harry B. Helmsley Charitable Trust

Kentucky Spinal Cord Injury Research Center

University of Louisville Foundation

Title: Lumbosacral spinal cord epidural stimulation enables step like patterns during BWST stepping in motor complete paraplegics

Authors: *C. A. ANGELI[1,2], S. HARKEMA[2,1];
[1]Frazier Rehab Inst., Louisville, KY; [2]Univ. of Louisville, Louisville, KY

Abstract: Epidural stimulation of lumbosacral spinal cord, combined with activity based training, enabled four motor complete paraplegics to progressively regain full weight bearing standing and achieve voluntary movement of their lower extremities. The aim of this study was to investigate the effects of different stimulation parameters on EMG patterns during stepping. Four participants with a motor complete injury implanted with an epidural electrode array over the L1-S1 segments of the spinal cord participated in this study. EMG, kinematics and ground reaction forces were recorded during stepping on a treadmill with body weight support. In this study we showed that three out of four individuals with a clinically motor complete spinal cord injury had the capacity to voluntarily modulate the motor output during stepping when the lumbosacral spinal cord was stimulated with individual-specific parameters optimal for stepping. Modulation of EMG activity was also shown during the stand to step transition highlighting the role of sensory information and the capacity of the spinal cord to interpret this information. We assessed the individual specificity of stimulation parameters by testing the stimulation configurations optimal for stepping across participants. In all cases, EMG was modulated differently when an individual was stimulated using parameters specific for other participants. Stimulation parameters selectively modulated the lumbosacral neural networks during stepping. EMG patterns observed during stepping are dependent on stimulation strength, frequency and electrode selection. These results have important implications with respect to: 1) how lumbosacral neural networks can be selectively modulated by varying the epidural stimulation parameters, and 2) identifying strategies that are likely to be most efficacious in enabling improved motor function for stepping after motor complete paralysis.

Disclosures: C.A. Angeli: None. **S. Harkema:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS091723

Craig H. Neilsen Foundation

Title: Acute pain after SCI exacerbates progressive hemorrhagic necrosis

Authors: *M. K. BRUMLEY, J. D. TURTLE, J. M. FORSBERG, J. W. GRAU;
Texas A&M Univ., College Station, TX

Abstract: Noxious input after SCI exacerbates progressive hemorrhagic necrosis
Vascular damage is a major factor in the pathogenesis of traumatic spinal cord injury (SCI). Progressive hemorrhagic necrosis (PHN), a devastating phenomenon that occurs soon after SCI, is mediated through the activation of sulfonylurea receptor 1-transient receptor potential melastatin 4 (Sur1-Trpm4) channel. PHN is characterized by capillary fragmentation and petechial hemorrhaging at the lesion site, and contributes to tissue loss and glial scarring after SCI. We have previously shown that peripheral noxious stimulation administered soon after SCI induces a recovery deficit. Further, noxious input increases the amount of red blood cells at the lesion site, which may indicate significant vascular damage. The present study assessed the impact of nociceptive stimulation on the development of PHN following SCI. Hemorrhage, capillary segmentation, and SUR1-Trpm4 upregulation were all assessed as markers for the development of PHN. To assess hemorrhage, adult rats received a laminectomy and a moderate contusion injury at the T12 vertebra. After a 24-hour recovery period, half of the subjects received noxious input in the form of six minutes of electrical stimulation applied to the tail. At 3 hours post-treatment, subjects were sacrificed and a 1-cm section of lesion tissue was collected, sectioned, and stained with hematoxylin and eosin. The amount of hemorrhage was determined by a blinded observer as a percentage of the total section area. Subjects that received shock had over twice as much hemorrhage at around the lesion site compared to subjects that received SCI alone. To determine the effect of noxious input on the expression of the SUR1-Trpm4 channel, we used co-immunoprecipitation to pull down SUR1 and western blotting to identify Trpm4. Noxious input resulted in the upregulation of SUR1-Trpm4 channel. Additionally, in a subset of subjects, tissue was stained with FITC-tomato lectin to visualize lesion-site vasculature. Ongoing studies are examining the degree of capillary fragmentation at the lesion site. Based on these findings, acute pain appears to induce PHN after contusive SCI.

Disclosures: **M.K. Brumley:** None. **J.D. Turtle:** None. **J.M. Forsberg:** None. **J.W. Grau:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: NIH (NS091723)

Craig H. Neilsen Foundation

Title: Spared fibers promote the development of secondary spinal injury in response to acute pain

Authors: ***J. A. REYNOLDS**, J. D. TURTLE, Y.-J. HUANG, M. M. STRAIN, J. W. GRAU; Texas A&M Inst. for Neurosci., Texas A&M Univ., Bryan, TX

Abstract: Events soon after spinal cord trauma alter spinal cord function and drastically impact functional outcomes. Guided by our work in a transection model of spinal learning, we hypothesized that uncontrollable noxious input (C fiber activation) undermines spinal function by engaging a pro-inflammatory state (central sensitization). In a clinically relevant model of spinal cord injury (SCI), C fiber input exacerbates inflammatory processes within the lesion, increases cell death, fosters the development of progressive hemorrhagic necrosis (M. Brumley, SfN, 2016), and impairs locomotor recovery (Grau, 2004, *J Neurotrauma* 21: 1795). The present study examined how spared fibers impact the development of hemorrhage and cell death at the lesion site, and the degree to which noxious stimulation affects tissue outside of the injured region. Adult male Sprague-Dawley rats received a spinal contusion injury at the T12 level. 18 hours following injury, locomotor function was assessed using the BBB scoring method. Half the subjects received a spinal transection at T2. Six hours following secondary surgery, half the subjects in each condition received electrical stimulation of the tail at an intensity that engages C fibers. Protein extracts of the spinal cord segments were analyzed with immunoblotting, targeting the pro-inflammatory cytokines IL-18 and IL-1β. Replicating ongoing studies, noxious stimulation increased cytokine expression and hemorrhage in rats that received a contusion injury alone. Spinal transection prior to stimulation blocked the detrimental effects of C fiber input. Ongoing studies are exploring the regional distribution of these effects. Our results show that spared descending fibers enhance the adverse effect that noxious stimulation has on spinal injury.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Craig H. Neilsen Foundation

NIH (NS091723)

Title: Spinal block with lidocaine: An effective treatment for reducing secondary injury after SCI and noxious stimulation

Authors: *J. TURTLE, M. M. STRAIN, Y.-J. HUANG, J. A. REYNOLDS, M. K. BRUMLEY, J. W. GRAU;
Texas A&M Univ., Bryan, TX

Abstract: Over 90% of spinal cord injuries (SCI) are caused by trauma and are often associated with peripheral tissue damage and pain. Our lab has shown that nociceptive stimulation increases lesion volume, induces neuropathic pain, and undermines functional outcomes in a rat model of SCI (Grau, 2004, *J Neurotrauma 21*: 1795). The current standard of care for the treatment of acute pain after trauma is poorly defined and opiates are commonly prescribed due to their ease of use and commonality. Unfortunately, treatment of nociceptive stimulation after SCI with morphine does not prevent the detrimental effects of stimulation (Hook, 2007, *Beh Brain Res, 2*: 281) and morphine treatment per se impairs outcomes after SCI (Hook, 2009, *J Neurotrauma 26*: 741). Here, we examined a new therapeutic strategy for the treatment of acute pain after SCI. In the first experiment, we examined the impact of epidural lidocaine on molecular indices of inflammation, cell death, and hemorrhage. Subjects received a moderate contusion injury to the lower thoracic cord. Twenty-four hours later, locomotor function was assessed and subjects were randomly assigned to one of four groups (lidocaine or vehicle crossed with shocked or unshocked). Subjects received a lidocaine or vehicle injection under isoflurane anesthesia. Thirty minutes later, shocked subjects were restrained in Plexiglas tubes and received six minutes of intermittent uncontrollable electrical stimulation to the tail. Controls were restrained, but did not receive stimulation. Three hours later, one centimeter of tissue from the lesion was collected and analyzed. Lidocaine treatment blocked stimulation-induced increases in TNF, IL-1β, IL-18, caspase 3, and hemorrhage. We then examined whether epidural lidocaine would also block the

effects of stimulation on behavioral recovery. Subjects were treated identically, but locomotor function and weight were assessed for a six-week recovery period. At the completion of the experiment, locomotor function (beam and ladder) and pain (mechanical reactivity, tail flick, girdle) were further examined. As was seen in the prior experiment, epidural lidocaine blocked all deficits induced by nociceptive stimulation. These results demonstrate that noxious stimulation adversely affects recovery after SCI and show that this effect is blocked by epidural lidocaine. This treatment could be readily translated to clinical use.

Disclosures: J. Turtle: None. **M.M. Strain:** None. **Y. Huang:** None. **J.A. Reynolds:** None. **M.K. Brumley:** None. **J.W. Grau:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Supported by Ray W. Poppleton Endowment (PGP)

Title: CX3CR1-deficient microglia and macrophages enhance endogenous repair, axon sprouting and synaptogenesis after spinal cord injury in mice.

Authors: *C. M. FRERIA, J. C. HALL, P. WEI, D. M. MCTIGUE, P. G. POPOVICH; Dept. of Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Impaired signaling via CX3CR1, the fractalkine receptor, promotes recovery after traumatic spinal contusion injury in mice, a benefit achieved by reducing macrophage-mediated injury at the lesion epicenter. Here, we tested the hypothesis that CX3CR1-dependent changes in microglia and macrophage function will enhance neuroplasticity. Eighty-five male and female $CX3CR1^{+/+}$, $CX3CR1^{-/-}$, $CX3CR1^{+/-}$ mice were anesthetized and subjected to moderate SCI (75kdyn, IH device). After 4, 14, 28 or 56days, mice were euthanized and their spinal cords processed for immunohistochemistry, Golgi-Cox staining and transmission electron microscopy. *In vitro* assays were used to evaluate microglia/macrophage-mediated neuron pathology. RT-PCR was used for analysis of macrophage phenotype. New data indicate that in the presence of inflammatory stimuli, CX3CR1-deficient microglia and macrophages adopt a reparative phenotype and increase expression of genes that encode neurotrophic and gliogenic proteins. At the lesion epicenter (T8 spinal level), the microenvironment created by $CX3CR1^{-/-}$ microglia augments NG2 cell responses and increases axon sparing and sprouting of serotonergic axons. Several segments below the lesion, in lumbar spinal cord, reduced inflammatory signaling in

$CX3CR1^{-/-}$ microglia is associated with reduced dendritic pathology as well as improved axonal and synaptic plasticity on ventral horn motor neurons. Together, these data indicate that CX3CR1, a microglia-specific chemokine receptor, is a novel therapeutic target for enhancing neuroplasticity and recovery after SCI. Genetic deletion of CX3CR1 allows microglia and macrophages to remain responsive to inflammatory signaling but with a reduced capacity for neuronal killing. Moreover, loss of CX3CR1 signaling improves the ability of microglia to repair or remodel the spinal cord. Whether these microglia effects on axons and synapses are direct or indirect is unknown; however, these data have significant implications for recovery after SCI. Indeed, genetic polymorphisms of CX3CR1 or immune modulatory interventions that specifically target microglia could enhance endogenous repair and spontaneous recovery and also could provide a neural substrate that is more responsive to rehabilitation.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Title: Proliferating $NG2^{+}$ cells are required for glial and fibrotic scar formation and maintenance of tissue integrity after spinal cord injury in mice

Authors: *Z. C. HESP[1], R. SUZUKI[2], A. NISHIYAMA[2], D. M. MCTIGUE[1];
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Abstract: Spinal cord injury (SCI) is characterized by an initial wave of cell death, followed by significant cell proliferation, gliosis, and the formation of astrocytic and fibrotic scars, the latter of which are critical in limiting lesion expansion. Our prior work and that of others reported dense NG2 accumulation around and within the lesion acutely after SCI, as well as chronically increased NG2 in the surrounding spared tissue. The significant increase in NG2 after SCI can

stem from two endogenous cell populations: $NG2^+$ glia and $NG2^+$ immature pericytes, both of which proliferate in response to injury. Considering the widespread increase in NG2 after SCI, particularly around/within the glial scar, we asked the question: What is the contribution of proliferating $NG2^+$ cells to scar formation and tissue repair after traumatic CNS injury? Several studies have used NG2-null mice or NG2-neutralizing antibodies in SCI paradigms, but these models only impair the expression of the NG2 molecule without killing cells. To answer this question, we used a thymidine kinase/ganciclovir (Tk/GCV) paradigm in a novel mouse line (*NG2-Tk* mice) to selectively ablate proliferating $NG2^+$ cells in the first 1-2wpi after SCI. In this model $NG2^+$ cells express HSV1 Tk which converts GCV into a cytotoxic triphosphate that is incorporated into the DNA of dividing cells, causing cell cycle arrest and apoptosis. *NG2-Tk* and wildtype mice received a moderate C5 hemicontusion injury followed by subcutaneous implantation of a minipump filled with GCV or saline whose guide cannula was inserted into the right lateral ventricle for continuous infusion directly into the CSF. Two cohorts of mice received GCV for 7 and 11 days post-SCI before sacrifice, and a third cohort received GCV or saline until 14dpi, at which point pumps were removed and mice survived until 21dpi. Tissue was processed for IHC, and spinal cords were labeled for neurofilament, NG2, GFAP, Mac1, PDGFRβ, and laminin. Overall, the data show that loss of proliferating $NG2^+$ cells over the first 7-11dpi caused significant edema, prolonged hemorrhage, and lesion expansion. It also caused pronounced disruption of glial scar formation and near complete abolishment of the fibrotic scar and intra-lesion ECM deposition, which began to show signs of recovery only after a week of GCV removal. An apparent benefit of breakdown of the glial and/or fibrotic scar was enhanced neurite growth into the lesion core by 21d in *NG2-Tk* mice. These data reveal novel ways in which proliferating $NG2^+$ cells play a fundamental role in tissue protection and repair following SCI through promotion of vascular integrity, glial scar compaction, and ECM deposition.

Disclosures: Z.C. Hesp: None. **R. Suzuki:** None. **A. Nishiyama:** None. **D.M. McTigue:** None.

Poster

323. Spinal Cord Injury Models and Mechanisms

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 323.29/BB7

Topic: C.09. Brain Injury and Trauma

Support: Craig H. Neilsen Foundation

The Ray W. Poppleton Endowment (PGP)

NIH NINDS

Title: Gut dysbiosis impairs recovery after spinal cord injury

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Abstract: The trillions of microbes that exist in the gastrointestinal tract (microbiome) have emerged as pivotal regulators of normal mammalian development and physiology. Disrupting the microbiome, i.e. dysbiosis, can cause or contribute to neurologic disease, but whether dysbiosis affects recovery or lesion pathology after spinal cord injury (SCI) is unknown. New data in this report show that traumatic SCI increases intestinal permeability and bacterial translocation from the gut. These changes are associated with activation of immune cells in gut-associated lymph tissues (GALT). Bacterial 16S ribosomal RNA (rRNA) gene sequencing data reveal SCI-dependent changes in the composition of gut microbiota. These changes are initiated during the first week post-injury and continue throughout the first month. To test the whether this post-injury dysbiosis affects recovery and pathology after SCI, we completed a "gain-of-function" study in which intestinal dysbiosis was induced prior to SCI. This was accomplished using a cocktail of broad-spectrum antibiotics. Experimental induction of dysbiosis before SCI impaired functional recovery and exacerbateed spinal cord pathology. Conversely, when the microbiota was protected from the effects of SCI using a commercial probiotic (VSL#3), we noted significant neuroprotection and improved functional recovery with evidence of a protective immune response in GALT. VSL#3-mediated protection was associated with significant changes in the composition of the gut microbiota (as shown by 16 rRNA gene sequencing). These novel data indicate that the gut microbiome play a pivotal but previously unappreciated role in regulating recovery of neurologic function and neuropathology after SCI.

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Poster

323. Spinal Cord Injury Models and Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Missouri State Spinal Cord Injury Research Fund

NIH PREP Grant for University of Missouri Columbia

Title: Spinal cord injury-induced pain and motor deficits in rats

Authors: *T. NDAM[1,2], J. CUI[1,2], Z. QU[1,2], M. BEKEMEIER[4,2], D. K. MILLER[4,2], A. SIMONYI[3,2], W. R. FOLK[3], G. Y. SUN[3,2], Z. GU[1,2];
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Abstract: Spinal cord injury (SCI) affects more than one million people in the United States and leads to varying degrees of sensory abnormality including both musculoskeletal and neuropathic chronic pain as well as motor deficits. SCI-induced chronic pain is the most common symptom in up to 70 % of SCI patients and has a major impact on their quality of life. SCI leads to marked changes in the synaptic circuits in the dorsal horn cells and inflammation of the spinal cord caused by activation of microglial cells through the release of inflammatory factors. Most rodent models of SCI are limited to reduced motor functions and sensory deficits, and because of variations in impact of the unilateral contusion/paralysis often occurs, and thus at-level or below-level neuropathic pain can be poorly controlled. To be able to control the severity of injury and pain after SCI, we have developed a contusion SCI model in rats where the lamina and spinous process at T10 were removed and replaced with a plastic chip. Graded injuries were induced through an electromagnetic impactor with various velocities at a depth of 2 or 3 mm. Assessments of both motor and sensory behaviors were conducted before and after SCI and in sham control rats via measures of locomotor function, mechanical allodynia, thermal hyperalgesia and anxiety-like behaviors. Rats were sacrificed 22 days after SCI and spinal cords were evaluated for tissue damage using H&E staining. SCI rats exhibited impaired motor function on hind limbs, although some recovery was observed across 22-day period. These rats showed an increased hyper-sensitivity to thermal and mechanical stimuli, relative to sham control rats. H&E staining showed mild damage of the spinal cord with gliosis infiltration. These findings demonstrate graded contusion SCI induces hyper-sensitivity to thermal and mechanical stimuli as a reproducible chronic pain model in rats. Our study with this model may lead to a better understanding of the underlying mechanisms of inflammation in musculoskeletal and/or neuropathic pain after SCI, as well as development of biomarkers and treatment for patients with SCI.

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Poster

324. Peripheral Nerve Injury

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 324.01/BB9

Topic: C.09. Brain Injury and Trauma

Title: Anti allodynic effects of medicinal herbs in a rat model of oxaliplatin induced neuropathic pain through the suppression of spinal glial activation

Authors: *J. LEE[1,3], W. KIM[1], H. YOON[3], H. BAE[2], S. KIM[2];
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Abstract: Activation of spinal glial cells, such as microglia and astrocytes, and increase of pro-inflammatory cytokines levels in the spinal cord play a crucial role in the pathogenesis of neuropathic pain. A single injection of oxaliplatin (6 mg/kg, i.p.), a widely used anticancer drug against metastatic colorectal cancer, can induce acute peripheral neuropathy. Gyejigachulbu-tang (GBT), herbal formula consisting of *Cinnamomi Cortex, Aconiti Tuber* and other medicinal herbs, has been used in East Asia to treat various pain symptoms. This study investigated whether and how GBT, *Cinnamomi Cortex*, or *Aconiti Tuber* alleviates oxaliplatin-induced cold and mechanical allodynia in Sprague Dawley rats. The behavioral signs of cold and mechanical allodynia were evaluated by a tail immersion test in cold water (4 ℃) and a von Frey hair test, respectively. Significant pain behaviors were observed three days after an oxaliplatin injection. GBT (400 mg/kg), *Cinnamomi Cortex* (200 mg/kg) or *Aconiti Tuber* (200 mg/kg) was orally administrated for five consecutive days after oxaliplatin injection. Behavioral studies reveal that GBT, *Cinnamomi Cortex* and *Aconiti Tuber* have potent relieving effects against oxaliplatin-induced cold and mechanical allodynia by increasing the tail withdrawal latency to cold stimuli and mechanical threshold. Immunohistochemistry studies show that both GBT and *Cinnamomi Cortex* suppress activation of spinal microglia and astrocytes, whereas *Aconiti Tuber* suppresses only activation of spinal astrocytes. Increased pro-inflammatory cytokines, interleukin-1 β and tumor necrosis factor, after oxaliplatin injection were decreased by GBT, *Cinnamomi Cortex* and *Aconiti Tuber*. In summary, GBT, *Cinnamomi Cortex* and *Aconiti Tuber* have potent anti-allodynic effect on oxaliplatin-induced neuropathic pain via attenuating activation of spinal glia and release of pro-inflammatory cytokines. This study suggests that GBT could be an alternative therapeutic agent on oxaliplatin-induced neuropathy, and that *Cinnamomi Cortex* and *Aconiti Tuber* may play a major role in this efficacy of GBT.

Disclosures: J. Lee: None. **W. Kim:** None. **H. Yoon:** None. **H. Bae:** None. **S. Kim:** None.

Poster

324. Peripheral Nerve Injury

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Topic: C.09. Brain Injury and Trauma

Support: Texas Biomedical Device Center

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Title: Vagus nerve stimulation paired with rehabilitation improves functional recovery following peripheral nerve injury.

Authors: *E. MEYERS, R. GRANJA, R. SOLORZANO, G. BENDALE, P. GANZER, N. ROBERTSON, K. ADCOCK, M. ROMERO-ORTEGA, M. KILGARD, R. RENNAKER, S. HAYS;
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Abstract: Peripheral nerve injuries affect an estimated 20 million Americans and frequently result in long-term loss of motor control in the upper extremities. Patients often undergo extensive physical rehabilitation but most fail to achieve substantial recovery of function, highlighting the need for improved rehabilitative interventions. Reorganization of neural circuitry, or neuroplasticity, throughout the central nervous system is believed to be largely responsible for recovery. Previous work from our lab has shown that pairing vagus nerve stimulation (VNS) with a motor movement drives substantial neuroplasticity observed by an increase in the cortical representation of the paired movement. Additionally, we have shown that pairing VNS with rehabilitation improves motor function in rat models of ischemic stroke, hemorrhagic stroke, traumatic brain injury and spinal cord injury. We hypothesized that pairing VNS with rehabilitation would improve functional recovery following peripheral nerve injury. Adult female Sprague-Dawley rats were trained on the isometric pull task, an automated, quantitative measure of forelimb strength. Upon reaching task proficiency, peripheral nerve injuries of the median and ulnar nerves in the right forelimb were performed. Following 6 weeks of homecage recovery, animals were randomly assigned to either the rehabilitation only group (Rehab), or the group receiving vagus nerve stimulation paired with rehabilitation (VNS+Rehab). Animals underwent rehabilitative training for an additional 7 weeks and the VNS+Rehab group received VNS paired with successful pull attempts. At the conclusion of behavioral testing, intracortical microstimulation (ICMS) of the motor cortex was performed in all animals, and Von Frey sensory testing of the forepaws.
VNS paired with rehabilitative training significantly improved recovery of forelimb function after PNI compared to rehabilitative training without VNS. ICMS motor maps revealed a significantly greater cortical area that evoked forelimb grasp responses in the VNS+Rehab group

compared to the Rehab group. Von Frey testing revealed decreased withdrawal response thresholds to a mechanical stimulation in the VNS+Rehab group, suggesting partial recovery of mechanical sensation.
Peripheral nerve injuries remain a serious condition, and injuries to the forearm and hand often lead to severe and life-long disabilities. Current treatment options in the chronic phase of injury are limited. Here we show that vagus nerve stimulation delivered as an adjunct to rehabilitative training has the capacity to promote functional recovery in a rat model of peripheral nerve injury.

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Poster

324. Peripheral Nerve Injury

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Title: The removal of proprioceptive IA afferent synapses from motoneurons after nerve injury occurs through a mechanism dependent on chemokine receptor CCR2

Authors: *T. M. ROTTERMAN, F. J. ALVAREZ;
Physiol., Emory Univ., Atlanta, GA

Abstract: Peripheral nerve injury results in axotomy of motor and sensory axons as well as a central immune response in which microglial cells become activated in the spinal cord ventral horn, migrate towards axotomized motoneurons and release a host of pro-inflammatory mediators. This inflammatory reaction results in a leaky blood-spinal cord barrier, allowing blood-born monocytes to infiltrate in a CCR2-dependent manner. These cells can then differentiate into phagocytic macrophages inside the spinal cord. In parallel, the central axons and synapses of axotomized proprioceptive IA afferents are permanently removed from the ventral horn causing the withdrawal of their monosynaptic connections with motoneurons (MNs) and ultimately the loss of the stretch reflex. To test the hypothesis that CCR2+ macrophages could be related to the degradation of central IA afferent collaterals and synapses we used

heterozygous “knock-in” CCR2 (RFP/+) and “knockout” (KO) homozygous CCR2 (RFP/RFP) transgenic C57Blk/6 mice to label infiltrating peripheral monocytes (RFP) and delete the receptor necessary for their recruitment. In these mice we retrogradely labeled lateral gastrocnemius MNs with Fast Blue, followed by a second surgery in which the sciatic nerve was transected and immediately repaired with fibrin glue. Mice survived 2 months following the injury, to allow peripheral axon regeneration and muscle reinnervation, at which point they were perfusion-fixed and the lumbar spinal cord sectioned and immuno-labeled for the vesicular glutamate 1 transporter (VGLUT1), a marker of IA afferent synapses. Fast Blue labeled MNs were confocal imaged and reconstructed in 3D using Neurolucida software to map their VGLUT1+ synapses on MN somas and proximal dendrites in heterozygous and KO mice. We found that sham control CCR2 het mice had a VGLUT1 somatic density of 0.74±0.11 (standard deviation) per 100 μm^2 and a linear dendritic density of 17.96±1.51 per 100 μm. Two months after injury CCR2 hets showed a 64% decrease in synaptic contact density around the soma (0.27±0.11) and a 40% decrease on the proximal dendrites (10.75±1.72). In contrast, in CCR2 KO mice, VGLUT1 contact density on the soma was partially preserved (0.43±0.06) and completely preserved on the dendritic arbor (16.41±0.92). Since the majority of IA afferent synapses are located on MN dendrites, these data suggests that deleting CCR2 preserves the majority of the IA afferent input that would be lost on MNs after nerve injuries.

Disclosures: T.M. Rotterman: None. **F.J. Alvarez:** None.

Poster

324. Peripheral Nerve Injury

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Topic: C.09. Brain Injury and Trauma

Support: ENDEAVOUR Scholarship Scheme (Malta)

Title: Enhancing peripheral nerve regeneration through combined tissue engineering and gene therapy

Authors: *F. BUSUTTIL[1], M. P. HUGHES[1], K. S. BHANGRA[2], P. J. KINGHAM[3], J. B. PHILLIPS[2], A. A. RAHIM[1];
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Abstract: Peripheral nerve injury (PNI) affects up to 5% of trauma patients and is associated with motor, sensory and autonomic impairment in the affected area. Following PNI, axonal regeneration is dependent on Schwann cells, which express regeneration-assisting genes. However, this pro-regenerative phenotype is not always maintained long enough to ensure functional recovery. Tissue engineered three dimensional (3D) constructs containing therapeutic cells that maintain the appropriate phenotype may help enhance nerve regeneration. An *in vitro* proof-of-concept study was carried out to deliver luciferase and green fluorescent protein (GFP) genes to rat Schwann cells (SCL4.1/F7) and to assess whether the transduced cells were still viable for the construction of 3D collagen constructs that can be used to enhance nerve repair. The Schwann cells were successfully transduced using the bicistronic lentiviral vector produced and the highest transduction efficiency was obtained at a multiplicity of infection of 100. This was confirmed by fluorescence microscopy, bioluminescence imaging and flow cytometry. Following incorporation into the collagen constructs, stereoscopic fluorescence microscopy and confocal microscopy showed that the transduced cells had distributed evenly throughout the constructs. Bioluminescence imaging confirmed that the Schwann cells were still viable after seeding into the constructs. No fluorescence or bioluminescence was observed in the untransduced cells or in the control constructs seeded with the untransduced cells. The results from this study suggest that Schwann cells can be transduced effectively using lentiviral vectors and then used to engineer tissue engineered nerve repair constructs. The approach has also been applied successfully to clinically relevant sources of therapeutic cells. This study provides the basis for potentially delivering genes that either maintain the Schwann cells in their pro-regenerative phenotype or which increase vascularisation following implantation. Additionally, the luciferase gene in the lentiviral vectors provides a powerful means to track cell fate *in vivo* during preclinical testing.

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Poster

324. Peripheral Nerve Injury

Location: Halls B-H

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Program#/Poster#: 324.05/BB13

Topic: C.09. Brain Injury and Trauma

Title: Analgesic effects of bee venom and bee venom derived phospholipase A_2 in a mouse model of oxaliplatin-induced neuropathic pain

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Abstract: Oxaliplatin, a chemotherapeutic drug for colorectal cancer, induces peripheral neuropathy characterized by dysesthesias of the hands and feet, which is a major dose-limiting side effect. Even a single administration of oxaliplatin can evoke this abnormal sensation, aggravated by cold and mechanical stimuli, to about 90% of treated patients. Bee venom (BV) has been traditionally used in Korea to alleviate pain and a recent clinical trial has suggested that BV may be effective for chemotherapy induced peripheral neuropathy. Here, we investigated the anti-allodynic effect of BV and further examined whether a co-administration of BV with morphine could have an additive on oxaliplatin-induced neuropathic pain in mice, as BV is known to be mediated by spinal noradrenergic and serotonergic receptors, whereas morphine by opioidergic receptors. The cold and mechanical allodynia signs were evaluated by acetone and von Frey hair test on the hind paw, respectively. The most significant allodynia signs were observed at three days after an injection of oxaliplatin (6 mg/kg, i.p.). BV (0.25, 1, and 2.5 mg/kg, s.c.) or morphine (0.5, 2, and 5 mg/kg, i.p.) alone showed dose-dependent anti-allodynic effects. The combination of BV and morphine at intermediate doses showed a greater and longer effect than either BV or morphine alone at the highest dose. Intrathecal pretreatment with the opioidergic (naloxone, 20 μg) or 5-HT_3 (MDL-72222, 15 μg) receptor antagonist, but not with α2-adrenergic (idazoxan, 10 μg) receptor antagonist, blocked this additive effect. Furthermore, we assessed the preventive and curative effects of BV derived phospholipase A_2 ($bvPLA_2$) on oxaliplatin-induced neuropathic pain in mice. Daily treatment with $bvPLA_2$ (0.2 mg/kg, i.p.) for five consecutive days prior to the oxaliplatin injection markedly inhibited the development of cold and mechanical allodynia, and suppressed infiltration of macrophages and the increase of IL-1β level in the DRG. Such preventive effects of $bvPLA_2$ were completely blocked by depleting regulatory T cells with CD25 antibody pre-treatments. Moreover, daily administration of $bvPLA_2$ (0.2 mg/kg, i.p.), after an oxaliplatin injection, for five consecutive days markedly attenuated cold and mechanical allodynia, which was more potent than the effect of BV (1 mg/kg, i.p.). In sum, these results suggest that BV can effectively attenuate cold and mechanical allodynia induced by an oxaliplatin injection, and that morphine can reinforce this effect. Also, $bvPLA_2$ may play an important role in this analgesic effect of BV, as it has shown a preventive and curative effect on oxaliplatin-induced neuropathic pain.

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Poster

324. Peripheral Nerve Injury

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Topic: C.09. Brain Injury and Trauma

Support: NIH

Title: MCT1 in Schwann cells expedites regeneration of injured peripheral nerves and is necessary for maintenance of aging sensory axons

Authors: *M. K. JHA, K. RUSSELL, A. SINGH, Y. LEE, J. D. ROTHSTEIN, B. M. MORRISON;
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Abstract: The proper functioning and maintenance of peripheral nerves, especially regenerating nerves, requires high metabolic provisions and heavily depends on monocarboxylates, principally lactate, as an energy source. Monocarboxylate transporters (MCTs), as downstream mediators, govern cellular levels and functional consequences of monocarboxylates. MCT1 is the predominant lactate transporter in the peripheral nervous system and is expressed mostly in Schwann cells, nerve perineurium, and DRG neurons. By employing MCT1 heterozygote null mice, we previously reported that nerve regeneration, in both sensory and motor axons, depends on MCT1 function. These findings led us to investigate the potential contribution of Schwann cell-specific MCT1 in nerve regeneration and axonal physiology. We confirmed that our laboratory-generated P0Cre::MCT1LoxP mice, which have selective knockdown of MCT1 in Schwann cells, were normal in development; unimpaired in anatomical and electrophysiological features; and devoid of any evidence of degeneration. The Schwann cell-specific ablation of MCT1 was sufficient to delay the sciatic nerve regeneration following crush. Furthermore, these mutant mice developed reduced distal amplitude of tail sensory nerves, but not sciatic motor nerves, with age compared to wild-type mice, indicating that Schwann cell-specific MCT1 is pivotal to maintain the functioning of sensory, but not motor, nerves during aging. Taken together, our results not only illuminate the role of lactate and its transporter MCT1 in regeneration and age-associated deterioration of peripheral nerve, but also advocate their implications as potential targets for the development of pharmacotherapies for peripheral neurodegeneration and axonal neuropathies.
Keywords: lactate; monocarboxylate transporter; Schwann cell; nerve regeneration; aging; peripheral neuropathy

Disclosures: **M.K. Jha:** None. **K. Russell:** None. **A. Singh:** None. **Y. Lee:** None. **J.D. Rothstein:** None. **B.M. Morrison:** None.

Poster

324. Peripheral Nerve Injury

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Topic: C.09. Brain Injury and Trauma

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Title: Excitability changes in aged regenerating axons

Authors: M. MOLDOVAN[1,2], S. ALVAREZ[1], D. CINTEZA[3], *C. KRARUP[2,1];
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Abstract: Following an interruption of continuity, peripheral axons have the ability to regenerate and reestablish functional connections with their targets. The internodal length remains short. Our previous experimental work in various species and clinical studies in humans indicated that distal to a nerve lesion, this increase in number of nodes leads to a persistent impairment of excitability where the increased voltage-gated Na^+ channel (VGSC) influx could reach neurotoxic levels during strenuous activity. Recently, we found that in aged mice there is an ectopic expression of the “sensory neuron specific” $Na_V1.8$ VGSC on motor axons leading to depolarizing shift in membrane potential which could aggravate these changes. The aim of this study was to chart the changes in regenerating motor axon excitability of aged C57BL/6 mice (more than 20 months old) after a unilateral sciatic nerve lesion. Regeneration at 5 months after section and reconstruction was markedly impaired in aged mice. Nevertheless, after a crush lesion, the recovery of plantar compound muscle action potentials evoked after tibial nerve stimulation at ankle was similar to mature mice. We therefore chose the ideal regeneration environment following the crush lesion to investigate the recovery of multiple measures of tibial motor axon excitability by “threshold-tracking” at ankle and at the sciatic notch. At ankle, regenerating motor axons showed persistent impairment in both voltage-dependent and passive membrane properties, consistent with previous reports on younger mice. The extent of these changes in the aged mice was additionally altered by a depolarizing shift in membrane potential. This was similar to the excitability differences with mature mice on the unlesioned side. Nevertheless, in contrast to mature mice, in aged mice, regeneration also aggravated the depolarizing excitability features at the sciatic level (proximal to the lesion). This changes could be attenuated acutely by the $Na_V1.8$ blocker Compound 31 (Bioorg. Med. Chem. Lett. 2010, 20,

6812; AbbVie Inc.). Taken together our data suggest that process of regeneration aggravates the VGSC expression changes in aged motor axons.

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Poster

324. Peripheral Nerve Injury

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Title: Multifunctional nerve conduit for peripheral nerve regeneration.

Authors: *H. AHN[1,2], M.-S. KIM[1,2], J.-W. KIM[1,2], J. HYUN[1,3,2];
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Abstract: Peripheral nerve injury is common and damaged peripheral nerves usually regenerate spontaneously, however the capacity for regeneration is not complete and remain motor and sensory dysfunctions and neuropathic pain in the clinical setting. Commercially available nerve conduits still cannot show better regeneration effects than autologous nerve graft. The aim of our study is to develop an ideal nerve conduits which act physical and biochemical cues for outgrowing axons after peripheral nerve injury and which function for axonal regeneration is comparable to autologous nerve graft.We developed a nerve conduit which contains numerous aligned microchannels and a selective permeable outer tube structure for physical cue, and sustained release of nerve growth factor within microchannels for biochemical cue. We performed *in vitro* study using a three dimensional culture system to detect neurite outgrowth within microchannels in the nerve conduit at first, and found that poly(lactic-co-glycolic acid)

(PLGA)-based microchannels were more effective for neurite outgrowth of neurons in dorsal root ganglion than polycaprolactone (PCL)-based microchannels, and tetraglycol was used as a solvent to make PLGA or PCL solution. Microchannels were developed using phosphate glass microfibers which were dissolved in distilled water, and heparin was used for the controlled release of nerve growth factor (NGF) within microchannels. *In vivo* studies were performed to the transected sciatic nerve of rats, and a 16mm-long NGF-containing PLGA nerve conduit was implanted. Autologous sciatic nerve and the PLGA or PCL nerve conduit without NGF were used as controls. We found that the length of axons from proximal stump was superior in PLGA nerve conduit-implanted group than PCL nerve conduit-implanted control at 2 weeks after implantation, and notably functional assessments including von Frey hair test and sciatic functional index, muscle fiber size, compound muscle action potential indicated that the NGF-containing PLGA nerve conduit is comparable to autologous nerve graft at 16 weeks. We concluded that our new nerve conduit is effective for axonal regeneration after peripheral nerve injury, and might substitute for autologous peripheral nerve.

Disclosures: **H. Ahn:** None. **M. Kim:** None. **J. Kim:** 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; Basic Research Program(2015R1D1A1A01059014). **J. Hyun:** 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 Grant; Mid-career Researcher Program (R-2015-01266), Basic Research Program (R-2015-01133).

Poster

324. Peripheral Nerve Injury

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Topic: C.09. Brain Injury and Trauma

Support: Roudebush VA IIMR Young Investigator Award (CLW)

IUCRG (KJJ)

Title: Benefits of combinatorial therapies for improving functional recovery in a rat model of facial nerve injury

Authors: ***E. M. RUNGE**[1,3], T. J. ASANTE[1], H. R. WELCH[1], C. L. WALKER[1,3], A. R. BEST[2], J. L. MULDOON[1], K. J. JONES[1,3];

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Abstract: Injury to the facial nerve due to trauma or surgical intervention for head and neck pathologies is common; however, few effective treatments eliciting full functional recovery following facial nerve injuries exist. Previous studies in our laboratory have found that single-agent therapies, while effective in improving functional recovery, are insufficient in promoting complete functional recovery after nerve injury. Consequently, combination therapies are an area of emphasis in our research. We have found that treating the repaired injured nerve with polyethylene glycol (PEG), a sealant that acts to fuse damaged cell membranes, and methylene blue (MB), an antioxidant, improves functional recovery in a rat model of facial nerve transection and suture. In particular, we observed significant recovery of vibrissae movement beginning at two weeks post-injury and suture that continued until the end of the study sixteen weeks later. We are also investigating the use of systemic testosterone propionate (TP) and direct electrical stimulation (ES) of the facial nerve in combination with PEG/MB in anticipation of improving the rate and extent of recovery beyond those observed for individual treatments. Given the ease in application of these various therapies, either singularly or in combination, we anticipate that these studies will aid translation of such methods for application in human facial nerve injury patients. Supported by a Roudebush VA IIMR Young Investigator Award (CLW) and IUCRG grant from IUSM (KJJ).

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Poster

324. Peripheral Nerve Injury

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Support: National Natural Science Foundation of China 31471030

Research foundation of Department of Science and Technology of Guangdong Province, P.R.China 2014A020212070

Research foundation of Department of Science and Technology of Guangdong Province 2015A030311014

Title: Distinct expression profiles of LncRNAs between ipsilateral and contralateral in adult rats following unilateral brachial plexus root avulsion

Authors: *Y. GUANGYIN[1], X.-Y. XU[2], Y. TANG[2], L.-L. LIU[2], X. CHEN[3], Z. QIU[4], Q. YAN[4], Q. ZHU[4], Z. WU[4], L. ZHOU[2,5];
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Abstract: Long noncoding RNAs (lncRNAs) are pervasively transcribed and have a critical role in genome regulation. Brachial plexus root avulsion (BPRA) induces multiple pathophysiological events consisting of altered levels of specific proteins, which collectively leads to dramatic motoneuron death. To identify the spinal cord lncRNAs involved in the BPRA, we describe the expression profile of lncRNAs in ipsilateral half compared to the contralateral half of the spinal cord using Arraystar Rat LncRNA/mRNA analysis. Unilateral BPRA resulted in significant alterations in lncRNAs expression. In the ipsilateral half of the spinal cord, on the 3rd day after the injury, 41 lncRNAs were upregulated, and 11 were downregulated (≥2.0-fold, $p<0.05$); 349 mRNAs were upregulated and 51 were downregulated (≥2.0-fold, $p<0.05$). And on the 14th day after the injury, 48 lncRNAs were upregulated, and 27 were downregulated (≥2.0-fold, $p<0.05$); 378 mRNAs were upregulated and 67 were downregulated (≥2.0-fold, $p<0.05$). Our previous studies have demonstrated that the spatial and temporal characteristic of Nos1 and Capn2 induced by avulsion injury is the crucial factor cause motoneurons death. Co-expression analysis shows that AF128540 and S69236 may target Nos1 and Capn2 mRNAs. In the present study, the upregulation of AF128540 and the downregulation of S69236 were highly in accordance with the Nos1(upregulation) and capn2(downregulation) mRNAs expressions. Thus, avulsion-induced activation of AF128540/Nos1 and S69236/Capn2 mechanisms may contribute to injured motoneuron degeneration. Pathway analysis indicated that 55 pathways corresponded to upregulated transcripts and that 10 pathways corresponded to down regulated transcripts (P-value≤0.05) on the 3rd day after the injury. And 52 pathways corresponded to upregulated transcripts and that 12 pathways corresponded to downregulated transcripts (P-value≤0.05) on the 14rd day after the injury. The expression of MRAK034299(downregulation, 4.1-fold, $p<0.05$) was highly in accordance with Adra1d (downregulation, 3.4-fold, $p<0.05$) gene expression. Pathway analysis the MRAK034299 target gene-Adra1d may be involved in calcium signaling pathway, cGMP-PKG signaling pathway, neuroactive ligand-receptor interaction, ect. Thus, the MRAK034299 may contribute to avulsion-induced motoneuron apoptosis or survival via Adra1d mechanism. Our present study show distinct expression profiles of LncRNAs in injured spinal cord following brachial roots avulsion. The data of the alternative lncRNAs and mRNAs in the spinal cords of the adult rats provide information and target genes for further research on the motoneuron diseases.

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Poster

325. Primary Olfactory Signal Transduction

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Program#/Poster#: 325.01/CC1

Topic: D.04. Olfaction and Taste

Support: FAPESP 2012/04026-1

VRERI UNICAMP 26/2016

CAPES Fellowship

Title: Detection of pup odors by non-canonical adult vomeronasal neurons expressing an odorant receptor gene is influenced by sex and parenting status.

Authors: *T. S. NAKAHARA[1], P. G. RIBEIRO[1], P. H. M. MAGALHAES[1], P. H. M. NETTO[1], X. IBARRA-SORIA[2], D. W. LOGAN[2], F. PAPES[1];
[1]Dept. Genet. and Evolution, Univ. of Campinas (UNICAMP), Campinas, Brazil; [2]Wellcome Genome Campus, Hinxton, Wellcome Trust Sanger Inst., Cambridge, United Kingdom

Abstract: Background: Olfaction is a fundamental sense through which most animals perceive the external world. The olfactory system detects odors via specialized sensory organs such as the main olfactory epithelium and the vomeronasal organ. Sensory neurons in these organs use G-protein coupled receptors (GPCR) to detect chemosensory stimuli. The odorant receptor (OR) family is expressed in sensory neurons of the main olfactory epithelium, while the adult vomeronasal organ is thought to express other types of receptors. Results: Here we describe *Olfr692*, a member of the *OR* gene family identified by next-generation RNA sequencing, which is highly upregulated and non-canonically expressed in the vomeronasal organ. We show that neurons expressing this gene are activated by odors emanating from pups. Surprisingly, activity in *Olfr692*-positive cells is sexually dimorphic, being very low in females. Our results also show that juvenile odors activate a large number of *Olfr692* vomeronasal neurons in virgin males, a situation in which infanticide is displayed. In contrast, activity substantially decreases in parenting males (fathers), where infanticidal aggressive behavior is not frequently observed. Conclusions: Our results describe for the first time a sensory neural population with a specific molecular identity that is involved in the detection of pup odors. Moreover, it is one of the first reports of a group of sensory neurons the activity of which is sexually dimorphic and depends on social status. Our data suggest that the *Olfr692* population is involved in mediating pup-oriented behaviors in mice

Disclosures: **T.S. Nakahara:** None. **P.G. Ribeiro:** None. **P.H.M. Magalhaes:** None. **P.H.M. Netto:** None. **X. Ibarra-Soria:** None. **D.W. Logan:** None. **F. Papes:** None.

Poster

325. Primary Olfactory Signal Transduction

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Program#/Poster#: 325.02/CC2

Topic: D.04. Olfaction and Taste

Support: NIDCD Grant R01DC013553

BRAIN Initiative Grant 5U01NS094296-01

Department of Defense, MURI Grant W911NF-12-1-0594

Kavli Foundation

Title: Native olfactory sensory neuron imaging with swept confocally-aligned planar excitation (scape) microscopy

Authors: *L. XU[1], W. LI[2], V. VOLETI[2], E. M. C. HILLMAN[2], S. J. FIRESTEIN[1];
[1]Biol. Sci., [2]Biomed. Engin., Columbia Univ., New York, NY

Abstract: Recording activity in olfactory sensory neurons (OSNs) has traditionally been done by using calcium imaging of individual dissociated cells or through electroolfactogram (EOG) field potential recordings of large numbers of cells. Both methods have considerable disadvantages. Many cells are damaged during the dissociation process and they are effectively axotomized. EOG uses an intact preparation but provides no cellular resolution and there is significant variability from preparation to preparation. It is also difficult to control stimulation in the EOG preparation.

We have developed a novel OSN imaging method that takes advantage of genetic tools and a recently developed microscopy technique, called Swept Confocally-Aligned Planar Excitation (SCAPE) microscopy. SCAPE-based OSN imaging enables widespread recording of cells in the olfactory epithelium of OMP-CRE$^{+/-}$GCaMP6f$^{-/-}$ mice, without damaging the nasal turbinate and preserving neuron innervations to the olfactory bulb by using a perfused half-head preparation. This method allows us to directly image large areas of the intact olfactory epithelium at over 10 volumes per second at cellular resolution. Compared with dissociated cell imaging, SCAPE provides increased sensitivity for detecting OSN responses, yielding more than two orders of magnitude more active neurons for similar olfactory stimuli. Cells remain healthy for longer, allowing more experimental conditions to be tested. The native tissue condition also enables us to draw a 3D response map for each odorant along the nasal turbinate for cross-comparison. Thus this method allows us to monitor odor-induced activity in large numbers of cells (as with the EOG) but with single cell resolution (as with dissociated cells) under precise delivery of controlled odor stimuli.

Disclosures: **L. Xu:** None. **W. Li:** None. **V. Voleti:** None. **E.M.C. Hillman:** None. **S.J. Firestein:** None.

Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: NIDCD grant DC 00997701-06

STAR 2014 grant 14-CSP3-C03-099

Title: A mathematical model for the response of olfactory sensory neurons to odor mixtures

Authors: ***A. MARASCO**[1], A. DE PARIS[1], M. MIGLIORE[2,3];
[1]Mathematics and Applications, Univ. of Naples Federico II, Napoli, Italy; [2]Natl. Res. Council, Palermo, Italy; [3]Yale Univ., New Haven, CT

Abstract: Most real world odors are complex mixtures consisting of dozens, often hundreds of components, and olfactory systems have evolved to recognize and discriminate them. The sensory process starts with the interaction of odorant molecules with the dendritic membrane of olfactory sensory neurons (OSNs). It has been widely demonstrated that, in most cases, the response of OSNs to (single) odor stimuli can be conveniently reproduced by a sigmoidal shape with concentration, usually implemented as a Hill function. However, the response to a mixture of odorants is not a simple function of the response to the individual components, and its properties are empirically classified as suppression, hypoadditivity, synergy, inhibition, and overshadowing. The models proposed in the literature to describe the responses of OSNs to single odors and binary mixtures cannot reproduce all of them. Here, we present a mathematical model able to predict all types of mixture interactions among N odorants ($N \geq 2$). The model has been validated against a number of different experimental findings. All the typical, experimentally measurable, characteristics of the response curves (i.e. steepness, midpoint, and asymptotic value) can be mathematically expressed by a non-linear combination of the parameters for the individual components. Furthermore, we show that it is possible to find suitable mathematical conditions for all parameters and concentrations, in such a way to make it possible to design mixtures with specific properties. The results suggest a way to predict the overall behavior of a receptor type in response to a mixture composed of any number of components, provided that the individual response curves and their relative concentration ratios are known. A rigorous analysis of the mathematical structure of the 3-dimensional *odor response*

space (*ORS*), suggests that any dose-response curve of an odor receptor can be obtained as a combination of three (out of four) "*primary odor responses*" with specific properties, opening the way to an objective procedure to obtain specific olfactory receptor responses by manipulating mixtures in a mathematically predictable manner.

Disclosures: A. Marasco: None. **A. De Paris:** None. **M. Migliore:** None.

Poster

325. Primary Olfactory Signal Transduction

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 325.04/CC4

Topic: D.04. Olfaction and Taste

Title: Effects of glycosylation in activity dependent cAMP mediated olfactory signaling

Authors: *S. RYU[1], T. SHIM[1], S. Y. KIM[1], J. GOLEBIOWSKI[2], C. MOON[1];
[1]department of Cognitive & Brain sciences, DGIST, Daegu, Korea, Republic of; [2]Inst. of Chem. CNRS, Univ. of Nice Sophia Antipolis, Nice, France

Abstract: Protein functions are often regulated by reversible post-translational modifications (PTMs) including phosphorylation, glycosylation, and ubiquitination. Odorant stimulation to odorant receptors (ORs) undergoes phosphorylation as a regulatory mechanism which is essential in the olfactory signal transduction including such as desensitization. Different from phosphorylation, little is studied about glycosylation of ORs in the olfactory signaling. Here, we demonstrated that glycosylation may have functional roles in cAMP mediated olfactory signaling in an activity dependent manner. We found increases in expression of glycosylation enzymes upon odorant stimulation in primary cultured olfactory sensory neurons (OSNs) of rat pups. In contrast, the overall glycosylated protein levels of OSNs were simultaneously decreased after odor stimulation. As the ORs are the key proteins in odorant stimulated olfactory signaling, we targeted ORs and performed site-directed mutagenesis of glycosylation sites within the OR sequence in order to examine the roles of glycosylation using HEK293T cells derived *in vitro* system. Glycosylation of ORs has an effect on cAMP mediated signaling, and also cAMP responsive binding protein (CREB) activity upon odorant stimulation. Our results suggest a novel regulatory mechanism in ligand-odorant receptor signaling. Moreover, we again underline the importance of odorant stimulation dependent regulatory mechanism in the olfactory sensory system.

Disclosures: S. Ryu: None. **T. Shim:** None. **S.Y. Kim:** None. **J. Golebiowski:** None. **C. Moon:** None.

Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: NIH Grant GG00900301

Title: What makes pineapple smell like a pineapple? Here's how medicinal chemistry would slice it.

Authors: *N. TAHIROVA[1], E. POIVET[2], L. XU[1], S. FIRESTEIN[1];
[1]Columbia Univ., New York, NY; [2]New York Univ., New York, NY

Abstract: Several hundreds of olfactory receptors (ORs) are expressed in the mammalian olfactory system. Receptor-ligand interactions are the first step in sensing the complex mixtures that initiate smell perception. Although it is clear that different ORs are selective toward different odorant ligands, there is a disparity in how odorants are classified. While some use traditional organic chemistry methods to group these molecules (a physics or molecular approach), others look entirely at the perceptual output of the subjects in response to the odors (a psychological approach). Both of these methods pay insufficient attention to the reaction of the olfactory sensory neurons (OSNs) – a *biological* level.
Medicinal chemistry, a pharmacological technique used widely in the pharmaceutical industry, emphasizes biological function over chemical form. Since each mature OSN expresses one type of OR, we can test the parameters of odorant recognition for odors related by medicinal chemistry principles using calcium imaging on dissociated OSNs and mouse behavioral assays. For example, flipping an ester group within a ligand while maintaining its position will not change the OR binding ability of the ligand. To this end, we have tested a panel of 6-carbon long esters and reverse esters with pineapple-like scents. By varying the position or direction of the ester group along the carbon chain of these molecules, we are modifying their hydrophobic chain length, a property that might change their interaction with the ORs. Testing this mechanism allows us to make certain predictions about the nature of odorant selection by the OR family. In our study, we find that while reversing the ester group has almost no effect on odorant binding, shifting its location along the carbon chain significantly reduces the OR activation overlap. Our results show that medicinal chemistry may be an efficient tool to slice through some of the mysteries of olfaction.

Disclosures: **N. Tahirova:** A. Employment/Salary (full or part-time): Full-time student at Columbia University. **E. Poivet:** A. Employment/Salary (full or part-time): NYU medical center. **L. Xu:** A. Employment/Salary (full or part-time): Full time student at Columbia University. **S. Firestein:** A. Employment/Salary (full or part-time): Full time professor at Columbia University.

Poster

325. Primary Olfactory Signal Transduction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 325.06/CC6

Topic: D.04. Olfaction and Taste

Title: Motile cilia in sensory organs: more than just generating flow?

Authors: *N. JURISCH-YAKSI, I. REITEN, S. FORE, R. PELGRIMS, M. HOFFMANN, E. YAKSI;
Kavli Inst. For Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: The cilium is a small cellular appendage projecting from the surface of most cells, like a tiny antenna. It can generate flow or play a structural, sensory or signaling role in many tissues. Hence, defects in cilia observed in ciliopathy patients affect multiple organs, including the brain, and result in hydrocephalus, anosmia and neurological disorders.
Motile cilia, which cover most epithelia and the brain ventricles, generate flow to move fluids, particles and molecules at even macroscopic scales. Interestingly, they can also sense and respond to their environment by changing their beating frequency. Even though their beating is extremely important for proper organ function, very little is known about intrinsic mechanisms regulating their beating frequency.
Using the olfactory epithelium of the zebrafish as model system, we are studying the cellular and physiological mechanisms regulating ciliary function and motility in vivo in a living animal. Our results suggest that ciliary beating is regulated by several environmental factors and genetic ablation of ciliary beating has important functional consequences for the ciliated cells as well as neighbouring cells in the epithelium, i.e. olfactory receptor neurons. Altogether we expect our work to set a framework for studying and characterizing human ciliopathies using zebrafish as a model organism. With its low cost, established genetic tools, small size and transparency, zebrafish is an excellent animal model that is amenable for high throughput genetic and in vivo drug screens.

Disclosures: **N. Jurisch-Yaksi:** None. **I. Reiten:** None. **S. Fore:** None. **R. Pelgrims:** None. **M. Hoffmann:** None. **E. Yaksi:** None.

Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: NIH Grant R01DC009597

NIH Grant R01DK092640

NSF Grant 0920668

Title: Hormonal modulation of pheromone detection enhances male courtship sucess

Authors: *H. LIN, J. W. WANG;
Section of Neurobio. Div. of Biol. Sci., UCSD, LA Jolla, CA

Abstract: During the lifespans of most animals, reproductive maturity and mating activity are highly coordinated. In *Drosophila melanogaster*, for instance, male fertility increases with age and older males are known to have a copulation advantage over young ones. The molecular and neural basis of this age-related disparity in mating behavior is unknown. Here we show that the Or47b odorant receptor is required for the copulation advantage of older males. Notably, the sensitivity of Or47b neurons to a stimulatory pheromone, palmitoleic acid, is low in young males but high in older ones, which accounts for older males' higher courtship intensity. Mechanistically, this age-related sensitization of Or47b neurons requires a reproductive hormone, juvenile hormone, as well as its binding protein Methoprene-tolerant in Or47b neurons. Together, our study identifies a direct neural substrate for juvenile hormone that permits coordination of courtship activity with reproductive maturity to maximize male reproductive fitness.

Disclosures: H. Lin: None. **J.W. Wang:** None.

Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: NIH Grant DC008119

Title: Inhibition of olfactory behavior in *Drosophila melanogaster* larvae through antagonism of the odorant receptor co-receptor (Orco) subunit

Authors: D. KEPCHIA, S. MOLIVER, K. CHOHAN, C. PHILLIPS, *C. W. LUETJE;
Mol. and Cell. Pharmacol. (R-189), Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Many insect behaviors are driven by olfaction, making odorant receptors (ORs) appealing targets for control strategies. Insect ORs, a family of odorant-gated ion channels, are heteromeric complexes of unknown stoichiometry that are composed of a representative from a large, variable family of odorant binding subunits and a highly conserved co-receptor subunit (Orco). Orco directed agonists and antagonists have recently been identified and we have shown that phenylthiophenecarboxamide structures competitively antagonize the Orco subunit. Importantly, Orco antagonists also act allosterically to inhibit OR activation by odorants.
To assess the utility of Orco antagonists as inhibitors of insect olfactory behavior, we focused on a series of phenylthiophenecarboxamides with halogen substitutions that increased volatility. In an electrophysiology assay, these compounds functioned as Orco antagonists when tested against ORs from *D. melanogaster*, *Anopheles gambiae* (malaria vector mosquito) and *Culex quinquefasciatus* (West Nile Virus vector mosquito). We then assayed *D. melanogaster* larvae in an olfactory chemotaxis behavioral assay to determine whether an airborne Orco antagonist could alter olfactory behavior. Larvae were placed in the center of a 10 cm Petri dish, flanked on either side by small filter paper disks at the dish perimeter. Highly diluted ethyl acetate (EA, a potent attractant) was placed on one filter and mineral oil (vehicle) was placed on the other. After a 5 min migratory period, the location of larvae was documented and attraction to EA was measured as a response index (RI). The RI towards EA was consistently robust. Genetically modified larvae lacking the EA responsive odorant binding subunit Or42b, or lacking Orco, were not attracted to EA. Larvae showed no preference when vehicle was placed on both filter disks, or when EA was placed on both filter disks. Attraction to EA was masked when EA was also added to a large filter disk attached to the inside of the dish lid, while vehicle in the lid filter disk had no effect. When a volatile Orco antagonist (halogen-substituted phenylthiophenecarboxamide) was placed in the dish lid filter, attraction to EA was abolished. In contrast, larval aversion to light was unaffected by the presence of the Orco antagonist. The ability of an airborne Orco antagonist to alter olfactory behavior in *D. melanogaster* larvae suggests that olfaction mediated behaviors of disease vector mosquitoes might also be manipulated through Orco antagonism.

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Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: JSPS Grant-in-Aid for JSPS Fellows Grant Number 14J06037

Title: The locally distinctive cGMP response in an olfactory sensory neuron of *Caenorhabditis elegans*.

Authors: *H. SHIDARA, K. ASHIDA, K. HOTTA, K. OKA;
Lab. of Biophysics and Neuroinformatics, Keio Univ., Yokohama-Shi, Japan

Abstract: Cyclic guanosine monophosphate (cGMP) has important roles in signal transductions of many animals as a second messenger. In *Caenorhabditis elegans*, the second messenger is required for sensing of odor, temperature and gas. Especially, for olfactory sensing, the cGMP in AWC sensory neuron plays two distinctive functions: odor sensation and adaptation. For odor sensation, odorants may bind to G protein-coupled receptors (GPCRs), which activate Gi-like proteins at cilia of AWC. Then, the activated Gi-like protein increases cGMP production to regulate cGMP-dependent channels (Bargmann, 2006). For odor adaptation, cGMP binds to cGMP-dependent protein kinase G (PKG), induces translocation of PKG into the nuclear, and changes cellular physiological functions (O'Halloran et al., 2009). Although many functions relating to cGMP in AWC have been supported by genetic and molecular studies, region-specific cGMP dynamics in AWC have not been mentioned yet. Because the cilia are far from the soma in AWC, the cGMP dynamics at each region may be different. In our study, we visualized local cGMP dynamics and showed the distinct cGMP response to odorants at each region in AWC. To elucidate the temporal and spatial cGMP activities, we used the genetic cGMP indicator, cGi500 (Russwurm et al., 2007), with str-2 promoter for expression in AWC. Transgenic worms were fixed in microfluidic devices and exposed to isoamyl alcohol (odor). In the soma, the odor exposure increased the cGMP level. After the odor was removed, the cGMP level returned to the basal level. On the other hand, in the cilia, the cGMP level decreased immediately after exposure to odor and returned to the basal level in about 10 sec. Interestingly, the cGMP response in the dendrite, which neighbors the cilia, was not similar to one in the cilia but in the soma. These results showed the local specific cGMP response in the cilia, the dendrite and the soma of AWC.

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Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: Barrett Foundation Grant

Mills College Research Grant

Title: Identifying and characterizing odor receptors in *Caenorhabditis elegans*

Authors: S. MAHER[1], M. HARWOOD[1], L. RESCH[1], C. DALTON[1], S. NATHAN[1], A. COX-HARRIS[1], R. MORTON[1], B. MOSQUEDA[1], E. JEROME[1], B. MEADOWS[1], V. THAKKER[1], W. MANKINS[1], L. ROST[1], H. RAGHUNATHAN[1], Y.-P. HSUEH[2], N. L'ETOILE[3], *J. J. YOUNG[1];
[1]Mills Col., Oakland, CA; [2]Academia Sinica, Taipei, Taiwan; [3]Univ. of California San Francisco, San Francisco, CA

Abstract: The nematode *Caenorhabditis elegans* uses a small number of sensory neurons to respond to a wide variety of odors. The identities of the odor receptors and an understanding of what they respond to and how they are organized is largely unknown. We are pursuing analysis of putative odor receptors. We are using translational GFP fusions to localize candidate proteins, generating knockout mutants using the CRISPR technique, and knocking down gene expression of candidates using RNA interference. Knockouts and RNA interference-induced worms will be tested in chemotaxis assays for odor responses.

Disclosures: S. Maher: None. **M. Harwood:** None. **L. Resch:** None. **C. Dalton:** None. **S. Nathan:** None. **A. Cox-Harris:** None. **R. Morton:** None. **B. Mosqueda:** None. **E. Jerome:** None. **B. Meadows:** None. **V. Thakker:** None. **W. Mankins:** None. **L. Rost:** None. **H. Raghunathan:** None. **Y. Hsueh:** None. **N. L'Etoile:** None. **J.J. Young:** None.

Poster

325. Primary Olfactory Signal Transduction

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Topic: D.04. Olfaction and Taste

Support: NIH R01 GM086456

NIH R01 GM108885

NSF IOS 1353075

Title: Biological sex modulates chemosensory function to bring about sex differences in *C. elegans* behavioral prioritization

Authors: *D. S. PORTMAN, K. A. FAGAN, E. WEXLER;
Ctr. for Neural Develop. and Dis., Univ. of Rochester, Rochester, NY

Abstract: Internally represented 'states' allow animals to dynamically and adaptively modulate their behavioral priorities. How and where these states are encoded within the nervous system, and how they reconfigure the properties of neural circuits, are central issues in behavioral neurobiology. Sex differences provide a unique example of natural variation in behavioral states and an ideal opportunity to understand their mechanistic underpinnings. Though the nematode C. elegans has a simple, compact nervous system, it exhibits robust behavioral states that are deeply influenced by biological sex. Our recent work has focused on understanding how biological sex modulates chemosensory circuits and behavior. Surprisingly, our results indicate that the modulation of sensory function itself is a key point of regulatory control. In adults, the two sexes of C. elegans (males and hermaphrodites, essentially self-fertile females) differ in several behaviors mediated by shared chemosensory circuits. For example, while hermaphrodites prioritize food detection, well-fed males tend to leave food in search of mates. In contrast, hermaphrodite-derived pheromones elicit weak repulsion in other hermaphrodites but strong attraction in males. We have found that the genetically determined sexual state of two pairs of sensory neurons in the worm's head are critical for implementing both of these differences. In the AWA neuron pair, genetic sex regulates expression of a key food chemoreceptor, odr-10, such that it is highly expressed in hermaphrodites but weakly expressed in males. Genetic 'sex-reversal' of this neuron is sufficient to reverse this expression pattern and alter behavioral prioritization. We have found that Insulin-like (daf-2) and TGFbeta (daf-7) signals are important contributors to this sex difference; moreover, these signals also regulate food detection as a function of nutritional state. Similarly, the ADF neurons are specialized by genetic sex to efficiently detect hermaphrodite pheromones only in males. Genetic masculinization of this neuron pair in hermaphrodites confers pheromone-sensitivity to them and generates pheromone attraction. Together, these studies identify sensory neurons as a key focus of sexual modulation, reinforcing the emerging idea that dynamic sensory function is an important contributor to state-dependent behavior. Understanding these regulatory interactions should shed light more generally on the mechanisms by which genetic sex modulates neural development and function.

Disclosures: D.S. Portman: None. **K.A. Fagan:** None. **E. Wexler:** None.

Poster

325. Primary Olfactory Signal Transduction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 325.12/DD1

Topic: D.04. Olfaction and Taste

Title: Characterization of odorant receptor expression and its glycosylated form in the olfactory and non-olfactory system

Authors: *T. SHIM, S. RYU, S. KIM, G. V. RONNETT, C. MOON;
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Abstract: The initial step in odor detection occurs by the interaction of odorous molecules with odorant receptors (ORs) in the olfactory cilia of olfactory sensory neurons (OSNs). OR protein is a member of large G protein coupled receptor (GPCR) family, and the amino acid sequence of each OR proteins are variable. Numerous studies have been done by targeting specific regions within the OR sequence to elucidate particular roles or regulatory mechanisms of ORs. In contrast, studies to examine the expression of broad range of ORs and general distribution of OR proteins in the olfactory system have not been thoroughly performed. Thus we here have employed sequence-specific rabbit polyclonal antibodies against ORs from BLAST analysis, and the amino acid sequences we chose appear to be highly specific to ORs from various species. The sequence-specific peptide antibodies have recognized ORs localized in the rat olfactory cilia, and pre-absorbed antibodies with the sequence specific peptide showed significantly decreased immunoreactivity in both immunohistochemistry and immunoblot, indicating that this antibody detects ORs specifically. Interestingly, the antibodies appeared to detect glycosylated form of ORs as well as ORs in non-olfactory tissues, indicating novel regulatory mechanisms of ORs during olfactory signal transduction and ectopic expression of ORs. Our studies demonstrate that employment of sequence specific OR antibodies in cellular biological studies is feasible, and open intriguing possibilities for roles of ORs besides olfaction.

Disclosures: **T. Shim:** None. **S. Ryu:** None. **S. Kim:** None. **G.V. Ronnett:** None. **C. Moon:** None.

Poster

325. Primary Olfactory Signal Transduction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 325.13/DD2

Topic: D.04. Olfaction and Taste

Support: NIH NIDCD 1R01DC012829

Title: Cyclophosphamide induced loss in olfactory cell populations.

Authors: ***N. AWADALLAH**[1], K. B. JOSEPH[1], K. PROCTOR[2], R. DELAY[1], E. DELAY[1]; [1]Biology/Neuroscience, [2]Med. Lab. & Radiation Sci., Univ. of Vermont, Burlington, VT

Abstract: Chemotherapy patients often experience chemosensory changes during and after drug therapy. Cyclophosphamide (CYP) is one of the first chemotherapy drugs with known cytotoxic and destructive effects, specifically on the taste cell cycle. **Similar to the taste system, the sense of smell is dependent on olfactory neurons that undergo rapid replacement. Therefore, we asked if a single injection of CYP would affect olfactory neurons in a similar form?** Due to a lack of knowledge on how CYP affects olfactory neurons, we examined the effects of CYP on olfactory neurons located in the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). We used an antibody to Ki6, a protein expressed solely in cells undergoing division. ~100 male mice were given a single, IP injection of CYP (75 mg/kg) and sacrificed from 1 day to 125 days' post-injection. Mice were perfused with 4% paraformaldehyde, decalcified with EDTA, cryo-protected, sectioned and incubated with a Ki67 antibody (Thermo scientific). There were clear differences between the MOE and the VNO across all the time points. At 1-day post injection, the MOE looked damaged, especially in the dendritic region while the VNO was structurally unaffected. Both tissues showed a decrease in Ki67 protein label compared to controls. During the peak days' post injection, Ki67 labeling either surpassed or were equal to levels of labeling of control animals. By day two, neither tissue showed any Ki67 labeling. Between days four and six post injection there was a surge in Ki67 labeling, but this dramatically decreased by day 14. Recovery was noticeable complete by 30 in the MOE and 45 days in the VNO. However, labeling levels were almost absent by day 60. Interestingly, we see an increase in Ki67 labeling starting at 75 days' post injection and a recovery of proliferation by day 125. We are continuing on to observe the effects of CYP on the horizontal and globose basal cells using Keratin 5. So far, our data suggest that the olfactory tissue in the main olfactory epithelium was more affected by CYP than the VNO.

Disclosures: **N. Awadallah:** None. **K.B. Joseph:** None. **K. Proctor:** None. **R. Delay:** None. **E. Delay:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.01/DD3

Topic: D.05. Audition

Support: R01DC015138

Title: Neural coding and discrimination of high-order sound statistics in the inferior colliculus

Authors: *F. KHATAMI, M. SADEGHI, H. L. READ, I. H. STEVENSON, M. A. ESCABI; Univ. of Connecticut, Storrs, CT

Abstract: Natural sounds exhibit high-order statistical regularities that are critical for neural coding and perception. Recently, it has been demonstrated that high-order statistical regularities in the spectrograms of sound textures, such as water, fire, and other environmental sounds, are critical for discrimination and identification (McDermott and Simoncelli, 2011). Furthermore, it is well known that neural responses in the central auditory system can be modulated by a variety of high-order sound statistics, including modulation and correlation statistics. Here we test the hypothesis that neural responses in the inferior colliculus are modulated by high-order statistical regularities in sounds and that statistical features of the neural response can ultimately be used to discriminate and categorize sounds. We performed recordings in inferior colliculus of unanesthetized rabbits in response to an ensemble of texture sounds (water, fire, speech babble etc.). We synthetically manipulated each sound by selectively adding or removing high-order statistics using the synthesis algorithm developed by McDermott and Simoncelli (2011). We show that neural response statistics, including spike timing precision and firing reliability, were modulated with manipulations of the sound statistics. Using neurometric and ideal observer analysis we demonstrate that neural response statistics can be used to discriminate sounds and that removing the high-order sound statistics decreases neural classification performance. These findings suggest that neural response statistics in the inferior colliculus have the capacity to convey information about statistical regularities in sounds that are critical for sound categorization.

Disclosures: F. Khatami: None. **M. Sadeghi:** None. **H.L. Read:** None. **I.H. Stevenson:** None. **M.A. Escabi:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

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Program#/Poster#: 326.02/DD4

Topic: D.05. Audition

Support: NeuroBasic PharmaPhenomics grant (AgentschapNL of the Ministry of Health, Welfare and Sports of the Netherlands)

Marie Skłodowska-Curie Individual Fellowship (European Commissions).

Title: Chronic Ca^{2+} imaging reveals strong suppressive effects of anaesthesia on spontaneous and sound-evoked responses in dorsal inferior colliculus

Authors: *A. B. WONG, J. G. G. BORST;
Dept. of Neurosci., Erasmus MC, Rotterdam, Netherlands

Abstract: The inferior colliculus (IC) is a major auditory processing center, which receives input from major brainstem auditory nuclei. The non-lemniscal regions of the IC, dorsal cortex (DCIC) and lateral cortex (LCIC), are major targets for feedback projections from the cerebral cortex, making them likely susceptible to anaesthetic effects. Moreover, a large part of the DCIC lies superficially, making it a favorable structure for chronic *in vivo* two-photon imaging. Using Ca^{2+} imaging, we measured spontaneous and sound-evoked activities of the same IC neuronal population in mice when the animals were awake and during anaesthesia with ketamine/xylazine. GCaMP6s was expressed in IC neurons through the injection of AAV-vector. Pure-tone stimuli of various frequencies and intensities were presented while fluorescence of GCaMP6s was recorded by two-photon imaging. In an awake animal, a substantial portion of cells showed inhibitory response to pure tone stimuli, as indicated by a decrease in GCaMP6s fluorescence. We also observed excitatory responses to tone offset in some cells. Upon ketamine/xylazine anaesthesia, we noticed a decrease in baseline GCaMP6s fluorescence, which we interpret as a decrease in spontaneous activity. Inhibitory and offset responses were greatly diminished. Onset excitatory responses often showed an increase in threshold or a decreased amplitude. Some cells showed a shift in their characteristic frequency during anaesthesia. The same neurons were imaged after the animals recovered from anaesthesia to confirm that the changes were temporary and due to anaesthesia. Pupil size, whisker movement and body movement of the animals were recorded to further probe the effect of alertness. Post-hoc histology was performed on imaged cells to relate their neurochemical properties to their response characteristics. Our findings suggest the presence of tonic excitation on IC neurons in awake animals, which is suppressed during anaesthesia.

Disclosures: **A.B. Wong:** None. **J.G.G. Borst:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.03/DD5

Topic: D.05. Audition

Support: National Science Foundation of China Grant 91332122

Title: Cell type specific connectivity and function in auditory midbrain

Authors: ***C. CHEN**[1], M. CHENG[1], T. ITO[2], M. ONO[3], S. SONG[1];
[1]Tsinghua Univ., Beijing City, China; [2]Univ. of Fukui, Fukui, Japan; [3]Kanazawa Med. Univ., Ishikawa, Japan

Abstract: The auditory midbrain or inferior colliculus (IC) is believed to be the first integration center in the auditory pathway, with information from lower brainstem nuclei, contralateral IC and auditory cortex. Different sources of input create functional domains in the IC in which excitatory and inhibitory neurons receive specific combination of inputs. However, the connectivity and function of different excitatory and inhibitory cell types is still unknown. Here, we combined the monosynaptic rabies virus tracing, Cre transgenic mice and *in vivo* two photon calcium imaging methods to address those questions. To exam the inputs to specific cell type, rabies virus were injected in the IC of 5 Cre transgenic mouse lines, labeling excitatory VGlut2, inhibitory VGAT, parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP) positive neurons. Four IC subregions were labeled by rabies virus, include the dorsal, central and external part of IC (ICD, ICC, and ICX). In all IC subregions, inhibitory neurons received significant lower portion of excitatory inputs than excitatory neurons, and no significant difference was found in the inhibitory inputs to both cell type. For both cell types, the fraction of subcollicular inputs was nearly equal with that of commissural IC inputs, but significant less than that of local inputs. All cell types examined, i.e. VGlut2, VGAT, PV, SOM and VIP had commissural, ascending or descending projections, suggesting that these molecules are not the marker of interneuron in the IC. To exam the functional differences between excitatory and inhibitory neurons, we injected AAV-flex-GCaMP6 to the IC of VGlut2-Cre or VGAT-Cre mice, and performed in vivo two photon calcium imaging to monitor the auditory feature that encoded by excitatory and inhibitory neurons. Spectral and temporal coding properties were resolved by pure tones and sinusoidal amplitude modulation (SAM) stimulus, respectively. In contrast to the broad tuning width of inhibitory neurons which had been widely observed in the cortex, the results show that inhibitory neurons in the IC had sharper spectral tuning width than excitatory neurons. For temporal coding properties, the inhibitory neurons usually preferred the low modulation SAM stimulus, while the excitatory neurons had preference for modulation frequency as high as 500Hz. For both cell types, the response profiles of neighboring neurons

were similar in general, but neuron pairs with quite heterogeneous response were always found. The physiological differences of excitatory and inhibitory cell types could be partially explained by their biased excitatory bottom-up and feedback inputs based on anatomy.

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Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

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Program#/Poster#: 326.04/DD6

Topic: D.05. Audition

Support: R01DC015138

Title: Nonstationary correlation statistics allow robust sound category identification

Authors: M. SADEGHI, I. H. STEVENSON, *M. A. ESCABI;
Univ. of Connecticut, Storrs, CT

Abstract: Natural and man-made sounds, including both background and vocalized sounds, can vary on multiple time and frequency scales and exhibit unique statistical regularities in the sound spectrogram. Recently, it has been demonstrated that humans perceive and utilize high-order statistical regularities to categorize and discriminate sounds. Furthermore, neurons in the central auditory system can respond selectively to various high-order sound statistics, including the modulation spectrum and correlation structure of the sound. Here we test the hypothesis that nonstationary high-order statistics derived from computational auditory cochlear model enable discrimination and identification of sound categories from a computational standpoint. We used a catalogue of natural and man-made sounds to test the information carrying content of various high-order sounds statistics. Time-varying statistics related to the sound correlation structure and modulation spectrum where extracted for each sound at time-scales comparable to perceptual and neural (auditory cortex) integration times. For each sound category we construct a high-dimensional prior over sound statistics. Then using naïve Bayes classification, we show that the these high-order statistics allow categorizing sounds at time-scales comparable to neural and perceptual integration.

Disclosures: **M. Sadeghi:** None. **I.H. Stevenson:** None. **M.A. Escabi:** None.

Poster

326. Auditory Processing: Subcortical

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Topic: D.05. Audition

Support: NIDCD R00-DC009635 (RCF)

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Hirschl/Weill-Caulier Career Research Award (RCF)

Sloan Research Fellowship (RCF)

Title: Learning and performance variability in a rodent model of multi-channel cochlear implant use

Authors: *J. KING[1], I. SHEHU[2], M. A. SVIRSKY[1], R. C. FROEMKE[1];
[1]New York Univ. Sch. of Med., New York, NY; [2]Hunter Col., New York, NY

Abstract: Cochlear implants (CIs) are neuroprosthetic devices that can restore meaningful hearing to the profoundly deaf. However, asymptotic speech recognition and time to reach asymptotic levels are variable, ranging from 0 to 100% speech recognition and from <6 months to >2 years, respectively (Tyler et al., 2000; Chang et al., 2010). To determine neural mechanisms that underlie individual differences in the time course of adaptation to CI stimulation as well as in steady state outcomes, we developed a rat model of CI use. Female Sprague-Dawley rats are first trained on a self-initiated broadband (half-octave pure tone spacing) and narrowband (quarter-octave pure tone spacing) frequency detection task. Once animals have learned both tasks (d'>1.7), they are bilaterally deafened with a combination of ototoxic drugs and intracochlear trauma (>50 dB hearing loss), and unilaterally implanted with an 8-channel array in the scala tympani. Following surgical recovery and objective sound processor programming, the animals are initially tested on the broadband frequency detection task using CI stimulation as their exclusive input; this is their first day of stimulation. They then receive further targeted training to improve their behavioral performance. Day 1 initial performance is highly variable (n=3; d': -0.18, 0.21, and 0.86) but overall relatively poor; over a period of weeks, while learning trajectory and peak performance differ across animals (n=3; d':

0.94, 1.15, and 2.38), all animals improve significantly compared to their initial performance ($p<0.01$). This model of initial and learning variability captures some key features of clinical outcome variability with regards to vowel and word recognition with a CI in post-lingually deaf users.
In order to explore the neural correlates of these phenomena, we are using chronic micro-electrocorticography (μECoG) in CI animals throughout the behavioral testing and learning period with the CI. In collaboration with the Viventi lab, the Froemke lab has developed a μECoG system for recording from auditory cortex in awake, freely moving animals (Insanally et al., 2016). The 60-contact arrays can record stable tonotopic maps over a period of weeks to months. We are expanding this setup to include chronic cortical recordings within the behavior boxes during CI learning and performance. We expect that cortical representations of CI channels will be overlapping or indistinguishable in the initial phase, correlating with relatively poor initial performance, and that the CI channel representations will become more defined with training and experience, correlating to improved behavioral performance with the CI.

Disclosures: **J. King:** None. **I. Shehu:** None. **M.A. Svirsky:** None. **R.C. Froemke:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.06/DD8

Topic: D.05. Audition

Title: The intrinsic physiology of inhibitory brainstem neurons changes during auditory development

Authors: ***B. J. CARROLL**[1], R. BERTRAM[2], R. L. HYSON, 32301[3];
[1]Florida State Univ., Tallahassee, FL; [2]Mathematics, [3]Psychology, The Florida State Univ., Tallahassee, FL

Abstract: Developmental changes in membrane properties promote the ability of excitatory neurons in the brainstem to code aspects of sound, including the timing and amplitude of a stimulus. Some of these changes coincide with the onset of hearing, suggesting that sound-driven activity in the nervous system produces developmental plasticity of ion channel expression. While it is known that the coding properties of excitatory neurons are modulated by inhibition in the mature system, it is unknown whether there are also developmental changes in the membrane properties of brainstem inhibitory neurons. We investigated the primary source of inhibition in the avian auditory brainstem, the superior olivary nucleus (SON), which displays sound-driven activity in mature animals. The present studies test the hypothesis that, as in excitatory neurons,

the membrane properties of these inhibitory neurons also change during the time of hearing onset. We examined SON neurons at different stages of auditory development: embryonic days 14-15 (E14-15), a timepoint at which cochlear ganglion neurons are just beginning to respond to sound, later stages of embryonic development (E18-19), and after hatching (P0-P1). We used *in vitro* whole-cell patch electrophysiology to explore neuronal excitability, measured as a neuron's ability to sustain tonic firing over a range of current steps. The results showed that SON neurons in young embryos were less likely to display tonic firing compared to those of older embryos and hatchlings, particularly at higher current injections. Biophysical models and voltage-clamp recordings were employed to examine how passive and active membrane properties contribute to the observed age-related differences.

Disclosures: **B.J. Carroll:** None. **R. Bertram:** None. **R.L. Hyson:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.07/DD9

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NWO grant #823.02006

FES #0908 'NeuroBasic PharmaPhenomics project'

Title: Resistance to spike depression of a rat central axon terminal during *In vivo* high-frequency firing

Authors: ***M. C. SIERKSMA**, J. G. G. BORST;
Dept. of Neurosci., Erasmus MC, Rotterdam, Netherlands

Abstract: By controlling calcium influx, the shape of the presynaptic action potential (AP) has a large impact on neurotransmitter release. Because of the small size of most terminals in the CNS, little is known about the regulation of their shape during natural firing patterns *in vivo*. The calyx of Held is a giant axosomatic terminal in the auditory brainstem, whose accessibility to patch-clamp recordings in slices has led to a detailed knowledge of its biophysical properties. Here, we report on whole-cell and juxtacellular recordings from calyceal terminals in newborn rat pups. Upon hyperpolarizing current injections the terminal revealed a depolarizing sag. Depolarizing current injections showed substantial outward-rectification and triggered a single, overshooting AP. Already at postnatal day (P)2, the calyx showed a characteristic burst firing pattern during spontaneous activity, which has previously been shown to originate from the cochlea.

Remarkably, despite the young age of the animals (P2-6), even at frequencies over 100 Hz the AP showed little or no depression nor broadening. In contrast, APs that were evoked by constant-current injections did depress and broaden. The rate of rise of the AP depended strongly on the preceding membrane potential, and the membrane potential in between the APs of a high-frequency train predicted whether the APs would depress or even potentiate. Instead of a slow depolarizing after-potential, as previously observed in slices, *in vivo* an AP was often followed by a hyperpolarization, which effectively prevented spike depression. Our findings imply that the *in vivo* shape of the calyceal AP minimizes activity-dependent changes, and show that the calyx of Held is reliably showing high-frequency activity already soon after its formation.

Disclosures: M.C. Sierksma: None. **J.G.G. Borst:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.08/DD10

Topic: D.05. Audition

Title: The role of nitric oxide in modulating neuronal activity in the ventral cochlear nucleus

Authors: *A. HOCKLEY[1,2], J. I. BERGER[1], P. A. SMITH[2], M. N. WALLACE[1], A. R. PALMER[1];
[1]MRC IHR, Nottingham, United Kingdom; [2]Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Tinnitus chronically affects an estimated 10-15% of adults and is characterised by the perception of sound independent of external stimuli. Nitric oxide synthase (NOS) expression has been studied in guinea pig ventral cochlear nucleus (VCN) where it is located in a sub-population of each cell type. Following unilateral acoustic over-exposure, a within-animal asymmetry of NOS expression was found exclusively in the 75% of animals that developed tinnitus (Coomber et al., 2015). The decrease in NOS expression in the contralateral VCN was observed as soon as 1 day after acoustic-over exposure, and the asymmetry in NOS expression was strongest at eight weeks after noise exposure. This provided evidence for a role of nitric oxide (NO) in tinnitus, and not simply as a biomarker for hearing loss.
Here, we describe the use of iontophoresis to apply the NOS inhibitor L-N^G-Nitroarginine methyl ester (L-NAME) to units within the VCN of the anaesthetised guinea pig. Upon identification and characterisation of a single unit, hour-long, pure tone pulse-trains were presented at the characteristic frequency (200 ms tone pip, 800 ms silence, 3600 repeats). The number of spikes per one second sweep were counted, allowing analysis of the changes in auditory-driven or spontaneous activity. An 80nA ejection current was applied through an

iontophoresis barrel containing 50mM L-NAME during a 20 min. period starting 15 min. after the start of the pulse-train; allowing assessment of the impact of blocking NO production on identified neuronal types.
Reducing NO production through NOS inhibition caused a significant increase in spontaneous and auditory-driven firing rate in 20% (2/10) of our VCN unit sample. This effect was found in both chopper and primary-like units. These results indicate that NO has a role within the VCN of reducing neuronal excitability. This effect of NO on excitability may be altered in tinnitus animals, producing a change in transmission with potential to contribute to the 'increased central gain' thought to be present in tinnitus animals.
The next stage will involve application of L-NAME to VCN neurons in guinea pigs following noise exposure and behavioural confirmation of tinnitus, therefore allowing us to determine the functional role of NO in tinnitus.

Disclosures: A. Hockley: None. **J.I. Berger:** None. **P.A. Smith:** None. **M.N. Wallace:** None. **A.R. Palmer:** None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 326.09/DD11

Topic: D.05. Audition

Title: Nitrergic Signalling in the bullfrog IC

Authors: *A. W. STAFFORD;
Biochem. and cellular and molecular biology, Univ. of Tennessee, Knoxville, TN

Abstract: Nitric Oxide (NO) is a gaseous molecule that functions as a retrograde messenger subserving long-term potentiation in the hippocampus where it plays a significant role in learning and memory. Activation of glutamate N-methyl-D-aspartate (NMDA) receptors stimulates NO production via the activity of nitric oxide synthase (NOS). NO is released and subsequently enhances the presynaptic release of glutamate. Staining for β nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) as well as immunohistochemical studies have revealed the presence of NOS-labeled neurons in a number of vertebrate brain structures including the inferior colliculus (IC), an important auditory processing center. These neurons presumably produce and release NO. However, the function of NO in auditory processing at the level of the IC is not known. Here we address this issue using single-unit recording combined with microiontophoresis to investigate the role of NO in the analysis of acoustic signals by neurons in the IC of the American bullfrog *Lithobates catesbeiana*. Of particular interest was

how NO modulates the responses of IC neurons to natural conspecific and heterospecific calls. *In vivo* iontophoretic application of L-NAME (a NOS inhibitor) was used to evaluate the effect of NO on the sound-evoked responses of neurons (n=8) in the IC. No change in best frequency, threshold, or tuning curve of units was seen during L-NAME application. In contrast, unit responses increased to conspecific bullfrog calls while remaining relatively unchanged in their responses to heterospecific calls during L-NAME application. Our data suggest a role for NO in gain control in the IC that may influence the output of neural circuits engaged in the analysis of behaviorally relevant acoustic signals, such as speech.

Disclosures: A.W. Stafford: None.

Poster

326. Auditory Processing: Subcortical

Location: Halls B-H

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Topic: D.05. Audition

Support: NSF - IOS - 1010193

NIH - T32 - DC00046

Title: Sensory and motor activity in the superior colliculus of the actively orienting bat

Authors: *M. J. WOHLGEMUTH, III[1], C. F. MOSS[2];
[2]Psychological and Brain Sci., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: The superior colliculus (SC) is a multimodal structure involved in integrating sensory signals into motor commands for species- specific orienting behaviors. The SC is laminated, with superficial sensory layers sending excitatory projections ventrally into sensorimotor and motor layers. Prior work has investigated the SC in visuomotor orientation in primates, but it is still an open question how sensory signals guide motor commands for species- specific orienting. To bridge this gap, we collected multichannel recordings from the SC of the echolocating bat as it performed natural orienting tasks. The echolocating bat serves as an excellent model for investigating sensorimotor processes in the SC because of the temporally discrete active sensing system it uses to probe the environment. The bat's spatial orientation behaviors involve the production of sonar vocalizations that return sonar echoes, and these echoes then influence motor planning for the next vocalization. Whereas primate studies often use "delay- period" activity before the initiation of a saccade to study how sensory activity affects motor planning, the orienting behaviors of echolocating bat naturally include delay-

periods between the reception of the echo and production of the next sonar vocalization. Furthermore, the timing of echo arrival and sonar vocal onset provide millisecond resolution for analyzing behaviorally relevant neural signals in the SC. We hypothesize that the orienting behaviors of the bat are tied to coordinated activity patterns across pools of SC neurons, such that superficial layer neurons are active during echo arrival, intermediate layer neurons are active for echo arrival and sonar vocal production, and deep layer neurons are active for sonar vocal production. To test this hypothesis, we designed a behavioral paradigm where the bat tracked and intercepted prey from a stationary position while SC recordings were collected simultaneously across laminae. We find that superficial neurons are indeed active during echo arrival, intermediate neurons are active before vocal onset and again at echo arrival time, and the deep layer neurons are active before vocal onset. This sensorimotor- timed activity suggests that a cascade of activity travels through the dorsal/ventral axis of the SC. In addition, the timing of individual neuronal activity indicates functionally relevant connections for sensorimotor integration in the SC. Our data present a novel data on population level activity related to sensation and motor action for spatial orienting behaviors in the SC; and also pose new questions on the nature of sensorimotor signals propagating throughout the SC laminae.

Disclosures: M.J. Wohlgemuth: None. **C.F. Moss:** None.

Poster

327. Auditory Processing: Coding and Theory

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Title: Neuronal activity packets as basic units of neuronal code

Authors: *A. LUCZAK[1], B. L. MCNAUGHTON[1], K. D. HARRIS[2];
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Abstract: Neurons are active in a coordinated fashion, for example, an onset response to sensory stimuli usually evokes a 50-100ms long burst of population activity. Recently it has been shown that such 'packets' of neuronal activity are not randomly organized, but rather composed of stereotypical sequential spiking patterns. It has been shown that such packets are ubiquitous feature of stimulus evoked as well as of spontaneous network activity, and are present across different brain states. Although these packets have a generally conserved sequential spiking structure, the exact timing and number of spikes fired by each neuron within a packet can be modified depending on the stimuli. Here we present evidence that packets can be a good candidate for basic building blocks or 'the words' of neuronal coding, and can explain the mechanisms underlying multiple recent observations about neuronal coding, such as: multiplexing, LFP phase coding, and provide a possible connection between memory preplay and replay.

Disclosures: **A. Luczak:** None. **B.L. McNaughton:** None. **K.D. Harris:** None.

Poster

327. Auditory Processing: Coding and Theory

Location: Halls B-H

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Program#/Poster#: 327.02/DD14

Topic: D.05. Audition

Support: NIH Grant DC014765

Title: Multi-channel open-loop thalamo-reticular architectures support thalamocortical wave propagation

Authors: *J. W. BROWN[1], D. A. LLANO[2];
[1]Col. of Med., [2]Neurosci. Program, Dept. of Mol. and Integrative Physiology, Beckman Inst., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Although the function of the thalamic reticular nucleus (TRN) remains poorly understood, both experimental studies and computational models have potentially implicated this elusive structure in phenomena ranging from attention and multi-sensory integration to epileptiform activity. It was recently demonstrated that a single-channel “open-loop” thalamo-reticular model, in which the TRN neuron is not reciprocally excited by thalamocortical (TC) neuron it inhibits, can paradoxically enhance TC output and consequently cortical activity across a broad parameter space defined by the input frequencies to the TC and TRN neurons. Here, we extend this open-loop architecture to include five thalamo-reticulo-cortical columns, representing distal, sequentially interconnected loci within a thalamocortical network. The most basic version

of this circuit, based on elaborating the single-column model developed by Willis et al. (2015), is able to sustain the propagation of spindle-like waves across the length of the network at a constant velocity. We demonstrate that the spatiotemporal profile of this wave propagation, including its periodicity and decay parameters, can vary robustly as a function of TC/TRN input profiles (e.g., Poisson-based, periodic) and adding and tuning intra- and inter-columnar synapses, including both GABAergic and electrical connections between TRN neurons. These findings are related to the physiology underlying both normal and pathological processes and compared to closed-loop models capable of approximating the same phenomena.

Disclosures: J.W. Brown: None. **D.A. Llano:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: NIH Grant DC012557

NYU Provost Academic Diversity Fellowship

Title: Nominally non-responisve sensory and frontal cortical cells encode task-relevant variables

Authors: *M. INSANALLY[1], I. CARCEA[1], B. ALBANNA[2], R. FROEMKE[1];
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Abstract: Single-unit activity recorded from behaving animals can often have heterogeneous response profiles. A fraction of recorded cells typically exhibit trial-averaged responses with obvious task-related features, such as pure tone frequency tuning in the auditory cortex, or ramping activity in secondary motor areas. However, a substantial number of cells do not appear to fire in a task-related manner and require different analytical methods. We analyzed single-units recorded from rats during a frequency recognition task in order to identify to what extent task variables are reflected in individual spike trains of every recorded neuron - independently or as part of small ensembles of cells. Adult rats were trained on a go/no frequency recognition task that required them to nosepoke to a single target tone for food reward and withhold from responding to multiple nontarget tones. Using multielectrode arrays we recorded from 66 single-units in the auditory cortex and 57 single-units in the frontal cortex (FR2). While the trial-averaged responses of some cells exhibited obvious and statistically significant task-related features, many cells were nominally non-responsive (31/66 AC cells and 35/57 FR2 cells from

six animals had neither significant tone-modulated activity or ramping activity; $p < 0.05$, 5,000 bootstraps). This variable activity is the only information available to downstream cells and circuits and must be decoded by other brain regions in real time on single trials. Accordingly, we devised a novel spike-timing based algorithm for trial-by-trial decoding. We found that: 1) Nominally non-responsive neurons represent behavioral variables. Our analysis shows that, in fact, the activity of cells that seem unresponsive when trial-averaged can and often do reflect basic differences in sensory stimulus encoding and decision-making. 2) We identified many 'multiplexed' neurons that simultaneously represented both the sensory input and the upcoming behavioral decision (43% of cells were multiplexed, with accuracy index ≥ 0.05 for both stimulus category and choice). 3) Frontal cortex has a better representation of task-relevant auditory stimuli than auditory cortex. Auditory cortex reliably responds to pure tones in untrained animals. However, when tones take on behavioral significance, this information is encoded more accurately in frontal cortex, suggesting that this region is critical for identifying the appropriate sensory-motor association.

Disclosures: **M. Insanally:** None. **I. Carcea:** None. **B. Albanna:** None. **R. Froemke:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: Wellcome Trust NIH PhD Studentship

Title: Top-down and bottom-up control through distinct phase-amplitude couplings in macaque auditory cortex

Authors: ***C. D. MÁRTON**[1,2], M. FUKUSHIMA[3,2], S. SCHULTZ[1], B. B. AVERBECK[2]; [1]Neural Coding Lab, Imperial Col. London, London, United Kingdom; [2]Lab. of Neuropsychology, Unit on Learning and Decision Making, NIH/NIMH, Bethesda, MD; [3]Lab. for Marmoset Neural Architecture, RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Cross-frequency coupling of neuronal oscillations is well-documented as a phenomenon across different tasks and regions. However, it remains poorly understood with regards to its function in information processing (Aru et. al., Curr Opin Neurobiol, 31:51-61, 2015). Here we analyzed local field potential signals from simultaneous multi-site recordings in the macaque auditory cortex to systematically investigate the prevalence of cross-frequency couplings (CFC) across the auditory hierarchy. Three monkeys were implanted with micro-

ECoG arrays, and electrodes were partitioned into four sectors along a caudorostral axis in the supratemporal plane (STP). Recordings were made while awake monkeys listened to 20 different types of conspecific vocalizations (Fukushima et. al., J Neurosci, 34(13): 4665-4676, 2014). Quantifying phase-amplitude CFC using multivariate regression techniques, we find Theta to Alpha & Low Gamma and Alpha to Low & High Gamma couplings significantly more pronounced ($p<0.01$) in the top-down direction, and Alpha to Theta & Beta ($p<0.01$) in the bottom-up direction (*Figure 1).* The results depicted here are from one monkey. The pattern of higher Alpha to Gamma remains consistent ($p<0.01$), albeit less widespread among Gamma bands, and mostly restricted to specific sector pairs in the remaining two animals. The findings raise the need to incorporate top-down effects into current models of Theta to Gamma CFC (Hyafil et. al., eLife, 4: 3958, 2015). Moreover, selected CFCs (black box, *Figure 1*) were found to allow classification of coo- vs. non-coo type calls with an average loss rate of ~7% (SVM classifier, leave-one-out validation), suggesting CFCs play a role in stimulus processing. Top-down phase-amplitude CFCs (from regions higher in the auditory hierarchy to lower ones) may allow for enhanced stimulus processing in early areas (Luczak et. al., Nature, 16(12):745-755, 2015; Giraud & Poeppel, Nature Neurosci., 15(4): 511-517, 2012).

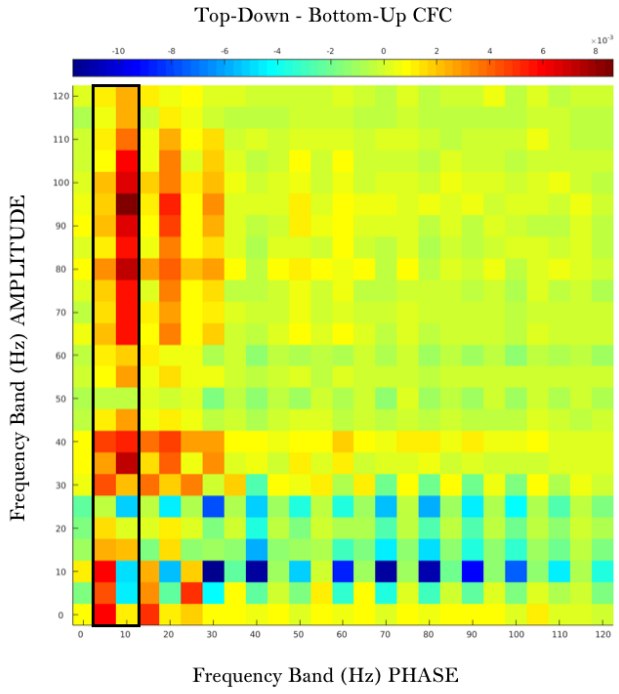

Figure 1: Difference between top-down and bottom-up cross-regional phase-amplitude coupling (i.e. Theta (Sector 4, located at rostral end of STP) to Gamma (Sector 1, located at the caudal end of the STP), depicted as a mean across all possible cross-regional combinations between the four sectors in the STP. The repetitive bands reflect harmonic effects of low-frequency phenomena.

Disclosures: C.D. Márton: None. **M. Fukushima:** None. **S. Schultz:** None. **B.B. Averbeck:** None.

Poster

327. Auditory Processing: Coding and Theory

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 327.05/DD17

Topic: D.05. Audition

Title: Coding sound loudness by pulse amplitude or pulse duration in cochlear implants: does it matter for cortical neurons?

Authors: *V. ADENIS[1], P. STAHL[2], D. GNASIA[2], B. GOURÉVITCH[1], J.-M. EDELINE[1]; [1]Neuro-Psi, Orsay, France; [2]Neurelec / Oticon Med., Vallauris, France

Abstract: Improving the coding strategies in cochlear implants (CI) is still the subject of intense investigations: the stimulation mode, the pulse shape and grounding schemes can lead moderate to important effects on the spread of excitation, electrode discriminability and nerve excitability. For example, several strategies are currently used to code loudness such as increasing the pulse amplitude, the pulse duration or the pulse rate. This study aims at comparing the responses obtained from the eighth nerve and the auditory cortex to stimulations delivered through a cochlear implant and for which increases in sound loudness were coded either by pulse amplitude or by pulse duration. Experiments were performed in urethane anesthetized guinea pigs (6-18months old). A map of the primary auditory cortex (AI) was first established by inserting an array of 16 electrodes (2 rows of 8 electrodes separated by 1mm and 350µm within a row) and quantifying the tonotopic gradient in AI based on multiunit recordings. A dedicated electrode-arrays (400µm) was then inserted in the cochlea (4 electrodes inserted in the 1st basal turn) and its connector was secured on the skull. The electrode array was placed back in the auditory cortex at the exact same location. A dedicated stimulation platform developed by Neurelec/Oticon was used to generate electrical current delivered by the implanted electrode. The eight nerve fibers were then stimulated with 20 levels of pulse amplitude or 20 levels of pulse duration generating similar charges. The patterns of cortical sites activated corresponded to the CI stimulating electrode. Furthermore, little differences in the cortical responses were found when the pulse duration was used rather than the pulse amplitude. The cortical responses evoked by electrical stimulations were often of shorter duration than the acoustic responses; the firing rate evoked by both the pulse duration and pulse amplitude strategies was usually higher than the one evoked by pure tones. These data suggest that, at the level of the primary auditory cortex, equivalent cortical activation can be achieved by coding sound loudness with pulse amplitude or with pulse duration.

Disclosures: **V. Adenis:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neurelec/Oticon Medical. **P. Stahl:** A. Employment/Salary (full or part-

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Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: NIH Grant 1355065

Title: Distinct timescales for neural discrimination of sound envelope shape in three auditory cortical fields.

Authors: *A. OSMAN[1], C. LEE[2], M. ESCABI[3], H. READ[4];
[1]Biomed. Engin., [2]Behavioral Neurosci., [3]Biomed Engin., Electrical Engin., [4]Behavioral Neuroscience, Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Mammals discriminate sounds based on perception of timbre and loudness due to temporal shape cues including duration and rate of change in sound envelope amplitude (Iverson and Krumhansl 1993; Irino and Patterson 1996, Drullman, Festen et al., 1994; Geffen et al., 2011). Mammals need cortex to detect temporal sound cues but the underlying cortical circuits and coding mechanisms for this ability remain unknown. Here we propose that cortical neuron spike timing patterns can be used to discriminate differences in sound envelope shape cues in three auditory cortical fields of the rat.
Single neuron response spike trains were recorded from layer 4 neurons in surgically exposed primary (A1), ventral (VAF) and caudal suprarhinal (cSRAF) auditory cortical fields of the rat as the physiology and corresponding thalamocortical pathways have been well described (Polley et al., 2007, Storace et al., 2010, 2011, 2012, Higgins et al., 2010). Spiking responses were probed with fifty-five unique noise burst sequences that varied in noise burst duration but had fixed noise burst repetition rate. To evaluate temporal patterns, spike trains were convolved with an exponential kernel having an evaluation window or time constant, Tc (van Rossum, 2001). A sound sequence discrimination index (d-prime) was computed from the evaluated spike train responses (Zheng and Escabi, 2012, Gai and Carney, 2008). Next, for each pair of spike trains, the Tc was varied between 1 and 256 ms to determine time window yielding optimal (aka maximal) sound sequence discrimination.
In all fields, we find a rank order increase in the synchronized response duration with A1 < VAF < cSRAF to any given sound shape (Lee et al., 2016). Similarly, here we find a rank order increase in Tc yielding best discrimination with: A1 < VAF < cSRAF (logarithmic means: A1:

33 ms (1.03), VAF: 39 ms (1.02), cSRAF: 44 ms (1.03), $p < 0.001$). A1 neurons discriminate small differences in sound envelope shape (e.g. 2Hz vs 4Hz). Whereas, VAF and SRAF are better than A1 at discriminating large differences in sound envelope shape (e.g. 2Hz vs 64Hz). These results indicate that cortical neuron spike-timing patterns can be used to discrimination of sound envelope temporal shape cues. Furthermore, distinct response timescales allow for complementary sound shape discrimination in primary versus non-primary auditory cortices. The multi-scaled organization could in theory provide a behavioral advantage to mammals for discriminating temporal shape cues in the sound envelope.

Disclosures: **A. Osman:** None. **C. Lee:** None. **M. Escabi:** None. **H. Read:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: NIH Grant 5R01DC013906

Title: Interactions of simultaneous sound representations in the primate inferior colliculus

Authors: ***S. M. WILLETT**, V. C. CARUSO, S. T. TOKDAR, J. M. GROH;
Duke Univ., Durham, NC

Abstract: When multiple stimuli activate overlapping populations of neurons, it is unclear how the brain preserves information about each stimulus. Two possible mechanisms that might support the accurate perception of multiple stimuli involve sorting neurons into subpopulations either across time or based on their tuning properties. Such mechanisms limit the number of stimuli encoded by any given neuron at any given time, thereby, enhancing the degree to which different stimuli are encoded by different neurons. We have previously found evidence of the sorting-across-time mechanism in the inferior colliculus (IC) by using multiple simultaneous sounds of similar frequencies, so that each individual sound activated similar populations of neurons (Caruso et. al, 2015, Soc. Neuro Abstr DP07.04/DP04). Here, we tested whether this sorting-across-time mechanism (time-division multiplexing) also occurs for more dissimilar sound frequencies eliciting a lower degree of overlap in neural representations. In this case an alternative coding mechanism could involve the refinement of the tuning of individual cells, leading to separate populations of neurons representing each sound. Thus, we investigated the responses of individual neurons in the IC in the presence of multiple sounds drawn from a larger range of frequencies. Single neurons were recorded in an awake, behaving rhesus macaque

during a dual sound localization task. The monkey was required to make either a single saccade to the location of one sound or a sequence of saccades to the locations of two simultaneously presented sounds. Frequency tuning was measured by presenting a fixed set of eight bandpass filtered noise frequencies alone or in combination with either a lower or higher frequency noise. As in our previous study, spike counts on single sound trials were modeled by Poisson distributions. The spike counts on dual sound trials were tested to see if they were best described by a mixture of the Poisson distributions observed on single sound trials, consistent with a sorting-across-time coding mechanism vs. other possibilities consistent with averaging, winner-take-all, or summation. We found that sorting-across-time was evident in some neurons even for sounds that were quite different from each other in frequency. Additionally, we found sharpening in neural tuning supporting an increased separation between representations of simultaneous sounds. Overall, these data suggest the IC could be using multiple mechanisms to increase encoding efficiency.

Disclosures: **S.M. Willett:** None. **V.C. Caruso:** None. **S.T. Tokdar:** None. **J.M. Groh:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: ERC Starter Grant (CHIME)

Title: Persistent activity in auditory cortex during passive listening

Authors: J. LEE[1], *J. E. COOKE[1], X. WANG[2], D. BENDOR[1];
[1]UCL, London, United Kingdom; [2]Johns Hopkins Univ., Balitmore, MD

Abstract: Persistent activity, the elevated firing of a neuron after the termination of a stimulus, is hypothesized to play a critical role in working memory. This form of activity is therefore typically studied within the context of a behavioural task, which includes a working memory component. Here we investigated whether persistent activity is observed in primary sensory cortex in the absence of any explicit behavioural task. We recorded spiking activity from single units in the auditory cortex (A1, R and RT) of awake, passively-listening marmosets. We observed persistent activity that lasted from a few hundred milliseconds to many seconds following the termination of the acoustic stimulus, in the absence of a task. Phasic persistent activity primarily occurred in units that showed strong phasic onset responses while sustained offset responses primarily occurred in units that showed sustained responses during the stimulus.

Long duration persistent activity, on the order of seconds, was observed primarily in units that showed dramatic suppression in during the stimulus. Given that these responses were observed in passively listening animals, persistent activity in sensory cortex may have functional importance beyond storing behaviourally relevant information in working memory. These findings also indicate that, despite the prevalence of balanced excitation and inhibition observed in A1 neurons, a range of unbalanced excitation-inhibition dynamics may exist in marmoset auditory cortex.

Disclosures: **J. Lee:** None. **J.E. Cooke:** None. **X. Wang:** None. **D. Bendor:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: NIH Grant R01DC008983

NIH Grant R01EY019049

David and Lucile Packard Foundation (Packard Fellowships for Science and Engineering)

Title: Predominance of dormant sensory neurons and learning-induced recruitment in auditory cortex

Authors: *X. CHOU[1,2], F. LIANG[1,3,5], H. LI[1,3,5], M. ZHOU[1,3], Q. FANG[1,2], H. W. TAO[1,4], L. I. ZHANG[1,3];
[1]Zilkha Neurogenetic Inst., Los Angeles, CA; [2]Neurosci. Grad. Program, [3]Dept. of Physiol. and Biophysics, [4]Dept. of Cell and Neurobio., USC, Los Angeles, CA; [5]Dept. of Physiology, Sch. of Basic Med. Sci., Southern Med. Univ., Guangzhou, China

Abstract: The general principle for how the external world is represented by neuronal populations in sensory cortex remains being actively investigated. In awake mouse primary auditory cortex, we surprisingly discovered that predominant neurons in layer 2/3 were dormant, showing no spike responses to sound stimuli of any fundamental category, while the remaining neurons responded to a broad variety of sound categories. Interestingly, dormant neurons all received sound-evoked synaptic inputs, but spiking failed due to a weak excitatory drive and low excitation/inhibition (E/I) ratio. The cortical sparseness developmentally emerged in an acoustic-experience dependent manner, along with differentiation of E/I ratio from a unimodal to bimodal

distribution. Furthermore, the sparseness can be down-regulated by associative learning in a stimulus-specific manner, through modulating layer 1 inhibitory circuits. Thus, the majority of supragranular neurons are on reserve for sensory representation, and recruitments of these under-committed cells can contribute importantly to functional evolution and plasticity of the brain.

Disclosures: **X. Chou:** None. **F. Liang:** None. **H. Li:** None. **M. Zhou:** None. **Q. Fang:** None. **H.W. Tao:** None. **L.I. Zhang:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: Seoul National University Boramae Medical Center research fund 03-2014-2

Title: Neurofilament heavy chain expression and neuroplasticity in rat auditory cortex after unilateral and bilateral deafness

Authors: ***M.-H. PARK**[1], H. LEE[1], S. OH[2];
[1]SMG-SNU Boramae Med. Ctr., Seoul, Korea, Republic of; [2]Seoul Natl. University, Col. of Med., Seoul, Korea, Republic of

Abstract: Deafness induces many plastic changes in the auditory neural system. For instance, dendritic changes cause synaptic changes in neural cells. SMI-32, a monoclonal antibody to neurofilament protein, reveals auditory areas and recognizes non-phosphorylated epitopes on medium- and high-molecular-weight subunits of neurofilament proteins in cortical pyramidal neuron dendrites. We investigated SMI-32-immunoreactive (-ir) protein levels in the auditory cortices of rats with induced unilateral and bilateral deafness. Adult male Sprague-Dawley rats were divided into unilateral deafness (UD), bilateral deafness (BD) and control groups. Deafness was induced by cochlear ablation. All rats were euthanized, and the auditory cortices were harvested for real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot analyses at 2, 4, 6, and 12 weeks after deafness was induced. Immunohistochemical staining was performed to evaluate the location of SMI-32-ir neurons. SMI-32 mRNA expression and SMI-32-ir protein levels were increased in the BD group. In particular, SMI-32-ir protein levels increased significantly 6 and 12 weeks after deafness was induced. In contrast, no significant changes were detected in the right or left auditory cortices at any time point in the UD group. Taken together, BD induced plastic changes in the auditory cortex, whereas UD did not affect

the auditory neural system sufficiently to show plastic changes, as measured by neurofilament protein level.

Disclosures: **M. Park:** None. **H. Lee:** None. **S. Oh:** None.

Poster

327. Auditory Processing: Coding and Theory

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Topic: D.05. Audition

Support: NIH R01 DC 011092

Title: Neuronal adaptation to background sound level statistics in the inferior colliculus of macaques.

Authors: F. ROCCHI, *R. RAMACHANDRAN;
Dept. of Hearing & Speech Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: The detectability of a target sound embedded in background noise is affected by the properties of the acoustic scene. The responses of auditory neurons change systematically depending on the spatiotemporal relationship between foreground and background sound levels. Recent physiological studies conducted on anesthetized animals suggested that responses of auditory neurons adapt to the statistics of the environment. It is generally accepted that changes of the mean sound pressure level lead to significant variations in the neuronal activity. However, it is still unclear how such adaptation to environmental statistics may influence signal detection and the underlying neuronal responses in awake behaving subjects. To answer that question, we measured the responses of well isolated single units in the inferior colliculus (IC) of nonhuman primates (*Macaca mulatta* and *Macaca radiata*) while these subjects performed a reaction time Go/No-Go detection task. Monkeys were trained to detect a 50 ms tone that was either simultaneously gated with a burst of noise or embedded within a continuous noise background, whose amplitudes were randomly sampled (every 50 ms) from a distribution with high and low probability regions. The mean of the distribution (centroid of the high probability region) matched the sound pressure level used in the gated noise condition, and was played simultaneously with the tone. Similar to anesthetized animals, awake macaque IC neuronal responses adapted to the mean noise amplitude by shifting their dynamic range to higher sound levels. However, neuronal adaptation was significantly modified by the addition of a foreground stimulus. Although behavioral and IC neurometric threshold shifts to tones were not affected by backgrounds statistics, the rate responses of IC neurons reflected the statistical noise properties.

Simultaneously gated noise caused higher baseline responses and higher rate compression compared to the noise distribution. Behavioral and IC neurometric thresholds did not vary with the width of the high probability region (variance). However, the slope of IC neurometric functions was observed to be significantly shallower when the variance was increased, suggesting that neuronal sensitivity might change as a function of the variability within the acoustic scene. Additionally, neuronal response to each 50 ms of noise amplitude was affected by the sound pressure level of the prior noise, implying that variables other than statistics may cause adaptation. Our results indicate that the mean of the sound pressure levels might not be the primary factor in the neuronal adaptation process.

Disclosures: F. Rocchi: None. **R. Ramachandran:** None.

Poster

327. Auditory Processing: Coding and Theory

Location: Halls B-H

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Program#/Poster#: 327.12/EE7

Topic: D.05. Audition

Title: A novel method for analyzing cortical steady state responses

Authors: *P. KRAUSS, A. SCHILLING, C. METZNER, K. TZIRIDIS, H. SCHULZE; Univ. of Erlangen-Nurnberg, Erlangen, Germany

Abstract: We present a novel method for analyzing and modeling high-dimensional data such as multichannel cortical recordings. Our method is derived from multidimensional scaling (MDS), a datamining method which aims to project N points from high-dimensional space onto a lower dimensional target space such that all mutual Euclidean distances are preserved. However, a fundamental shortcoming of classical MDS is the impossibility of assigning coordinates in target space to points that have not been taken into account for the previous scaling procedure. To do so requires performing a new run of the scaling procedure with all previous points plus the additional new point, thus making it impractical for applications where the coordinates of new points have to be estimated, since the computational complexity of MDS is of order $O(N) \sim N^2$. Overcoming this problem we project a grid from k-dimensional space to 2-dimensional target space while preserving all mutual distances. Thus new data points can directly be projected without performing new computations by simply mapping them to the nearest grid point. Furthermore, we generalize classical MDS to non-Euclidean distances such as the Mahalanobis distance. We investigate spontaneous and stimulus (pure tones lasting 120 seconds) driven steady state activity of auditory cortex using 16-electrode arrays. The temporal development of the spatial activity pattern across the channels corresponds to a trajectory in an abstract 16-

dimensional state space. Projecting trajectories corresponding to different conditions reveals attractor-like dynamics. In addition, we use our animal model to induce auditory phantom percepts (tinnitus). The development of tinnitus corresponds to an increasing resemblance of spontaneous activity to stimulus driven activity. Our method enables inferring the pitch of the tinnitus precept from recorded data, which we validate using a behavioral tinnitus assessment paradigm.

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Poster

327. Auditory Processing: Coding and Theory

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Support: Medical Research Council (UK) grant MR/L022311/1

Title: The impact of hearing loss on the neural representation of speech in noise in the gerbil auditory midbrain

Authors: *J. A. GARCIA-LAZARO[1], D. MCALPINE[2], R. SCHAETTE[1];
[1]Ear Inst., London, United Kingdom; [2]The Australian Hearing Hub, Macquarie Univ., Sydney, Australia

Abstract: The healthy human auditory system is capable of understanding speech in complex 'cocktail party' environments, even when the target speech may be quieter than the surrounding babble. However, understanding speech in the presence of noise is considerably degraded for those with hearing impairment, who struggle not only with a loss of audibility, but also with a loss of intelligibility. Even with hearing aids, they are often able to 'hear' speech in background noise, but they are unable to understand it. To determine which factors contribute to the loss of speech intelligibility, we used an animal model to investigate how a controlled acoustic noise insult impacts on the neural representation of speech-in-noise in the auditory midbrain. Hearing loss was induced in adult gerbils through exposure to octave band noise (2-4 kHz) at 105 dB SPL for 2 hours under anaesthesia. Control animals received a sham exposure. Auditory brainstem response (ABR) thresholds were measured before and after noise insult, and after a recovery period of 4 weeks. We performed *in vivo* electrophysiological recordings in the inferior colliculus (IC) under ketamine/xylazine anesthesia using 32-channel tetrode arrays. To investigate the neural representation of speech in noise, we presented 16 different vowel-

consonant-vowel (VCV) tokens from an adult female talker in speech-shaped noise (at either 60 or 75 dB SPL), with signal-to-noise ratios (SNRs) varying from -12 to +12 dB.
In noise-exposed animals, we observed a marked increase in neuronal response strength for the 60, but not the 75 dB SPL condition. To determine the effects on the neural representation of speech in noise, we used a neurogram-based distance metric and a PSTH-based classifier to assess how well the individual VCV stimuli could be discriminated based on the neural responses. In noise-exposed animals, there was a decrease in discrimination performance when background noise intensity was increased from 60 to 75 dB SPL. In contrast, performance increased slightly in the control group. These findings are consistent with noise-induced damage to low spontaneous rate fibers in the auditory nerve, and increased neuronal response gain in the central auditory system. Taken together, our results show how peripheral and central processes can impact on functional hearing.

Disclosures: J.A. Garcia-Lazaro: None. **D. McAlpine:** None. **R. Schaette:** None.

Poster

327. Auditory Processing: Coding and Theory

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Support: University of Virginia

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Title: Simultaneous estimation of receptive fields and intrinsic dynamics of auditory neurons using affine-invarient MCMC

Authors: T. D. ROBBINS[1], *C. MELIZA[2,3];
[1]Cognitive Sci. Program, [3]Dept. of Psychology, [2]Univ. of Virginia, Charlottesville, VA

Abstract: Quantitative models of neural responses to sensory input can yield insights into information flow and processing in the brain. For example, spectrotemporal receptive fields (STRFs) of neurons at successive stages of the auditory pathway are increasingly matched with species-specific vocal elements and learned acoustic features. STRF models typically represent spiking with a static non-linearity and a rate-based, inhomogeneous Poisson process, which may fail to represent many features of intrinsic neural dynamics and their contribution to information processing. Recently, statistical methods have been developed to infer parameters and

unmeasured states of dynamical neuron models by statistically assimilating responses to intracellular current injection. Here, we combine dynamical neuron models with linear STRFs to analyze extracellular recordings of auditory responses in the zebra finch pallium. This method takes a Bayesian approach to parameter optimization by using an affine-invariant Markov Chain Monte Carlo algorithm to estimate posterior distributions for each parameter, including the coefficients of the STRF. We validate the technique against simulated data from a range of neuron models and compare to rate-based estimation methods.

Disclosures: T.D. Robbins: None. **C. Meliza:** None.

Poster

327. Auditory Processing: Coding and Theory

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 327.15/EE10

Topic: D.05. Audition

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Title: Combining multi-unit recording and flavoprotein fluorescence imaging reveals field- and layer-specific sound-evoked neural responses in the rodent auditory cortex

Authors: *J. NISHIKAWA, T. HAGA, Y. TACHIBANA, Y. OHTAKA, Y. YANAGAWA, H. OSANAI, T. TATENO;
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Abstract: The auditory cortex (AC) processes complex sounds through its field and laminar structure, however, little is known about the relationship between the structure and functions. In this study, we combined multi-unit recording and flavoprotein fluorescence imaging to reveal field- and layer-specific sound-evoked neural responses in the rodent AC. First, we examined information flow of the AC in sound processing. To identify the primary auditory field (A1) and anterior auditory field (AAF), we conducted flavoprotein fluorescence imaging in urethane-anesthetized rats in response to several tone bursts. Next, we inserted a 16-ch silicon probe to the fields, and recorded single-units and local field potentials (LFPs) to various tone bursts. After cortical layers' identification using LFP recording, we calculated frequency response areas and spectro-temporal receptive fields (STRFs) of each single-units. As a result, the AAF had

significantly higher auditory threshold, longer latency and narrower band properties than the A1. In addition, layer 2/3 (L2/3) had significantly longer latency and narrower frequency-band properties than layer 4 (L4). Layer 5/6 (L5/6) had significantly longer latency and shorter duration than L4. These results indicate that the processed information of sound mainly flows from the A1 to the AAF and, in each field, from L4 to L2/3, and then to L5/6. These results will help us to understand the auditory processing on the hierarchical field and laminar structure in AC. Second, we investigated social-context dependency of the receptive fields. In our previous chronic recording in freely moving mice, success rate for observing auditory response was not high because of individual differences in the location of AC. To improve the success rate, we executed transcranial flavoprotein fluorescence imaging in anesthetized mice before the implantation, and identified the exact location of AC in each animal. Next, we surgically implanted a microdrive with a tungsten electrode exactly into the AC location. These procedures improved the success rate in the chronic neural recording from 26.3% to 100.0%. We recorded neural responses to various tone bursts in social context (with a stranger) and alone context (without a stranger) in their recording cage, and calculated STRFs in both contexts. Consequently, response areas of each STRF shrank both in frequency and time axes. These results suggest that such social context sharpens the tuning of AC neurons.

Disclosures: **J. Nishikawa:** None. **T. Haga:** None. **Y. Tachibana:** None. **Y. Ohtaka:** None. **Y. Yanagawa:** None. **H. Osanai:** None. **T. Tateno:** None.

Poster

328. Motion Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 328.01/EE11

Topic: D.06. Vision

Title: how grit can overcome oculomotor insufficiencies and change gpa

Authors: *T. GORJI[1,2], D. LARRANAGA[3], A. S. HOCHMAN[2], J. R. MIER[4], S. A. DREW[5]; [1]Psychology, California State University, Northridge, Tarzana, CA; [2]Psychology, California State University, Northridge, Northridge, CA; [3]Psychology, California State University, Northridge, Nothridge, CA; [4]California State University, Northridge, Northridge, CA; [5]California State Univ. Northridge, Northridge, CA

Abstract: Introduction: In performing near work tasks individuals may experience symptoms of visual discomfort. Symptoms may include headaches, eyestrain, double vision, blurred vision, and sensitivity to light. Two systems contributing to near work performance are the: (1) Accommodative, in which the lenses thickens or thins while focusing on the target and (2)

Vergence, focusing on the rotation of the eyes inwardly while focusing on the target. Insufficiencies in both these ocular systems can occur and several surveys have been developed to measure visual discomfort associated with these conditions. **Methods**: We administered the Visual Discomfort Survey (VDS) and the Convergence Insufficiency Symptom Survey (CISS), which are closely associated with accommodative insufficiency and convergence insufficiency, respectively. We also administered the Academic Problems Survey (APS) to assess students' academic difficulties as well as a survey to assess grit, the perseverance and passion for long-term goals (Duckwoth et al., 2007). **Results**: We used a Sobel test and multiple linear regressions to evaluate the relationship between oculomotor symptoms (VDS & CISS), APS, and Grit. Our results indicate that Grit acts as a mediator between symptoms and GPA. Grit is also a predictor of APS. **Discussion**: This suggests that Grit will help students to overcome their oculomotor insufficiency symptoms and achieve higher GPA. A Sobel Test revealed that Grit significantly accounts for a portion of the variance in oculomotor symptoms and GPA.

Disclosures: T. Gorji: None. **D. Larranaga:** None. **A.S. Hochman:** None. **J.R. Mier:** None. **S.A. Drew:** None.

Poster

328. Motion Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 328.02/EE12

Topic: D.06. Vision

Title: Apparent motion extrapolates size, shape and brightness.

Authors: *C. CHUNHARAS[1,2], V. RAMACHANDRAN[1];
[1]UCSD, La Jolla, CA; [2]Med., Chulalongkorn University, KCMH, Bangkok, Thailand

Abstract: A large, 2.5 degree, shaded disc A was flashed on the screen followed by a small, 1 degree, disc B shifted horizontally. Disc A was seen to simultaneously move and shrink as expected. Intriguingly, the disc was seen to become 10% smaller at its destination in frame 2 than its true size; a new illusion we call "size extrapolation". We found the effect to be almost as compelling with homogeneous discs which we used in a more formal experiment. To demonstrate that size expansion was correlated with the strength of perceived motion, we varied the SOA between the flashing discs resulting in different magnitude of apparent motion ("Korte's law"). Control experiments showed that the effect was not due to either size contrast, size adaptation or figural after effect. This effect is reminiscent of "visual inertia" in apparent motion, in which a spot in apparent motion has a tendency to continue its trajectory when confronted with a choice. A similar but less compelling effect was observed when the disc

changed shape or brightness rather than size. The extrapolation was then along the corresponding dimension. Curiously, the effect was compelling only for the direction of shrinkage compared to expansion. Our results are a striking example of the principle that perception involves making "educated guesses" or predictions based on the immediate past; and using them to resolve ambiguity in interpreting both the currently ongoing onslaught of sense impressions as well as the immediate future.

Disclosures: C. Chunharas: None. **V. Ramachandran:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: NIH Grant EY022443

University of Wisconsin-Madison School of Medicine and Public Health

Wisconsin Alumni Research Foundation

Title: Neural representation of multiple moving stimuli with competing features in cortical area MT is drastically altered by spatial arrangement of visual stimuli

Authors: *S. WIESNER, X. HUANG;
Neurosci., Univ. of Wisconsin Madison, Madison, WI

Abstract: Objects in the natural world usually have multiple visual features that each can vary in signal strength and the spatial layout of the objects may also vary, making it important to study how the visual system encodes multiple visual stimuli and whether spatial arrangement affects the neural representation. We have shown that neuronal responses in extrastriate area MT elicited by two overlapping, moving stimuli can be described as a weighted sum of the neuronal responses elicited by the individual stimulus components plus a nonlinear interaction term, and the response weight is stronger for the component that has a higher signal strength (Xiao et al., 2014, J Neurophysiol). These results are consistent with the idea of normalization. Here we investigate how MT neurons respond to multiple moving stimuli that have competing visual features and the effect of spatial arrangement of visual stimuli.
We recorded from neurons in area MT of fixating monkeys and used motion coherence and luminance contrast as two competing features. Visual stimuli were two random-dot patches moving simultaneously in different directions separated by 90°. The diameter of each patch was

3°. One patch moved at a high coherence (100%) with a low contrast (36%) (HCoh/LLum), whereas the other moved at a low coherence (60%) with a high contrast (77%) (LCoh/HLum). The two patches were placed within the receptive fields (RFs) of MT neurons, and either overlapped, or were separated with at least 1° gap in-between. We varied the vector-averaged direction of the two patches to characterize direction tuning curves.
We found that although MT response elicited by HCoh/LLum patch alone was significantly stronger than that elicited by LCoh/HLum patch, MT response elicited by both patches was strongly biased to the component response elicited by LCoh/HLum patch when the two patches overlapped. The average response weight for LCoh/HLum patch was 0.9, significantly larger than the weight of 0.2 for HCoh/LLum patch. As the spatial arrangement changed from overlapping to non-overlapping, the mean weight for LCoh/HLum patch reduced to 0.6 and the weight for HCoh/LLum patch increased to 0.8. MT response was now biased to the response elicited by HCoh/LLum patch. The effect of spatial arrangement on response weights was highly significant. When overlapping, the two stimulus patches compete within the RFs of both V1 and MT neurons, whereas when not overlapping, the two patches can only fit and thus compete within the RFs of MT neurons, but not V1 neurons. The effect of spatial arrangement on MT responses may reflect response normalization occurring at different stages of visual processing that favors different stimulus features.

Disclosures: **S. Wiesner:** None. **X. Huang:** None.

Poster

328. Motion Processing

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Support: NIH NEI EY023371

NSF IOS 145704

Alfred P. Sloan Foundation

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Brain Research Foundation

Title: The Spatial temporal filter for motion integration for smooth pursuit eye movements

Authors: *T. MUKHERJEE, C. SIMONCINI, L. C. OSBORNE;
Univ. of Chicago, Chicago, IL

Abstract: Motion processing is integral to how our brain perceives and responds to the natural visual world. Motion is used to identify objects, to track and intercept moving targets, to determine the body's movement through space, to perceive depth, and other behaviors. Despite the enduring interest in motion integration, a direct measure of the space-time filter that the brain imposes on a visual scene has been elusive. This is perhaps because of the challenge posed by measuring a three dimensional function from perceptual reports in psychophysical tasks. We take a different approach. We exploit the close connection between visual motion estimates and smooth eye movements to measure stimulus-response correlations across space and time, computing the space-time filter directly. In pursuit, the eyes rotate smoothly to stabilize the retinal image of a moving target, thereby improving visual acuity. Pursuit can be driven by the motion of small objects against a background and by spatially distributed motion such as random dot kinetograms. In this sense, pursuit bridges two different scales of spatial motion processing, one operating on local motion contrast and another that sums motion over larger visual regions. Motion integration need not be accomplished by a fixed function, so we explore both the commonalities and differences across stimulus forms in both humans and monkeys. These results allow us to evaluate theories of motion integration that have arisen from psychophysical studies and analyses of cortical physiology. We find that motion is not weighted equally within the visual field. The most weight is given to motion a few degrees from the fovea depending on the aperture size, and in front of and slightly above the direction of eye movement. Convolving the filter with an optic-flow based representation of the stimulus allows us to predict eye acceleration for novel stimulus forms. Our data are not consistent with perceptual surround suppression observed in prior studies, hypothesized to arise from the preferential activation of neurons with center-surround receptive fields. Rather we find that uniform textures are processed distinctly from non-uniform textures, in agreement from recent physiological analysis of V1 neuron behavior.

Disclosures: T. Mukherjee: None. **C. Simoncini:** None. **L.C. Osborne:** None.

Poster

328. Motion Processing

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Program#/Poster#: 328.05/EE15

Topic: D.06. Vision

Support: ERC grant Parietalaction

Title: Human motion-responsive regions in intracerebral recordings

Authors: *P. CARDELLICCHIO[1], P. AVANZINI[1], F. CARUANA[1], V. PELLICCIA[2], G. CASACELI[2], G. LO RUSSO[2], G. RIZZOLATTI[1], G. A. ORBAN[1];
[1]Dept. of Neurosci., Univ. of Parma, Parma, Italy; [2]Ctr. per la Chirurgia dell'Epilessia "Claudio Munari", Niguarda Hosp., Milan, Italy

Abstract: Most human fMRI studies have used the contrast moving vs static flow fields to map the motion response regions in the human brain (Tootell et al 1997, Sunaert et al 1999). We investigated the motion responsive regions in the human brain by means of intracerebral recordings made for diagnostic purposes in 55 hemispheres (L=30, R=25) of 51 patients with drug resistant epilepsy. We calculated the optic flow from video clips (240 videos each 2.5 s) depicting various tool and hand actions (Peeters et al., 2009) presented to the participants. We cross-correlated the speed extracted from the optic flow with the intra-cerebrally recorded high gamma power (50-150Hz), and generated correlation maps using the mapping procedure by Avanzini et al 2016. Only regions sufficiently explored (2 leads per disc) and consistently responding (at least 20% significant correlation) were visualized. The motion responsive regions were more extended in the left than the right hemisphere, consistent with a stronger flow in the right than left hemifield. Motion responsive regions in the left hemisphere included regions expected from earlier imaging studies, notably the MT cluster, extending into the LO complex and into the posterior middle temporal gyrus (pMTG), V3C/D and neighboring ventral intraparietal sulcus (VIPS), dorsal intraparietal sulcus anterior/medial (DIPSA/DIPSM) and a retro-insular region (Sunaert et al 1999). Additionally, motion responses were observed in hV4/VO1 and collateral sulcus, in SPL dorsal from DIPSA, in an occipito-parietal cluster extending from PGp, and in a precentral
gyrus site. For each correlating lead, two indices were computed comparing the correlation with hand vs tool flow, and correlation with contralateral vs bilateral optic flow. Most of the extra-occipital regions were more correlated with the bilateral than the contralateral flow, indicating that most RFs in these regions were bilateral. Very intriguingly, several motion regions in left hemisphere fell apart into dorsal sectors dominated by hand videos and ventral ones dominated by tool videos: in pMTG, in the hV4/fusiform region, and most notably, in the PPC, SPL and VIPS being dominated by hand videos and DIPSA/DIPSM and the occipito-parietal cluster by tool videos. These results underscore the importance of the time resolution of intracerebral recordings, reveal motion responsiveness over a wider extent than previously known, and suggest a sorting of tool and natural effector dynamic signals.
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Poster

328. Motion Processing

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Topic: D.06. Vision

Support: DFG Grant EXC307

Max-Planck Society, Germany

Title: A 9.4T human fMRI study reveals differential laminar responses for visual motion in eye- and world-centered reference frames in area V3A

Authors: *F. MOLAEI-VANEGHI[1,2,3], K. SCHEFFLER[1], A. BARTELS[1,2,3];
[1]Max-Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [2]Vision and Cognition Lab, Ctr. for Integrative Neuroscience, Univ. of Tübingen, Tübingen 72076, Germany; [3]Dept. of Psychology, Univ. of Tübingen, Tübingen 72076, Germany

Abstract: Neural mechanisms underlying a stable perception of the world during pursuit eye movements are not fully understood. Both, perceptual stability as well as perception of real-world motion are the product of multi-modal integration between retinal motion (visual motion signals) and efference copies of eye movements (non-visual motion signals). The comparison between these two signals allows differentiating between self-induced motion and external, real-world motion. Recently, pursuit-paradigms revealed that human area V3A responds to motion predominantly in a world-centered rather than in a retina-centered reference frame (Fischer et al., 2012). This indicates that V3A integrates retinal motion with non-retinal eye-movement signals. In this study we combined ultra-high-field (9.4T) human fMRI, state of the art pulse sequences, and laminar analysis to find out if there is a differential involvement of cortical layers in the processing of real world motion compared to retinal motion in area V3A. We used a 2D GE EPI sequence with 0.8 mm isotropic resolution to measure BOLD signal at different cortical depths while subjects performed a visual pursuit task. The paradigm involved a 2x2 design containing real motion and visual pursuit, which allowed separating responses to retinal and real motion while fully controlling for pursuit-related effects. A laminar surface-based analysis method was used to study the relationship between spatial localization and activation strength as a function of cortical depth by sampling the BOLD signal from the superficial, middle, or deep cortical lamina in area V3A.
The results show that signals related to retinal motion were evenly spread across layers with a bias towards deep layers of V3A while real-motion responses had a gradient with a peak in superficial layers. The differential laminar response profile is compatible with differential local processing for the two motion types. The stronger involvement of superficial layers during real motion processing may be indicative of feedback related processing, possibly through mediation

of efference-copy related signals from higher-level regions such as parietal cortex or smooth pursuit fields of the frontal eye fields with which V3A has direct connections. Future studies are needed to clarify the reasons for differential laminar responses and to identify communication pathways leading to V3A.

Disclosures: F. Molaei-Vaneghi: None. **K. Scheffler:** None. **A. Bartels:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: NARSAD

Title: Resolving the apparent motion paradox by rTMS

Authors: *J. CSATLOS[1,2], S. SHIMOJO[3], R. J. BUCHANAN[4,5], Z. NADASDY[6]; [1]Eötvös Lóránd Univ., Budapest, Hungary; [2]Dept. of Theory, Complex Systems and Computat. Neurosci. Group, Wigner Res. Ctr. for Physics, Hungarian Acad. of Sciences, Budapest, Hungary, Budapest, Hungary; [3]Div. of Biol. and Biol. Engin., CALTECH, Pasadena, CA; [4]Neurosurg., Seton Brain and Spine Inst. and UT Austin, Austin, TX; [5]Univ. of Texas at Austin Dell Med. Sch., Austin, TX; [6]St. David's Neurosci. and Spine Inst., Austin, TX

Abstract: Apparent motion (AM), more specifically beta motion as defined by Wertheimer in 1912, is a percept of continuous motion induced by the quick succession of static images. Although the effect is the key principle behind motion pictures and animation, the experience demonstrates a still unresolved paradox. Namely, the observer perceives the motion before the second image is exposed, while the direction of motion, in theory, cannot be resolved until the second image is exposed. This paradox can be approached by two types of models: one type posits that apparent motion is postdictive, i.e. motion percept is generated retrospectively, after the second image is flashed, and the experience is subjectively backdated to an earlier time. We considered an alternative model and hypothesized that AM may rely on a spreading activation to neurons between the cortical representations of the motion inducers. When spreading ring-waves emanating from two cortical sources meet, they generate an interference pattern, which activates neurons along the likely motion trajectory, even though no stimuli were presented between the AM inducers. We tested this latter hypothesis with an experiment done on normal human subjects (n=21), which determined the psychometric function for near-threshold stimuli flashed between two AM inducers. The subjects' task was to discriminate between a low luminance

triangle and a square flashed briefly during the AM sequence between the AM inducers. We showed that the detection threshold between the AM inducers is (i) an exponential function of the distance between the two AM inducers (ANOVA $F=399.75$, $P<0.001$) and (ii) it is periodic. The spatially periodic modulation of threshold is consistent with the proposed interference model. Next, we demonstrated by using rTMS, that this interference in V1-V2 is susceptible to a 100 ms excitatory train of 40 Hz pulses (signed rank test: 17.500, $p = 0.0292$, $df=20,1$). In summary, our results confirm that the spreading activation model can explain the AM paradox. Second, we were able to unravel the causal role of cortical excitatory waves in the modulation of detection threshold along the path of motion perception. Third, our rTMS protocol proved that the spreading activation underlying apparent motion is localized to the V1-V2 areas. Because the cortical interference model necessitates the exposure of second stimulus before the interference is induced, this model is partly consistent with the postdictive model. However, in contrast with the postdictive model, the cortical motion interpolation and concomitant motion readout may start before the presentation of the second phase of the AM inducer.

Disclosures: **J. Csatlos:** None. **S. Shimojo:** None. **R.J. Buchanan:** None. **Z. Nadasdy:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: National Natural Science Foundation of China Project (grant number: 31471048)

Title: Selective computation of path-dependent and -independent rotations in macaque parietal cortex

Authors: ***Z. CHENG**, B. LIU, Y. GU;
Inst. of Neuroscience, CAS, Shanghai City, China

Abstract: We experience complex visual stimulation patterns due to our different kinds of self-motion through the environment. Whether the brain can use the visual information to compute both the heading directions and trajectories of body during natural navigation remains debating and unclear. In the current study, we use optic flow stimuli containing motion parallax information to simulate three self-motion conditions: (1) Path-dependent-rotation condition, where subjects were moving along a curved path without any rotations around the body axis; (2) Path-independent-rotation condition, where subjects were moving along a straight path while rotating one's body either clockwise or counter-clockwise; and (3) Curvilinear motion condition

resulted from matched path-dependent and -independent rotations, where subjects were moving along a curved path while rotating one's body such that the gaze was always kept aligned with the path. We then collected extracellular activities from three cortical areas in which multisensory translation and rotation signals have been reported previously: the dorsal portion of the medial superior temporal area (MSTd), ventral intraparietal area (VIP), and visual posterior sylvian fissure (VPS). We found that the path-dependent rotation signals did not significantly alter the tuning properties of parietal neurons in response to translation stimuli, while the path-independent rotation signals led to significant gain modulation, bandwidth change and preference shift. In the curvilinear motion condition, the responses followed more closely with those in the path-independent rotation. Our data indicates that path-dependent and –independent rotations are selectively represented in the parietal cortex. Therefore, parietal neurons cannot distinguish path-independent rotations versus curvilinear motion based on the visual inputs alone.

Disclosures: Z. Cheng: None. **B. Liu:** None. **Y. Gu:** None.

Poster

328. Motion Processing

Location: Halls B-H

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Program#/Poster#: 328.09/FF1

Topic: D.06. Vision

Support: Hellman Faculty Scholars

Title: Hierarchical effects of contrast and motion coherence in human visual cortex

Authors: *D. BIRMAN, J. GARDNER;
Psychology, Stanford Univ., Stanford, CA

Abstract: Contrast and motion coherence are fundamental visual attributes that control the perceptual strength of random dot displays. Past studies of the representation of these attributes in humans have examined only a few specific cortical regions and have used manipulations in which both contrast and motion coherence changed simultaneously. As human visual cortex is highly sensitive to changes in contrast, isolating responses to motion coherence may have been confounded by contrast changes. Here we independently varied the contrast and coherence of random-dot displays while participants performed an unrelated task and asked how topographically mapped cortical areas throughout the visual hierarchy represent these visual attributes. Methods. We measured BOLD responses in human participants to random dot displays with contrasts of 25-100% and motion coherences of 0-100%. Retinotopic mapping was first performed with drifting bars to identify visual areas. In subsequent sessions participants

were shown two 11.5x14 deg patches of dots, centered 7.75 deg to the left and right of fixation, while performing a fixation task. The patches consisted of equal proportions of black and white dots (21 dots/deg, linearized gamma) which increased in contrast, coherence, or both for 2.5 s before returning to a constant incoherent background of 25% contrast for 2.65 - 11.5 s. BOLD response to each contrast and coherence condition were estimated using a finite impulse response model averaged across voxels in each cortical area. Contrast and coherence sensitivity was determined by finding the Naka-Rushton or linear function, respectively, that best fit the measured BOLD responses. Results. Later cortical areas were less sensitive to changes in stimulus contrast compared to earlier areas (V1-V3), as expected from previous findings. In contrast, a subset of later areas (V3A, MT) showed consistent sensitivity to motion coherence, but earlier areas (V1-V3) did not. The neural response to motion coherence in our data appeared as an increasing function of stimulus coherence, consistent with previous models of MT neural responses (Rees et al., 2000; Simoncelli and Heeger, 1998). Our data confirm that even when attention is directed away from stimuli, MT and V3A parametrically increase response as a function of motion strength. Independently manipulating contrast and motion coherence allows for experimental designs to study how these two ways of changing the strength in visual stimuli determine motion perception.

Disclosures: **D. Birman:** None. **J. Gardner:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: NIH NEI EY023371

NSF IOS 145704

Alfred P. Sloan Foundation

Whitehall Foundation

Brain Research Foundation

Title: Synergistic encoding of multiple visual features in MT neurons and implications for natural vision

Authors: *M. V. MACELLAIO, B. LIU, L. C. OSBORNE;
Neurobio. Dept., Univ. of Chicago, Chicago, IL

Abstract: Most sensory neurons encode information about more than one stimulus parameter, complicating the brain's decoding task. For example, neurons in cortical area MT encode both object motion direction and speed, and the brain must seemingly obtain independent estimates of both in order to guide behavior. Here we examine the computational consequences and benefits of multiplexed sensory information in the context of direction and speed representation in MT. We performed extracellular single-unit recordings in area MT of monkeys performing a fixation task. Random dot patterns moved coherently within apertures scaled to the classical receptive field size. We generated 2D direction-speed, direction-time, and speed-time tuning curves for each neuron from motion steps with pseudorandom direction-speed pairings. We also computed the information about direction, speed, and time encoded by each unit collectively and individually. We find that the encoding is synergistic, meaning that more information is encoded about combinations of variables, e.g. direction+speed, than the sum of the information about each individually. Synergy is often thought to arise based on anisotropies in feature selectivity, such that the tuning for one parameter is influenced by the value of the other. However, we find that the direction-speed tuning for most MT units is separable over the first 200ms of their response to a motion step (index values > 0.95). In comparison, 2D direction-time and speed-time tuning is much less separable (index values > 0.6). Contrary to intuition, separability and synergy are anticorrelated, as we demonstrate analytically with Poisson models. Temporal patterning within spike trains also cannot account for our result: although two-spike synergy is present, it is a small contribution. We find evidence that the brain uses synergy to its advantage by decoding direction and speed jointly to drive smooth pursuit eye movements, causing local fluctuations in eye direction and speed to be correlated. Joint encoding of direction, speed, and time may also benefit motion perception in natural vision: we discover that MT cells preferentially code for qualities of natural motion, especially changes in direction and speed.

Disclosures: **M.V. Macellaio:** None. **B. Liu:** None. **L.C. Osborne:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: JSPS KAKENHI Grant 16K16081

Title: Neural response to object motion-in-depth independent of vergence eye movements

Authors: *A. WADA[1,2], Y. SAKANO[1,2], H. MIZUSHINA[3], H. ANDO[1,2];
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Abstract: Perceiving an object moving toward or away from self has ecological importance including avoidance of approaching objects. The retinal image contains motion components derived by both the object's actual motion and self-originated movements (e.g., eye movements). Therefore, a computation to dissociate these different sources of retinal motion is necessary to accurately perceive object motion in the external world. The current study investigated the still unexplored neural mechanism underlying the detection of object motion in the depth dimension. We conducted a preliminary fMRI experiment, aimed at identifying neural responses to object motion-in-depth defined by binocular disparity that are invariant to the presence of vergence eye movement. Object motion stimuli were random dots that sinusoidally oscillated in depth provided by changes in binocular disparity. We also controlled vergence eye movement, of which target trajectory in depth was set identical to that of random dots to cancel the retinal motion induced by object motion. The experimental paradigm of Fischer et al. (2012) was adapted, in which the presence of each of the object motion and eye movement were combined to yield four experimental conditions: (A) object motion with vergence, (B) no object motion with vergence, (C) object motion without vergence, and (D) no object motion without vergence conditions. Here, retinal motion induced by either vergence or object motion was present in conditions B or C, respectively. On the other hand, retinal motion was either canceled by vergence or absent in conditions A or D, respectively. The design enabled us to separately identify the effect of object (A and C vs. B and D) and retinal (B and C vs. A and D) motion-in-depth. Six normal subjects (four males) participated in the fMRI experiment. We also ran two functional (motion and retinotopic) localizer experiments to identify visual areas V1, V3A, V3B, and hMT+. A region-of-interest analysis identified significant effect of both object and retinal motion-in-depth in V3A, V3B, and hMT+, whereas only V3A among the assessed regions exhibited a significantly larger effect of object compared to retinal motion-in-depth. In contrast to previous studies that reported the involvement of hMT+ in estimating visual motion-in-depth from binocular information, our present results may further suggest a distinct role of V3A in computing object motion-in-depth by discounting the retinal motion component originating in vergence eye movements.

Disclosures: **A. Wada:** None. **Y. Sakano:** None. **H. Mizushina:** None. **H. Ando:** None.

Poster

328. Motion Processing

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Title: Synchronous human motion is processed by two distinct mechanisms in the brain

Authors: *N. ALP[1], A. NIKOLAEV[2], N. KOGO[2], J. WAGEMANS[2];
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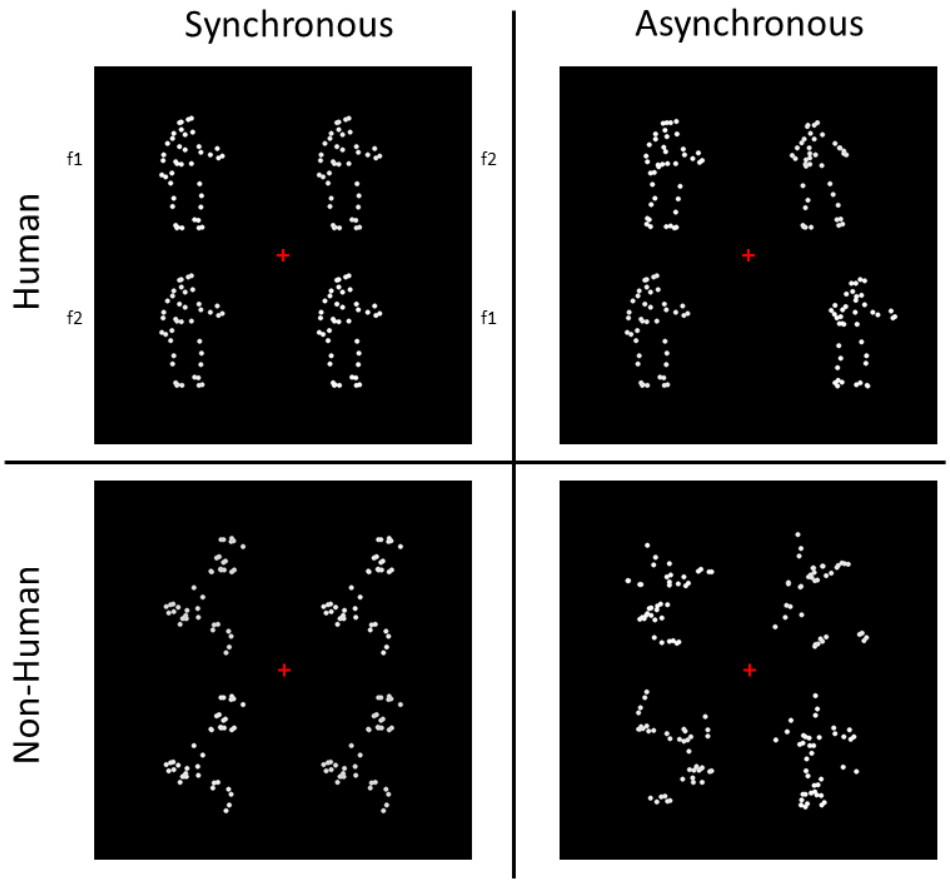
Abstract: The brain has dedicated networks for perception of motion synchrony and perception of interacting human bodies. However, it is not clear to what extent the corresponding perceptual processes in the visual system are similar or distinct. Here, we manipulated the temporal coherence of the motion patterns (synchronous vs asynchronous) and the biological agency (human vs non-human) of the multiple point-light displays (PLDs) in a 2X2 experiment.



For this purpose, the frequency-tagging technique was applied in combination with EEG recording. In the synchronous human motion condition, the motions of the PLDs were synchronous as in a group of dancers. In the asynchronous human motion condition, this synchronous motion was destroyed by starting each PLD with a different frame. In the synchronous non-human motion condition, we used identical cluster-shuffled PLDs, which started their motion from the same frame and followed the same motion sequence. In the asynchronous non-human motion condition, we used different cluster-shuffled PLDs and each cluster started their motion from different frame and followed a different motion sequence. The luminance intensity of the point lights was modulated with different frequencies for the different PLDs (PLD1=f_1 and PLD2=f_2 and vice versa). In the frequency spectrum of the steady-state visual evoked potentials, we found two emergent frequency components, which suggested distinct mechanisms for different levels of interactions between PLDs. The integration of motion synchrony is reflected in the second-order component (f_1+f_2) while the integration of multiple human configurations was found in the third-order component ($2f_1+f_2$). These findings suggest that the process of integrating the visual information of motion synchrony is distinct from the integration process of multiple human bodies. We speculate that the differentiation on frequency order may reflect that integration based on synchronous
motion happens at a lower level, while integration based on human configuration happens at a higher level.

Disclosures: **N. Alp:** None. **A. Nikolaev:** None. **N. Kogo:** None. **J. Wagemans:** None.

Poster

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NIH Grant F32 EY025121

NIH Grant T32 EY007031

Title: GABA and visual context processing in autism spectrum disorders

Authors: ***M.-P. SCHALLMO**, A. V. FLEVARIS, A. M. KALE, R. A. BERNIER, S. O. MURRAY;
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Abstract: Previous work has demonstrated altered visual processing in autism spectrum disorders (ASD). Notably, individuals with ASD have shown substantially reduced motion duration thresholds (measured psychophysically) compared to neuro-typical (NT) observers. We replicated this finding using an unmedicated sample of young adult ASD and NT participants. Further, we show how the standard normalization model of early visual processing can account for these psychophysical results. Specifically, the model suggests reduced normalization strength in ASD, a proposal that is consistent with prominent excitation/inhibition hypotheses of ASD. We further investigated the possible role of the neurotransmitter γ-aminobutyric acid (GABA) by using magnetic resonance spectroscopy to measure GABA concentrations in medial occipital lobe (early visual cortex), lateral occipital lobe (including area MT), and dorsal parietal lobe (sensorimotor cortex). While our preliminary results showed that overall GABA concentrations did not differ between ASD and NT in any region, we did find that across all subjects, the strength of spatial context effects in our motion discrimination task correlated with the concentration of GABA in lateral occipital cortex (but not in other regions).

Disclosures: **M. Schallmo:** None. **A.V. Flevaris:** None. **A.M. Kale:** None. **R.A. Bernier:** None. **S.O. Murray:** None.

Poster

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Support: R01MH106520

F32 EY025121

Title: Effects of stimulus size and contrast on motion perception: Comparing psychophysics and fMRI

Authors: *A. V. FLEVARIS, M.-P. SCHALLMO, A. KALE, S. O. MURRAY;
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Abstract: In a well-known effect of spatial context, more time is needed to discriminate the direction of motion for a high contrast pattern as its size increases. It has been proposed that this effect reflects surround suppression in area MT, whereby stimulation in the surround of the classical receptive field (CRF) suppresses the response to a stimulus in the CRF, making it more difficult to perceive. At low contrast, on the other hand, increasing stimulus size reduces the amount of time necessary to discriminate motion. This effect is assumed to reflect spatial summation in MT, wherein larger stimuli evoke an enhanced response. In order to link these psychophysical effects to the underlying neural mechanisms, we compared psychophysical motion duration thresholds to high (98%) and low (3%) contrast drifting gratings with fMRI responses to the same drifting gratings. Gratings were presented in three different sizes: 1°, 2°, and 12° in diameter. In the psychophysical task, participants reported the direction of motion of the grating and stimulus duration thresholds were measured for each contrast and stimulus size. In the fMRI experiment, participants ignored the stimuli and instead attended to a stream of shapes at fixation and pressed a button whenever a target shape appeared. Stimulus conditions were blocked and percent signal change was measured for each contrast and stimulus size in visual areas MT and V1. MT and V1 were each localized separately for each participant. Psychophysically, we replicated prior results showing suppression at high contrast and summation at low contrast. The fMRI results in areas MT and V1 were similar to psychophysical results overall but showed distinct patterns between areas. In V1, we found suppression at high contrast, but we did not see summation at lower contrast. In MT we found summation at low contrast but no evidence of suppression. These results suggest that the differences in motion perception across stimulus size may not be explained solely by activity in area MT. Instead, a combination of activity across areas including V1 and MT may underlie the psychophysical effects.

Disclosures: **A.V. Flevaris:** None. **M. Schallmo:** None. **A. Kale:** None. **S.O. Murray:** None.

Poster

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Topic: D.06. Vision

Support: Human Frontier Science Program

Title: Response properties of global motion sensitive neurons in the zebra finch vestibulocerebellum

Authors: ***A. H. GAEDE**, D. L. ALTSHULER;
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Abstract: Visual guidance is a key component of avian flight that has been relatively unexplored. A flying bird perceives large-field visual motion, or global optic flow, as it moves through its environment, and this strong visual signal is processed in the accessory optic system. This signal is passed from the retina to two midbrain nuclei, one of which, the lentiformis mesencephali (LM) is hypertrophied in hummingbirds, suggesting an important role in stabilization. Information from the LM converges in the cerebellum; the LM projects both directly and indirectly (via a climbing fiber pathway through the medial column of the inferior olive) to the vestibulocerebellum, providing an expedited route from eye to premotor areas. Extensive research in the pigeon vestibulocerebellum has revealed parasagittal functional zones that can be identified with neurochemical staining and responses to global motion. Complex spike activity of Purkinje cells in the flocculus (lateral regions) of the vestibulocerebellum responds to optic flow resulting from self-rotation about the vertical or horizontal axes. Because zebra finch LM neurons prefer higher pattern velocities than pigeons and have a broader speed tuning width than hummingbirds, we hypothesized that further velocity tuning occurs in the zebra finch flocculus. Using standard electrophysiological techniques and computer-generated stimuli (a single plane of random dots), we characterized the response properties of these motion-sensitive cells in the flocculus of zebra finches (*Taeniopygia guttata*), which are small birds that do not hover and do not have a hypertrophied LM. As in pigeons, we found that zebra finch complex spike activity in the flocculus is directionally selective, as demonstrated by increased firing in response to motion in the preferred direction and suppression of spontaneous firing in the anti-preferred direction. Furthermore, we found that climbing fiber-mediated complex spike activity is tuned to visual stimulus velocity. In this study, we examine the distribution of preferred velocities in the flocculus, as well as the width of the velocity-tuning

response. In zebra finches, the speed tuning width of complex spike activity was narrower than the speed tuning width of LM cells. Furthermore, the preferred velocity of optic flow sensitive Purkinje cells recorded in the flocculus extended across a lower range than that of the LM. The level at which velocity tuning to a given visual stimulus occurs could differ across avian species that have different flight behaviors. How the climbing fiber-mediated response to visual stimuli in the flocculus compares to the response in the LM has yet to be investigated.

Disclosures: **A.H. Gaede:** None. **D.L. Altshuler:** None.

Poster

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Topic: D.06. Vision

Support: PACE Marie Slodovska Curie project MSCA-ITN-2014-642961

Title: Voluntarily tracking moving clouds Effects of spatial frequency bandwidth on human smooth pursuit

Authors: ***K. MANSOUR POUR**[1], L. PERRINET[2], G. MASSON[2], A. MONTAGNINI[2];
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[2]Neurosciences, Inst. de Neurosciences de la Timone, UMR7289, CNRS & Aix-Marseille Univ., marseille, France

Abstract: The properties of motion processing for driving smooth eye movements have been extensively investigated using simple, artificial stimuli such as gratings, small dots or random dot patterns. We know however very little about this processing in the context of complex, natural images. We have previously investigated the human ocular following responses to a novel class of random texture stimuli of parameterized naturalistic statistics: the *Motion Clouds*. In Fourier space, these dynamical textures are designed with a log normal distribution of spatial frequencies power multiplied by a pink noise power spectral density that reduces the high frequency contents of the stimulus (Sanz-Leon et al. 2011). We have previously shown that the precision of reflexive tracking increases with the spatial frequency bandwidth of large (>30° diameter) patterns (i.e. the width of the spatial frequency distribution around a given mean spatial frequency; Simoncini et al. 2012). Now, we extend this approach to voluntary tracking and focused on the effects of spatial frequency bandwidth upon the initial phase of smooth pursuit eye movements. Participants were instructed to pursue a large patch of moving clouds (mean speeds: 5, 10 or 20°/s) embedded within a smoothing Gaussian window of standard

deviation 5°. The motion stimuli were presented with four different spatial frequency bandwidths and two different mean spatial frequencies (0.3 and 1 cpd). We observed that smaller bandwidth textures exhibit a stronger spectral energy within the low spatial frequency range (below 1cpd), yielding to shorter latency of smooth pursuit eye movements. A weak and less consistent effect was found on initial eye acceleration, contrary what was previously observed with OFR. After 400ms, the steady-state tracking velocity matched the mean visual motion speed and pursuit performance was comparable with that observed with a control, small dot motion. Motion clouds offer an efficient tool to probe the optimal window of visibility for human smooth pursuit through the manipulation of both the mean and the variability of spatial frequency.

Disclosures: **K. Mansour Pour:** None. **L. perrinet:** None. **G. masson:** None. **A. Montagnini:** None.

Poster

328. Motion Processing

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Support: NEI Intramural Program

Title: High spatial frequency components of white noise stimuli suppress initial disparity-vergence responses in humans

Authors: ***B. M. SHELIGA**, C. QUAIA, E. J. FITZGIBBON, B. G. CUMMING;
Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: We recorded horizontal disparity-vergence responses (DVRs) to vertical 1D noise stimuli, and vertical DVRs to horizontal stimuli, in two human subjects. For a given disparity we compared DVRs to white noise with those to white noise after removing certain spatial frequency (SF) bands (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* DVR magnitudes (measured as total displacement in the first 50ms of the response), and the SFs associated with the greatest reduction were close to those that produced the maximal response when presented alone. This suggests that low SF components contribute to the DVR drive. In contrast, removing higher-SF components *increased* DVR magnitudes. This suggests that high SF components suppress the DVR. These components presented alone produced small responses. As disparity increased, the optimal SF for both effects - reduction and increase - shifted towards lower SFs. We previously demonstrated a similar phenomenon in ocular following produced by

moving noise stimuli. There the effect of high SFs could be explained as an artifact of discrete temporal sampling by the display, since high SF components moved with high temporal frequencies. Such an explanation cannot be applied to disparity, and so these results suggest that the suppression produced by high SFs is not an artefact, but reflects a computation that is applied in natural viewing. After the initial 50ms the effect of high SF components reverses - removing them reduces the late response - indicating that the interaction between SF components changes over time. This may reflect "Coarse to Fine" processing of disparities.

Disclosures: **B.M. Sheliga:** None. **C. Quaia:** None. **E.J. FitzGibbon:** None. **B.G. Cumming:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: National Natural Science Foundation of China (31571078)

Title: The neural mechanisms in MST underlying Pinna- Brelstaff rotational visual illusions

Authors: ***J. LUO**, X. LI, K. HE, J. YIN, I. ANDOLINA, Y. GU, W. WANG;
Inst. of Neurosci., Inst. of Neurosci., Shanghai, China

Abstract: The Pinna illusion is a striking example of the perception of rotation in the absence of real physical motion. Upon physically approaching or receding from the Pinna-Brelstaff figure, the observer experiences vivid illusory counter rotation of the figure. This visual phenomenon of illusory rotary motion is a well-known example of integration of local cues to form a global percept. Using psychophysical tests and functional magnetic resonance imaging (fMRI) of visual cortices V1-V4v, MT, and MST of the dorsal and ventral visual pathways, we recently found that the Pinna-Brelstaff figure (illusory rotation) and a real physical rotation control stimulus both predominantly activated subarea MST in hMT+, each with a similar response intensity. However, the detailed neural mechanisms underlying the Pinna illusory rotation remain unknown. By manipulation of the physical characteristics of the Pinna-Brelstaff figure, we could generate 3 types of illusory motion: rotation, expansion and contraction. We performed single-unit recordings of MST in awake macaques, and found that up to two-thirds of MST neurons encode these illusory motions, the majority of whose responses corresponded to illusory expansion and contraction. The tuning preferences of these MST neurons were similar between conditions of real and illusory motions. We have preliminary data in recording neural responses

to the same stimulus in area V5/MT, and found only weak responses which predominantly related to real motion components. These results confirm that neurons in area MST respond equivalently to illusory or real rotations, expansions and contractions. Together all these findings suggest that the representation of illusory and real complex motion fields in primate MST relies on a similar cascade of neural integrative mechanisms as physical motion from earlier visual areas.

Disclosures: **J. Luo:** None. **X. Li:** None. **K. He:** None. **J. Yin:** None. **I. Andolina:** None. **Y. Gu:** None. **W. Wang:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: NIH Grant EY016178

NSF GRF Grant No. DGE-1419118

Title: Optic flow parsing in macaque monkeys

Authors: ***N. E. PELTIER**[1], D. E. ANGELAKI[3], G. C. DEANGELIS[1,2];
[1]Brain and Cognitive Sci., [2]Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY; [3]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: As we move through the world, our retinas are presented with a structured pattern of motion called optic flow. Typically, optic flow reflects some unknown combination of object motion and self-motion. It is essential to separate the sources of retinal motion in order to interact with objects and avoid obstacles as we navigate. A simple scheme that explains how we compensate for self-motion when estimating the motion of independently moving objects is called optic flow parsing (e.g., Warren and Rushton, 2009, Curr. Biology). According to the flow parsing hypothesis, we can detect the motion of an object relative to the world by identifying motion vectors that are inconsistent with the global pattern of optic flow due to self-motion. As a result, we can estimate object motion in the world by subtracting out the retinal motion that results from self-motion. The flow parsing hypothesis uniquely predicts perceptual biases that are not predicted by other models, and its mechanism is simple enough to be feasibly implemented by neurons. However, the neural mechanisms of flow parsing remain unknown. To explore neural correlates of flow parsing, we trained macaque monkeys to perform fine

discrimination of object motion (left or right relative to vertical), and we presented object motion together with optic flow fields that simulated forward self-motion, backward self-motion, or no self-motion (0% coherence). The monkey's perception of object motion was systematically biased by the presence of background optic flow, consistent with flow parsing. When we masked the optic flow surrounding the object, the bias in perceived object motion was slightly decreased but not extinguished. Even when optic flow was masked in the entire hemifield surrounding the object, we still observed systematic biases due to the direction of simulated self-motion. This suggests that the monkey may infer a global optic flow field and subtract the inferred optic flow vectors around the object to compute object motion.
Because flow parsing entails the subtraction of different motion vectors in each part of the visual field, we hypothesized that neural correlates of flow parsing may appear in retinotopic visual areas containing a motion map, such as the middle temporal (MT) area. We recorded neural activity from MT neurons, using 24-channel V-Probes, while the monkey discriminated object motion during simulated self-motion. Preliminary neural results show that the responses of some MT neurons to object motion are modified by the direction of self-motion simulated by optic flow. Our findings establish a robust animal model of flow parsing, and suggest that area MT may be part of the underlying neural mechanisms.

Disclosures: **N.E. Peltier:** None. **D.E. Angelaki:** None. **G.C. DeAngelis:** None.

Poster

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Topic: D.06. Vision

Support: CIHR MOP-115178

CIHR CGSD-121719

Title: Training alters the causal contribution of area MT to visual motion perception

Authors: ***L. D. LIU**[1], C. C. PACK[2];
[1]Neurol. and Neurosurg., [2]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Visual stimuli elicit neural activation in a large number of distinct cortical areas. In principle, visual perception could arise from a distributed process that combines information across these areas, or it could rely exclusively on the areas that are most specialized for each stimulus. We have examined these possibilities, using single-unit recording, reversible

inactivation, and microstimulation of the middle temporal (MT) area, while monkeys performed a motion discrimination task. Area MT of the visual cortex is highly specialized for processing visual motion.
Monkeys were trained to report the direction of motion of a moving grating, which has been shown to be represented in many different visual cortical areas. Following this training, fluctuations in single units MT activity showed little correlation with the animals' choices. Moreover, reversible inactivation of MT, using muscimol injections, had little effect on behavioral performance (22% increase in psychophysical thresholds). Microstimulation had a small, but significant bias on the monkeys' choices toward the preferred directions of the stimulation sites, and at the same time decreased the slope of the psychometric functions. These results suggest that the brain uses a distributed representation to make perceptual decisions, so that area MT is not necessary for motion perception of a moving grating.
The same monkeys were then trained over several weeks to report the motion direction of random dots embedded in noise. This stimulus elicits far stronger direction selectivity in MT than in most other areas, suggesting that it is a specialized probe of MT; as in previous studies, we found that MT inactivation devastated behavioral performance for this stimulus. Surprisingly, training on the dots task led to dramatic changes in the relationship between area MT and perceptual decisions. First, there was a significant increase in the correlation between MT neuronal selectivity and choice probability in single-unit responses to gratings. Second, following dots training muscimol injections led to a very large decrease in performance on the grating motion task (500% increase in psychophysical threshold). Finally, the bias induced by microstimulation was larger and the decrease in the slope was smaller than before training on the dots task.
Together, these results suggest that the readout of sensory information depends strongly on perceptual experience: Even for tasks that involve a common stimulus feature (e.g., motion), perceptual decisions can rely on different brain regions for different types of stimuli, consistent with the idea of a flexible, distributed process.

Disclosures: **L.D. Liu:** None. **C.C. Pack:** None.

Poster

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Topic: D.06. Vision

Support: JSPS KAKENHI Grant 16K13506

Title: Localization and functional characterization of human area prostriata using fMRI

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Abstract: In monkeys, the area prostriata is a small limbic region located between the anterior calcarine fissure and hippocampal formation. Although its function has been elusive since its description by Sanides (1969), a recent study on monkeys demonstrated its visual responsiveness, especially to high-speed visual motion stimuli appearing in the far periphery. The purpose of the present study was to find a human homolog of the area prostriata. To this end, we used functional magnetic resonance imaging (fMRI) in conjunction with a custom-made wide-field visual display system to investigate brain responses to visual motion in the far peripheral visual field.
Two types of experiments were conducted using 20 subjects. The first experiment was designed to locate the area prostriata. In a scanning run, moving bars (5-55°) were presented in the periphery with alternating static bars in a standard block-design paradigm. Throughout the scanning run, the subjects were required to perform a rapid serial visual presentation (RSVP) task at the fovea, in which a target letter had to be detected among distractor letters. In the second experiment, we employed a rapid event-related design to assess functional properties of the area prostriata with regard to its receptive field, speed tuning, and attentional processing. Visual stimuli were either high- (60°/sec) or low- (20°/sec) speed moving bars presented at eccentricities either 5-25° or 40-60°, leading to four trial conditions. One of the four stimuli was presented in each trial and the subjects were required to perform the central RSVP task during half of the scanning runs. In the other half of the scanning runs, the subjects were required to detect abrupt changes in motion.
The localizer experiment showed, as expected, that peripheral motion stimuli evoked responses in visual areas MT+ and V6, as well as in the periphery of V1. In addition to these areas, we found a robust activation in a small limbic region located at the isthmus of the cingulate gyrus. In view of its anatomical location, this region may be the human homolog of the area prostriata. According to the results of the second experiment, the human prostriata tended to show larger responses to far peripheral and high-speed motion stimuli, similar to what has been observed in monkeys. Interestingly, the human prostriata displayed a unique property regarding attention: its activity increased, not in the motion-detection task, but in the RSVP task, which is in clear contrast to attentional processing in MT+ and V6. These results help in understanding the visual cortical network necessary for peripheral vision and the functional roles of the human area prostriata.

Disclosures: **H. Yamamoto:** None. **Z. Lin:** None. **K. Okamoto:** None. **S. Ohno:** None. **S. Kanazawa:** None. **J. Wu:** None.

Poster

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Topic: D.06. Vision

Support: CIHR #MOP 123349

NSERC #RGPIN 121713-11

Title: Pupillary response to objects and perceived motion.

Authors: *S. BEUKEMA[1], B. JENNINGS[2], J. OLSON[3], F. KINGDOM[2];
[1]Neurosci., [2]Ophthalmology, [3]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: In two experiments investigating pupil-size changes to objects and textures, pupillary response differences were observed between various conditions. Experiment 1 had participants passively view static images that contained textures perceived as inherently static, or inducing a strong sensation of (illusory) motion. Despite literature reporting pupillary constrictions to the perception of coherent physical motion, our results indicate a pupillary dilation to the illusory motion textures as compared to the perceptually static textures - potentially implicating differences arising from tangible vs. intangible perceptual states. Experiment 2 had participants view images with elements forming objects, non-objects, and random patches. A greater pupillary dilation response was observed for the perception of objects relative to the other conditions, potentially outlining the recruitment of high-level cognition for top-down semantic processes inherent in object-recognition. Together, these experiments further highlight pupillometry as a useful means to explore high-level visual processes through the identification of physiological pupil-size differences to distinct perceptual states.

Disclosures: S. Beukema: None. **B. Jennings:** None. **J. Olson:** None. **F. Kingdom:** None.

Poster

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Support: Australian Research Council through Discovery Grants 7 DE120102210,DP140104533

the ARC Centre of Excellence for Integrative Brain 8 Function (CE140100007)

Title: A biologically-based computational model to deliver unambiguous motion information and overcome the X-junction illusion

Authors: P. ZAREI ESKIKAND[1], T. KAMENEVA[1], *M. R. IBBOTSON[2], A. BURKITT[1], D. GRAYDEN[1];
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Abstract: The end-points of an object, called intrinsic terminators, represent unambiguous motion information. They assist in overcoming the aperture problem and achieving an accurate estimation of motion in area MT of the visual cortex [1]. However, the extrinsic terminators formed at the intersection of two overlapping objects themselves result in ambiguous motion signals [1]. We investigate, using a computation model, how interactions between form and motion information may assist MT neurons to differentiate intrinsic from extrinsic terminators. The motion model has two stages: a model of V1 neurons, including standard complex neurons and end-stopped neurons, and MT, which has integration and segmentation neurons. Integration MT neurons propagate the motion information through excitatory interconnections from the terminators of the stimulus along the object. Segmentation neurons control the activity of the integration neurons by responding to the discontinuities in the stimulus and suppressing the activity of integration neurons via inhibitory connections. In the proposed model, MT neurons additionally receive form information from bipole neurons in V2. These neurons have two extensive receptive fields that result in the perception of coherency when both parts of the receptive fields are active simultaneously [2]. There are inhibitory interactions between bipole neurons that have receptive fields with opposite orientations, which suppress the activity of bipole neurons when an apparent terminator is formed by overlapping objects [1]. The excitatory connections between MT neurons are activated when the corresponding bipole neurons at the same locations are active. Therefore, motion signals are propagated along the orientation of an object when the corresponding bipole cells are active. In the case of an X-junction formed by two crossing bars moving in opposite directions, bipole cell activity is suppressed, which prevents the propagation of the ambiguous motion information from the extrinsic terminators. The results show that, despite the inability of end-stopped neurons to distinguish two different types of terminators, bipole neuron activity at the intrinsic terminators results in an accurate representation of motion by MT neurons. 1. Berzhanskaya, J., S. Grossberg, and E. Mingolla, Laminar cortical dynamics of visual form and motion interactions during coherent object motion perception. Spatial vision, 2007. 20(4): p. 337-95. 2. Von der Heydt, R., E. Peterhans, and G. Baumgartner, Illusory contours and cortical neuron responses. Science, 1984. 224(4654): p. 1260-1262.

Disclosures: **P. Zarei Eskikand:** None. **T. Kameneva:** None. **M.R. Ibbotson:** None. **A. Burkitt:** None. **D. Grayden:** None.

Poster

328. Motion Processing

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Program#/Poster#: 328.24/FF16

Topic: D.06. Vision

Support: NIH T32 EY015387

NIH R01 EY022087

Title: Retinal stabilization reveals limits of efference copy influence on heading tuning in the medial superior temporal area (MST)

Authors: ***T. MANNING**, K. BRITTEN;
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Abstract: The use of optic flow information in the calculation of heading direction and guidance of self-motion has been well established over the past few decades by a number of neurophysiological, computational, and behavioral studies. Eye movements performed while the body is moving, however, substantially distort the optic flow patterns falling on the retina. Observers are able to compensate for these movements perceptually, but the mechanism by which this occurs is unknown. One hypothesis for which there is psychophysical support states that heading representations are stabilized by efference copy signals related to ongoing eye movements. To test this hypothesis at the physiological level, we performed single-unit recordings from neurons in MST while a monkey performed a smooth pursuit task. Neurons in this region are selective for both the direction of heading and eye rotation, and their activity has been causally linked to the perception of heading. In the task, the animal fixated or pursued a small target in a virtual reality environment that simulated different observer translations through a three-dimensional cloud of dots. To reveal the presence of efference copy signals, two manipulations were made to the visual stimulus. First, we simulated the optic flow distortions on the retina that result from pursuit while the monkey fixated a stationary target. This stimulus is retinally identical to what results from translation with pursuit, but eliminates efference copy signals. Second, we counteracted the retinal effects of pursuit by counter-rotating the virtual camera using feedback from a scleral search coil eye tracker. This feedback system performed at frame rate (120 Hz) with a fixed single-frame lag. This manipulation produces a retinal stimulus identical to the one resulting from translation with fixation, but efference copy signals related to

pursuit would remain active. Based on the analysis of a preliminary sample of neurons, we find that both manipulations substantially change their responses to heading tuning in MST. The simulated and stabilized pursuit conditions resulted in opposite shifts of the neurons' preferred heading directions from the normal pursuit case. As expected from previous work, simulated pursuit produced substantial shifts in tuning in the direction of pursuit. Tuning under stabilized pursuit, however, closely matched the cells' preference while the eyes were stationary. These results suggest there is only a modest direct modulatory role of efference copy signals in area MST.

Disclosures: T. Manning: None. **K. Britten:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/ MINDS); the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT)

Title: Functional analysis of visual responses with an ultra high filed MRI in awake marmorets

Authors: *T. KANEKO[1], J. HATA[1], N. KISHI[1], H. OKANO[2,1];
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Abstract: Growing interest in viral and genetic manipulations on non-human primates brings neuroscientists' attention to marmosets(*Callithrix jaccus*), as their high reproductive efficiency and easy husbandry cost comparing to other primates. However, question remains to what extent the brain of marmosets shares functional architecture with that of other primates, such as humans and macaques. This is not only important to utilize marmosets as a model to develop therapeutic strategies for human psychiatric/neurological disorders, but also to understand the evolution of primate brain. Functional MRI is most suitable to this question as it allows whole brain analysis and also can accommodate various kinds of cognitive tasks. The goal of the present study was to develop functional MRI setup for awake animals, and to demonstrate feasibility of our system. We developed 8-channel phased array coil which was compatible to head-post system to restrain head motion of animals. Visual stimulus was presented on a screen located inside the scanner by which relatively large visual field (about 42 degree) can be stimulated. With this setup, we observed robust blood oxygen level dependent signal by visual stimulation. Significant signal

change was observed throughout various parts of cortical and subcortical areas. Most of these areas correspond with predictions derived from putative anatomical homology to other primate species. We also mapped visual motion sensitive areas by computing the contrast between static and drift grating stimulus. Visual motion activated at MT complex, and the activity extended to rostral on ventral part of lateral surface of the temporal cortex. The observed functional segregation at the temporal lobe is similar to those of other primate species such as macaques and humans. We also observed that foveal V1 showed biased activation to static stimulus, while peripheral V1 was activated more by motion stimulus. These features correspond well with reported physiological property of this area in macaques. Furthermore, we have not observed motion selective response around putative V2 and V3 so far. This is distinctive from the results of macaques, and might suggest the functional difference of these early visual areas among species. In summary, these results demonstrated the potential of the developed system to create functional map of the marmosets' brain at macro-level perspective, and to elucidate similarity and dissimilarity of functional architecture among primate species.

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Poster

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Topic: D.06. Vision

Support: NEI EY013644

Title: Neurons in macaque area MT signal depth from motion parallax by combining extra-retinal signals regarding both eye and body rotation

Authors: ***V. KOGAN**[1], D. E. ANGELAKI[2], G. C. DEANGELIS[1];
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Abstract: Motion Parallax (MP) refers to the relative image motion of objects located at different depths, caused by the translation of the observer. In the absence of pictorial depth cues, MP is ambiguous regarding depth sign (near vs. far). Theoretical work by Nawrot and Stroyan (2009) showed that the critical variable for disambiguating depth sign from MP is the rate of change of eye rotation relative to the scene (R_{es}), which is determined by the combination of eye rotation relative to the head (R_{eh}), head rotation relative to the body (R_{hb}), and body rotation relative to the world (R_{bw}): $R_{es} = R_{eh} + R_{hb} + R_{bw}$.

We showed previously (SfN, 2015) that macaques can discriminate depth sign from MP under some combinations of R_{eh} and R_{bw} that produce the same R_{es} ($R_{hb} = 0$ by design). Discrimination performance was robust when R_{eh} and R_{bw} had the same sign, mostly robust for when $R_{es} = R_{bw}$ ($R_{eh} = 0$), and quite erratic when R_{eh} and R_{bw} had opposite signs. This suggested that the brain relies on both R_{eh} and R_{bw} signals to disambiguate depth from MP, but that these signals are not ideally combined into an estimate of R_{es}.
Neurons in macaque area MT combine retinal image motion with extra-retinal signals regarding R_{eh} to signal depth sign from MP (Nadler et al., 2008, 2009). It remains unclear, however, whether MT neurons also incorporate vestibular rotation signals (R_{bw}) to compute depth sign. We recorded from single MT neurons while animals were translated back and forth by a motion platform. The animal's task was solely to maintain fixation on a world-fixed target, making smooth eye movements as needed. The motion platform could rotate the whole body around an axis through the eye, to generate various combinations of R_{bw} and R_{eh} that produce the same R_{es} ($R_{hb} = 0$ in all cases). In all conditions, image motion of a patch of random dots simulated a surface that appeared at one of several different depths, and we measured depth tuning curves of MT neurons under various combinations of R_{bw} and R_{eh}.
Preliminary results show that MT neurons use both vestibular rotation signals (R_{bw}) and eye rotation signals (R_{eh}) to compute depth sign from MP. Analogous to behavioral data measured separately, MT neurons have only slightly reduced depth-sign selectivity when $R_{es} = R_{bw}$ ($R_{eh} = 0$), indicating that vestibular rotation signals alone can generate selectivity. However, neural depth-sign preferences reverse (and weaken slightly) when eye and body rotations are in opposite directions (opposite signs of R_{bw} and R_{eh}), indicating that eye rotation signals dominate. Our findings show that neural computations of depth from MP incorporate both efference copy signals regarding eye rotation and vestibular signals regarding body rotation.

Disclosures: V. Kogan: None. **D.E. Angelaki:** None. **G.C. DeAngelis:** None.

Poster

328. Motion Processing

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Topic: D.06. Vision

Support: 1U01NS090449-01

Title: Visual processing of motion-selective information in the larval zebrafish brain

Authors: *C. RIEGLER[1,2], D. GUGGIANA-NILO[1], F. ENGERT[1];
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Abstract: The vertebrate retina extracts spatiotemporal features of the visual environment and diverse retinal ganglion cell (RGC) types carry extracted information into the brain. We studied how and where the direction of object motion, a behaviorally relevant feature, is represented, and further processed, in the retinorecipient arborization fields(AFs) of larval zebrafish. First, we created a functional map that describes the response properties of the RGCs that project to each AF. Of the 10 AFs, the largest is the optic tectum (homologous to the mammalian superior colliculus) and in addition there are 9 smaller AFs. Using 2-photon microscopy and GCaMP6 targeted to synaptic terminals, we recorded from all RGC terminals while showing behavior-relevant directional visual stimuli. One such visual stimulus was whole-field motion, which triggers the optomotor response. In this behavior fish turn their body and swim in the direction of perceived motion. Direction selective information is thus necessary for calculating the direction of perceived motion. Similarly, visually driven escape responses, triggered by an approaching black edge, lead to directed escape turns away from the edge. Therefore both behaviors require direction selective information. We found that the majority of OFF, ON and ON-OFF direction selective terminals are located in the optic tectum and one extratectal area termed arborization field 6 (AF6). Given this exclusivity, we asked if the same population of RGCs projects into both retinorecipient areas. Targeted laser ablation of individual AFs shows at least one subset of direction selective RGCs that both, projects to the posterior AF6, and is also responsible for conferring directional selectivity to the posterior optic tectum. Finally, the same ablation impairs all turning behaviors to whole-field visual motion but has a lesser effect on directed escape responses. These findings establish the entry into two different behavioral circuits that require similar but not identical directional information.

Disclosures: **C. Riegler:** None. **D. Guggiana-Nilo:** None. **F. Engert:** None.

Poster

328. Motion Processing

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Program#/Poster#: 328.28/GG2

Topic: D.06. Vision

Title: Ferret visual area PSS: A model system for studying functional development of higher order motion cortex

Authors: *A. A. LEMPEL[1,3], A. DANIELS[3], J. M. LAW[2], K. J. NIELSEN[1,3];
[1]Neurosci., [2]Krieger Sch. for Arts and Sci., Johns Hopkins Univ., Baltimore, MD; [3]Zanvyl Krieger Mind/Brain Inst., Baltimore, MD

Abstract: Due to their immature stage at birth, ferrets have become an important animal model for the study of visual system development. So far, most studies have focused on lower visual processing stages such as primary visual cortex (V1), leaving the functional development of higher order areas unexplored. Here, we begin to address this issue by investigating higher order area PSS.
PSS is located in the posterior bank of the suprasylvian sulcus. Previous studies demonstrated a high degree of direction selectivity in PSS, and impaired motion detection after PSS lesions. However, without determining responses to more complex motion stimuli, it remains an open question whether PSS indeed is a higher order motion area. In a first set of experiments, we therefore tested PSS responses to stimuli designed to reveal signatures of higher order motion processing. We chose two stimulus sets commonly used to compare processing in macaque areas V1 and MT, coherent plaids and transparent motion stimuli. Both test the integration of local motion signals. Plaids are constructed by superimposing two component gratings drifting in different directions. Perceptually, they appear as a coherent plaid drifting in a third direction. Our data demonstrate that neurons in ferret V1 respond only to the motion of the component gratings. In contrast, PSS contains a population of neurons that respond to the motion of the plaid pattern. Transparent motion stimuli are generated by superimposing two fields of dots moving in opposite directions. Perceptually, they appear as two surfaces sliding across each other. V1 neurons were largely unaffected by the addition of a second field of dots, but responses in PSS neurons were suppressed under these stimulus conditions. Our data therefore reveal motion integration processes in PSS that exceed those found in V1, and suggest PSS as a higher order motion area.
As a first step towards studying PSS development, we determined the developmental time course of PSS direction selectivity. Our data show a rapid development of direction tuning after eye opening (P32-34), with mature selectivity levels reached by P36-38. This parallels the time course established for direction selectivity development in V1. Our data also suggest that direction selectivity is higher in PSS than V1 throughout development.
In conclusion, PSS provides an exciting opportunity to investigate the development of higher order visual functions. A better understanding of their development at the neural circuit level is critical, as these functions are often impaired in developmental disorders.

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Poster

328. Motion Processing

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Topic: D.06. Vision

Support: NIH IRP

Title: Binocular contrast summation for visual motion processing in humans

Authors: *C. QUAIA, B. M. SHELIGA, L. M. OPTICAN, B. G. CUMMING;
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Abstract: Binocular contrast summation, the advantage conferred by seeing a stimulus through two eyes instead of one, is usually quantified by the ratio (binocular gain) between the detection threshold for static stimuli viewed binocularly and monocularly. It typically ranges between 1.4 and 2. When matching higher contrasts, the binocular gain is usually close to 1.0 - one eye is as good as two. Here we quantify binocular summation for the motion signals that drive reflexive eye movements. We recorded, in three human subjects, the short-latency ocular following responses (OFRs) that are induced by the sudden motion of a large visual stimulus. First, we measured the contrast response functions (CRFs) for sinusoidal drifting gratings (0.25 cpd, 20Hz) presented monocularly or binocularly in a large (28 deg diameter) circular aperture. We found that the responses were always much stronger for binocular than monocular stimuli. Binocular gain was as high as 10 at low (2.5%) contrast, and asymptotically approached 2 at high (80%) contrast. Perceptual gains for contrast matching with the same stimuli were much lower, similar to values reported for static stimuli. Next, we measured the CRFs for 1D random line stimuli (RLS) drifting at high speed (40 deg/s). We used two binocular conditions, presenting either the same RLS to both eyes (correlated condition), or different RLS to the two eyes (uncorrelated condition). Again OFRs to the binocular stimuli were considerably stronger than those elicited by the monocular stimuli, and the binocular gain decreased as contrast increased. The responses to correlated stimuli were always strongest, with responses to uncorrelated stimuli falling between those to correlated and monocular stimuli. At the highest contrast, the binocular gain was 2 for correlated stimuli, and around 1.4 for uncorrelated stimuli. A very simple two-stage model fit the data well. The first, monocular, stage is characterized by a strong contrast gain control, similar to that of magnocellular neurons in LGN. The second (binocular) stage, sums the outputs of the monocular stages from each eye, and has an input-output relationship that is expansive at low inputs, and slightly compressive at high inputs (similar to that of cortical neurons). The responses to uncorrelated RLS at all contrast levels were well described by multiplying the monocular outputs by 0.65 before feeding them to the binocular stage. We conclude that, unlike for contrast perception, there is a strong binocular advantage for motion processing: two eyes are much better than one. This implies that different neural substrates subserve these two binocular summation processes.

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Poster

328. Motion Processing

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Topic: D.06. Vision

Support: JSPS Grant-in-aid for JSPS Fellows

Title: Human white-matter pathway communicating parietal and posterior-insular cortex

Authors: *H. TAKEMURA[1,2,3], M. UESAKI[2,4,5], H. ASHIDA[4];
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Abstract: Human brain comprises cortical regions selective for sensory information in parietal and posterior-insular cortex. Previous fMRI study has reported concurrent activations in parietal and posterior-insular cortex during visual optic-flow stimulation, suggesting that these areas may involve with the integration of visual and vestibular information for self-motion perception (Cardin & Smith, 2010; 2011). This study aimed to clarify how these areas communicate through white-matter pathways by combining functional and diffusion MRI. Using functional MRI, we identified the cortical regions activated by optic-flow stimulation in parietal and posterior-insular cortex (VIP, p2V, PcM, PIC) in six participants. We then analyzed diffusion MRI data collected from identical participants, by using Ensemble Tractography method (Takemura et al., 2016). We generated whole-brain connectome by combining the output of probabilistic tractography with four different parameter settings (MRtrix, Tournier et al., 2012), and optimized the connectome using Linear Fascicle Evaluation (Pestilli et al., 2014). We then identified the fascicles having one endpoint near parietal cortex and the other endpoint near posterior-insular cortex. As a result, we consistently identified the white-matter fascicle connecting parietal and posterior-insular cortex in all hemispheres we analyzed. The anatomical shape and location of this fascicles are consistent with the fascicle, named *Stratum Proprium of Interparietal Sulcus (SPIS)*, reported in classical and recent fiber dissection studies (Sachs, 1892; Vergani et al., 2014). Using Virtual Lesion method (Pestilli et al., 2014), we also identified significant statistical evidence in support of SPIS. Furthermore, we also found that SPIS endpoints are near to cortical regions activated by optic-flow stimulation in the parietal cortex (VIP, p2V, PcM) and posterior-insular cortex (PIC). Taken together, these findings suggest that the sensory regions in parietal and posterior-insular cortex communicate throughout the SPIS. The SPIS may support

sensory integration underlying self-motion perception, which requires visual and vestibular information represented in parietal and posterior-insular cortex.

Disclosures: H. Takemura: None. **M. Uesaki:** None. **H. Ashida:** None.

Poster

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Max Planck Society

Title: Integration of visual and extra-retinal self-motion during voluntary head movements in the human brain

Authors: *A. SCHINDLER[1,2,3], A. BARTELS[1,2,3];
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Abstract: Our phenomenological experience of the stable world is maintained due to continuous integration of visual self-motion with extra-retinal signals. This mechanism is not only essential for locomotion and navigation but also a crucial prerequisite for virtually any successful interaction with our environment. Constraints in fMRI acquisition methods previously prevented the study of neural processing associated to integration of visual signals with those related to head-movement. Here, we developed a novel and ecologically valid fMRI paradigm that enabled us to study integration of optic flow with extra-retinal heading signals while observers performed voluntary head movements. Our results provide first evidence for the multisensory integration of head-motion in human regions MST, VIP, the cingulate visual area (CSv) and a region in pecuneus (Pc) that are known to process visual self-motion signals. In addition, we found multisensory heading integration in posterior insular cortex (PIC) that we suggest to be homolog to monkey visual posterior sylvian (VPS). In contrast, no integration was found in parieto-insular-vestibular cortex (PIVC). These results identify for the first time head-movement related

integration of visual heading signals in the human brain, and identify a clear functional segregation of the human posterior insular cortex.

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Poster

329. Visual Motion

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BUPA (NY)

Title: Dopamine preserves visual motion perception despite noise interference of human V5/MT

Authors: ***B. M. SEEMUNGAL**[1], N. YOUSIF[2], R. Z. FU[2], B. ABOU-EL-ELA-BOURQUIN[2], V. BHRUGUBANDA[2], S. R. SCHULTZ[3];
[2]Brain Sci., [3]Bioengineering, [1]Imperial Col. London, London, United Kingdom

Abstract: When processing sensory signals, the brain must account for noise, both in the stimulus and that arising from within its own neuronal circuitry. Dopamine receptor activation is known to enhance both visual cortical signal-to-noise-ratio ('SNR') and visual perceptual performance, however it is unknown if these two Dopamine-mediated phenomena are linked. To assess this link we used single pulse transcranial magnetic stimulation (TMS) applied to visual cortical area V5/MT to focally reduce the SNR, and hence disrupt visual motion discrimination performance to visual targets located in the same retinotopic space. The hypothesis that Dopamine receptor activation enhances perceptual performance by improving cortical SNR predicts that Dopamine activation should antagonise TMS-disruption of visual perception. We assessed this hypothesis via a double-blinded, placebo controlled study with dopamine receptor agonists Cabergoline (D2 agonist) and Pergolide (D1/D2 agonist), administered in separate sessions (separated by 2 weeks) in 12 healthy volunteers in a William's balance-order design. TMS degraded visual motion perception when the evoked-phosphene and the visual stimulus overlapped in time and space, in the Placebo and Cabergoline conditions but not with Pergolide. This suggests that Dopamine D1 (but not D2) receptor activation, enhances cortical SNR to

boost perceptual performance. That local visual cortical excitability was unchanged across drug conditions suggests the involvement of long-range intra-cortical interactions in this D1 effect. Since increased internal noise (and hence lower SNR) can impair visual perceptual learning, then improving visual cortical SNR via D1 agonist therapy may be useful in boosting rehabilitation programmes involving visual perceptual training.

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Poster

329. Visual Motion

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Topic: D.06. Vision

Support: Swedish Research Council (grant 621-2012-3673)

Russian Science Foundation (grant #14-35-00060)

Title: Frequency of visual gamma oscillations in adults with ASD: a pilot study.

Authors: ***E. OREKHOVA**[1,3], J. SCHNEIDERMAN[4], S. LUNDSTRÖM[2], B. RIAZ[4], S. RAJAEI[2], N. HADJIKHANI[5,2], O. SYSOEVA[3], T. STROGANOVA[3], C. GILLBERG[2]; [1]Gillberg Neuropsychiatry Ctr., [2]Gillberg Neuropsychiatry Ctr. (GNC), Univ. of Gothenburg, Gothenburg, Sweden; [3]Autism Res. Laboratory, Ctr. for Neurocognitive Res. (MEG Center), Moscow State Univ. of Psychology and Educ., Moscow, Russian Federation; [4]Inst. Neurosci & Physiol., MedTech West, Sahlgrenska Acad. and Univ. of Gothenburg, Gothenburg, Sweden; [5]Martinos Ctr. for Biomed. Imaging, Harvard Med. School, MGH/MIT/HST, Charlestown, MA

Abstract: An altered balance between excitation and inhibition (E/I) in neural networks is a likely underlying cause of neurobehavioral deficits in autism spectrum disorders (ASD). Frequency of gamma oscillations depends on the level of excitation of parvalbumin-containing inhibitory neurons and appears to be a useful index of inhibitory efficiency in ASD. Gamma oscillations in the visual cortex are reliably induced by high-contrast moving visual stimuli and their frequency is modulated by velocity of the motion. We have previously shown that modulation of gamma frequency by velocity is reduced in children with ASD and mild intellectual disability, putatively reflecting decreased inhibition in visual cortex (Stroganova et al. JND, 2015, 7:21). Here we investigated visual gamma frequency and its modulation by motion velocity in high-functional (HF) adults with ASD.

We recorded MEG in 11 ASD and 19 neurotypical (NT) subjects aged 19-50 years while they watched high-contrast circular gratings moving with velocities of 1.2 (Vel1), 3.6 (Vel2), or 6.0 (Vel3)°/s. The gamma frequency (e.g., fVel1) was defined as the maximal reliable stimulus-induced increase of sensor power between 40-100Hz in 0.4-1.2 sec post-stimulus.
The peak frequency of gamma oscillations increased with increasing velocity of visual motion in both NT and ASD subjects (p's<0.00001). Unlike our previous findings in children, modulation of gamma frequency by velocity was unremarkable in HF adults with ASD. In the NT group the fVel1, fVel2 and fVel3 decreased with age (p's<0.05), while no correlation with age was found in the ASD group (p's>0.7). The separate slope analysis revealed group differences in regression to age for fVel1 and fVel3 (p<0.05). When the age was accounted for separately in the ASD and NT groups, gamma frequency was higher (p<0.05) in the ASD participants in Vel1 and Vel3 conditions (NT: fVel1-3= 53.4, 64.4, 71.0; ASD: 54.7, 65.0, 71.9 Hz, at 30 years).
The absence of the normal age-related decrease of gamma frequency in adults with HF ASD on the group level points to the presence of pathological factors that affect visual gamma frequency to the greater extend than age. The atypically increased gamma frequency in participants with ASD suggests on average higher than normal excitability of the inhibitory interneurons in visual cortex, which may result in stronger inhibition. Most importantly, comparison of the present results in HF adults with those previously obtained in children with ASD and variable IQs using the same paradigm suggests that the extent and direction of the E/I abnormalities in individuals with ASD may depend on age and/or vary with severity of intellectual disability.

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Poster

329. Visual Motion

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Royal Society Wolfson Research Merit Award

Title: Selective cortical responses to relative object/background motion

Authors: ***M. I. SERENO**[1], C. OZOLINS[2], M. SOOD[2], C. GALLETTI[3], P. FATTORI[4];
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Abstract: During active vision, local motion signals capable of driving neurons in V1, layer 4B, are typically detected at many retinal locations. Sources include coordinated movements of scene contours due to self-motion as well as movement of objects -- such as other agents -- relative to the background. It has long been known that responses to preferred classical receptive field motion can be enhanced by presentation of non-preferred motion to the non-classical surround, even in V1 (review: Allman et al., 1985). In this study, we searched for human visual areas that selectively and retinotopically responded to local relative object/background motion. Color photographs of village street scenes (Eguisheim, Alsace) were continuously and smoothly moved around a central position by temporally smoothing sequences of small flat-random displacements (avoids long term drift). One or more rings of 10 superimposed colored objects (animal and tool images with transparent backgrounds) were similarly moved, with 9 objects moving along with the background and 1 object moving relative to it using a different random stream of similar-amplitude movements. Subjects fixated centrally during each run. The polar angle of the relatively moving object was varied in a periodic fashion (8 cycles in 512 sec). Since much of the retinotopy found in higher visual areas reflects sustained spatial attention (Saygin and Sereno, 2008), subjects had to perform a continuous attention-demanding task to report when any object slightly changed color. Color-change targets were randomly distributed in polar angle. Single objects were given a greenish (or reddish) cast for 130 msec by increasing green (or red) by 25% and by decreasing red (or green) by 25% in order to reduce spurious luminance-based apparent motion signals. Temporally jittered targets appeared once every 2 to 3 seconds. Wide-field direct-view experiments analyzed by phase-encoded methods and sulcus-based surface alignment (freesurfer, csurf) showed strong selective retinotopic responses in V3A, V6, and MSTd, with weaker responses in MT. The retinotopically balanced stimulus elicited no periodic response in early visual areas (V1-V3) and the attention-demanding task completely suppressed responses in occipital, parietal, and frontal visual attention areas. This suggests that relative motion is selectively computed by V3A, V6, and MSTd independent of endogenous attention.

Disclosures: **M.I. Sereno:** None. **C. Ozolins:** None. **M. Sood:** None. **C. Galletti:** None. **P. Fattori:** None.

Poster

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Title: (Mis-)perception of motion in depth originates from underestimation of binocular extraretinal signals

Authors: *T. MURDISON[1,2,3], G. LECLERCQ[4,5], P. LEFÈVRE[4,5], G. BLOHM[1,2,3];
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Abstract: Perception of motion in the depth dimension is essential to moving through the world. To estimate 3D motion, the brain uses extraretinal signals - including ocular vergence, horizontal and vertical version - to interpret binocular inputs. However, the extent to which these extraretinal signals contribute to perception of motion in depth is unclear. To answer this question, we asked 13 participants to estimate 3D motion without any visual references, forcing them to utilize extraretinal cues to reconstruct the spatial motion.
Participants sat in complete darkness while fixating one of 9 locations (horizontal version angles of -30°, 0° or 30° and vergence angles of 8.8°, 4.8° or 3°). We moved an LED around fixation along an arc in the horizontal depth plane with one of 36 possible trajectories. After target motion, participants were instructed to reproduce their perception of the motion in space using a touchscreen. After observing version-induced systematic errors in estimated trajectories relative to the spatial motion, we compared these perceptions to the output of a 3D kinematic eye-head-body (forward and inverse) model describing the predicted target motion either based solely on retinal motion or instead based on a transformation of retinal motion into spatial coordinates. For the transformation model, we varied the contributions of reference vergence, horizontal version and the gain of motion in depth to the inverse model and found the set of extraretinal parameters that resulted in a predicted trajectory best representing the reproduced motion.
Across participants, we found that perceived trajectories were best captured with a version estimate of 19.5° ± 6.0° (mean ± SD) for a true horizontal version of 30°, a gain term accounting for 65% ± 14% of the motion in depth, and a reference vergence angle of 6.7° ± 1.4°. These findings were consistent with previous studies reporting underestimates of horizontal version and depth changes, and point toward a partial transformation of retinal motion signals into spatial coordinates resulting in substantial errors in motion perception.

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Poster

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ANR-12-BSV4-0005

Marie-Curie-PIIF-GA-2011-298386

Title: Representation of egomotion in non-human primate

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Abstract: During locomotion, the central nervous system has to monitor the optic flow that falls on the retina. Electrophysiological recordings have led to the belief that in primate, visual areas MST and VIP have a central role in this process. Recent neuroimaging studies in human have however suggested that the cortical network processing egomotion-compatible optic flow might be more extended and complex than originally believed. The aim of this study is to characterize this network in non-human primate. fMRI recordings were performed at 3T in 3 awake behaving macaques using a specialist 8-channel coil positioned above the animal's head. The experimental protocol was similar to that of a previous human study: we used a block design paradigm where the stimuli alternated between a baseline (i.e. fixation point only), a single patch of egomotion-compatible optic flow ('EC') and an array of nine similar optic flow patches that was inconsistent with egomotion ('EI'). Eye position was monitored while the monkeys performed a fixation task and only the runs where the percentage of correct fixation was above 85% were processed. Data were analyzed using SPM12 and the cortical areas responding specifically to egomotion-compatible optical flow were obtained from the general linear model. Consistent with human data and single-cell studies in macaque, significant activations were found in dorsal MST, in area VIP and in the visual posterior sylvian area (VPS). Responses in all these areas, and also in the frontal eye field (FEF) and area FST, were significantly stronger for the 'EC' condition. We also found a preference for the 'EC' condition in area V6 and in the cingulate sulcus but it was weak, whereas these two regions are particularly responsive in human using the same protocol. Altogether, our results suggest that if monkeys also have extended cortical networks to guide their navigation, there are important differences from those observed in human.

Disclosures: **B.R. Cottereau:** None. **S. Rima:** None. **Y. Trotter:** None. **A.T. Smith:** None. **J. Durand:** None.

Poster

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ARC DE13100493

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Title: Fewer neurons in MT are direction selective to random dot stimuli after chronic V1 lesions in adult marmoset monkeys

Authors: ***M. A. HAGAN**[1], T. CHAPLIN[1], K. R. HUXLIN[2], M. G. P. ROSA[1], L. L. LUI[1]; [1]Dept. of Physiol., Monash Univ., Clayton, Australia; [2]Flaum Eye Inst., Univ. of Rochester, Rochester, NY

Abstract: Damage to the primary visual cortex (V1) results in a scotoma in the corresponding parts of the visual field. However, both humans and monkeys retain some unconscious visual faculties, or "blindsight", within cortically blind (CB) fields, presumably via pathways that bypass V1.

The motion-sensitive, Middle Temporal Area (MT) is thought to mediate blindsight, as MT neurons respond to stimuli inside the scotoma following V1 lesions in both Old and New World monkeys (Rodman et al., 1989; Rosa et al., 2000). However, these prior studies quantified MT responses 1-3 months after damage; there is little data looking at long-term effects on MT responses following V1 lesions. This is important, as visual abilities in humans take ~6 months to stabilize post-lesion after an initial period of spontaneous plasticity (Zhang et al., 2006).

We recorded from 88 MT cells in 3 anesthetized, adult marmosets, 7-11 months after unilateral V1 lesions. The size and location of each scotoma was determined by recording from remaining V1 neurons around the lesion border. Using moving, random dot stimuli, we found that MT receptive fields in the scotoma differed in shape (quantified by comparing the Gaussian peak of each receptive field to its centre of mass) from those outside the scotoma ($p=2.15x10^{-4}$, Wilcoxon's rank sum). We then compared direction selectivity of cells in V1 lesioned animals to data from two control animals. The majority of MT neurons in controls were direction selective

(80%, 40 total cells); V1 lesioned animals had fewer direction selective cells with receptive fields located inside (22.5%; $p = 5.55x10^{-7}$, Binomial distribution) and outside the scotoma (36%; $p = 3.28x10^{-5}$, Binomial distribution). Receptive fields outside the scotoma included cells within the lesion project zone, which had remapped post-lesion.
Our data suggest that MT responses exhibit dramatic changes after long-standing V1 lesions. The persistence of MT responses within the scotoma may account for the simple, residual motion perception reported in blindsight patients. The decreased proportion of directionally selective cells could also explain the compromised global motion perception in these same patients (Weiskrantz et al., 1995; Azzopardi and Cowey, 2001; Huxlin et al., 2009). However, a small number of direction selective neurons was maintained long-term. We posit that these cells may be recruited through training to recover global motion discrimination in CB fields (Huxlin et al., 2009; Das et al., 2014).

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Poster

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Topic: D.06. Vision

Support: ERC-2009-AdG 249425-CriticalBrainChanges

Title: The effect of a transient congenital visual deprivation on the neural systems for visual and sound motion processing

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Abstract: Permanently blind individuals have been shown to possess a superior sound-motion processing capability. Brainimaging studies have reported an activation of visual-motion sensitive areas (e.g. MT+) during the processing of auditory motion stimuli. In contrast, typical sound-motion related areas (as the posterior planum temporale, PT) seem to be less activated in permanently blind as compared to sighted controls. If sight can be restored, we would predict incomplete recovery of visual-motion processing along with persisting enhanced auditory motion

processing skills. Alternatively, it could be speculated that visual-motion processing recovery results in the loss of the superior auditory motion processing abilities. Behavioral studies have shown impaired global motion processing after sight restoration following a compete blindness. We used a combined behavioural and electrophysiologcal assessment of visual and auditory motion processing in individuals who were born with bilateral congenital cataracts (cc) which were removed a few months up to several years later. Two additional control groups were tested: a group of visually impaired individuals without a history of congenital blindness (developmental cataract, dc) and a group of matched heathy controls (mc). In experiment 1 the electroencephalogram was recorded in response to moving dots (1 sec) with four levels of motion coherence (30%, 50%, 70% and 90%). In a separate behavioral assessment, discrimination thresholds for global motion were assessed. In experiment 2 sound-motion stimuli (700 ms) with four difficulty levels (by manipulating the SNR) were employed. In both EEG tasks participants were asked to discriminate the motion direction of infrequent target trials. In experiment 1 the cc group displayed higher threshold than the other two groups in discriminating global motion stimuli. The cc group showed an overall significantly reduced alpha activity. In the mc, alpha oscillatory activity was modulated by the global visual motion coherence level. In experiment 2 the cc group outperformed both control groups in sound-motion discriminations; they displayed an overall enhanced beta activity compared to the two control groups. In each group the level of beta activity varied with sound-motion SRN. These results suggest persisting crossmodal plasticity (oscillatory activity changes) and crossmodal compensation (enhances auditory motion processing skills) due to congenital blindness despite on average several years since sight restoration.

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Poster

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Title: Gain adaptation with and without rate adaptation in cortical area MT

Authors: *B. LIU, M. MACELLAIO, L. OSBORNE;
Neurobio. Dept., Univ. of Chicago, Chicago, IL

Abstract: Sensory neurons respond to changes in stimuli with a modulation of their firing rate, but that response is highly context-dependent. The mean, variance, higher order stimulus statistics drive changes in a neuron's state that impact how information about the stimulus is encoded. These adaptive changes can result in an efficient representation of the incoming signal within the neuron's limited response bandwidth. In previous studies of cortical adaptation, changes in sensitivity to stimulus fluctuations (gain adaptation) were coupled to changes in firing rate, typically a fast transient rate change that relaxes more slowly over time with dynamics on many time scales (rate adaptation). Here we show that gain adaptation and rate adaptation are independent processes in the intact brain. We recorded extracellular activity of single units in area MT in alert monkeys using stochastic motion stimuli that varied in direction or speed on short (20ms) timescales, and stepped between different mean and/or variance levels at longer (250ms-2s) intervals. We find that step changes in variance of time-varying motion stimuli centered on the flank of the tuning curve, where neurons are most sensitive to direction and speed fluctuations, can produce very rapid changes in response gain without changes in firing rate. However, the same neurons driven by fluctuations along the preferred–null axis produce both the rate-independent fast gain shift and the slower rate adaptation observed in previous studies. We find that the dominant rate-adaptation time constants in MT neurons, determined by exponential fits to the firing rate relaxation, have a mean of 87ms for upward shifts in velocity variance, and 96ms for downward variance shifts, similar to reported time constants for synaptic facilitation and depression. The rate adaptation time constants for upward and downward variance steps were correlated, but were independent of the timescale of the transient onset responses to motion steps. The rapid gain changes were almost too fast to resolve, suggesting that neither intrinsic, conductance-mediated adaptation nor synaptic adaptation mechanisms can account for the observed neural behavior. An information theoretic analysis of coding in both the preferred-null and flank-centered cases indicates that the rapid gain rescaling alone is responsible for information recovery after a step change in motion variance.

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Poster

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Title: Efficient coding of optic flow can account for MSTd visual response properties

Authors: *M. BEYELER[1], N. DUTT[2], J. L. KRICHMAR[3];
[1]Computer Sci., Univ. of California Irvine Dept. of Computer Sci., Irvine, CA; [2]Dept. of Computer Sci., [3]Dept. of Cognitive Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Neurons in the dorsal subregion of the medial superior temporal (MSTd) area respond to large, complex patterns of retinal flow, implying a role in the analysis of self-motion. Some neurons are selective for the expanding radial motion that occurs as an observer moves through the environment ("heading"), and computational models can account for this finding. However, ample evidence suggests that MSTd neurons may exhibit a continuum of visual response selectivity to large-field motion stimuli. Furthermore, the underlying computational principles by which these response properties are derived remain poorly understood. Here we describe a computational model based on the hypothesis that neurons in MSTd efficiently encode the continuum of large-field retinal flow patterns on the basis of inputs received from neurons in MT with receptive fields that resemble basis vectors recovered with nonnegative matrix factorization (NMF). These assumptions are sufficient to quantitatively simulate several essential response properties of MSTd neurons, such as 3D translation and rotation selectivity, tuning to radial, circular, and spiral motion patterns, as well as heading selectivity. This finding suggests that these properties might emerge from MSTd neurons performing a biological equivalent of dimensionality reduction on their inputs. Furthermore, the model accurately captures prevalent statistical properties of visual responses in macaque MSTd, such as an overrepresentation of lateral headings that can predict behavioral threshold of heading discrimination and heading perception during eye movements. At the population level, model MSTd efficiently and accurately predicts a number of perceptual variables (such as heading and eye rotation velocity) using a sparse distributed code, consistent with ideas from the efficient-coding and free-energy principles. The present work offers a biologically plausible account of a wide range of visual response properties ranging from single-unit selectivity to population statistics, and thus provides

a further step towards a scientific understanding of the often noninuitive response properties of MSTd neurons.

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Poster

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ARC Centre of Excellence for Integrative Brain Function

ARC SRI in Bionic Vision

Title: Task- and time-dependence of population codes for motion in marmoset MT

Authors: ***E. ZAVITZ**, H.-H. YU, M. G. P. ROSA, N. S. C. PRICE;
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Abstract: Perception depends on decoding the activity of populations of sensory neurons. The firing rate of the down-stream neurons that contribute to this decoding is thought to be determined by a weighted sum of the inputs received from lower-order sensory neurons. Previous studies have suggested that the nature of the task may affect this weighting; yet work on adaptation-related perceptual aftereffects suggests that the flexibility of the read out is limited. To examine the necessity of a flexible readout, we compared the decoding performance and profiles of weights when linear decoders were specifically trained on identification and discrimination tasks, to performance and weights with a single, general model. We recorded spiking activity from 136 direction-selective single units in the middle temporal area (MT) of four sufentanil-anaesthetised marmoset monkeys (*Callithrix jacchus*). Visual stimuli comprised 500 ms of coherent dot motion, followed by 500 ms of blank screen, with 12 motion directions randomly interleaved. Using a generalized linear model, we learned each neuron’s optimal weights for solving 2- and 12-alternative forced choice direction identification tasks. A neuron's learned weight depends almost entirely on (1) the relationship between the neuron’s preferred

direction and the direction being decoded, and (2) the direction selectivity of the neuron. In a 12 AFC task, the optimal weights are highest when firing rates are highest, i.e. when the preferred direction is the same as the decoded direction. In a 2 AFC task, the weights are zero when the preferred direction of the neuron aligns with the discrimination boundary. Across neurons, the shapes of these functions are consistent, but the amplitudes vary widely. This variance is largely predicted by direction selectivity, as neurons that are more direction selective have higher weights. To assess how general these models are, we compared the decoding performance on the two types of task using the learned weights to performance using a reduced model that assigned weights based only on preferred direction and direction selectivity. Surprisingly, this reduced model completely accounted for decoding performance in 12 and 2 AFC tasks, but the template model learned in the 12 AFC task was equally effective in the 2 AFC task. These results demonstrate that a rigid, template-based population code produces sufficiently stable and high-quality decoding over time and across perceptual tasks, and can account for the existence of adaptation-induced perceptual aftereffects.

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Poster

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Title: Dynamics of population codes of stimulus features in primate area MT

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Abstract: The middle-temporal area (MT) of primate visual cortex plays a key role in the perception of motion. Neurons in area MT are generally thought to transform the signals of localised motion detectors into a representation of motion that is invariant to other image

attributes, including contrast and contour orientation. Electrophysiological studies have explored response properties of single neurons within area MT. This work suggests that the distinct motion sensitivity of area MT takes times to develop. Here, we explored how the encoding of direction by MT evolves over time in population responses. We performed extracellular recordings from populations of neurons in area MT of anaesthetised marmoset monkeys, during presentation of large moving gratings. Using multivariate classification analysis, we measured the stimulus-related information that was present in the spiking responses. Using Representational Similarity Analysis (RSA) we were then able to reveal the order in which different features of the moving gratings (spatial and temporal frequency, direction and orientation) are evident in the population response. We found a striking dependence of the population response on grating orientation at response onset, which shifted towards an orientation-invariant representation of direction over the first 100ms of response, consistent with single-unit work. In addition our analyses show that the contour-orientation dependent response and the shift toward invariance are most pronounced for stimuli of high spatial frequency and low temporal frequency. Our work demonstrates the dynamic encoding of motion primitives and emergent motion features (contour invariant responses) at the population level in MT neurons.

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Poster

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Support: ONR N00014-14-1-0359

Title: A neural model of how direction and disparity signals interact in MT and MSTd to extract object motion during self-motion

Authors: *O. W. LAYTON[1], B. R. FAJEN[2];
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Abstract: As humans move through the world, we must perceive the motion of objects that move independently from ourselves. Remarkably, our perception of an object's trajectory relative to the stationary environment is at best weakly affected by our self-motion. This suggests that the visual system recovers the world-relative motion of objects from the observer-relative pattern of motion that strikes the eye of the moving observer (i.e. sum of motion arising from the observer and objects). According to the flow parsing hypothesis, the visual system extracts

world-relative object motion by factoring out the component of the optic flow due to observer's self-motion (Rushton & Warren, 2005). While studies have established the essential role of the global pattern of motion, consistent with the flow parsing account, it remains unclear how the visual system robustly recovers world-relative object motion under a wide range of circumstances. In particular, humans are capable of perceiving the world-relative trajectory, whether the object is stereoscopically defined and spatially separated from the background environment (Warren & Rushton, 2007) or appears at the same depth as the background (Warren & Rushton, 2008). Previously, Layton & Fajen (2015, SfN) built a neural model of cortical areas MT and MSTd that demonstrates how the visual system may rely on a temporal solution to recover world-relative object motion. While MT responses may initially reflect the retinal motion of an object, the response shifts over time through feedback from MSTd cells that respond to the global pattern of motion and local interactions with MT cells tuned to opponent motion. We expanded the model to clarify how motion and disparity signals may interact to recover an object's world-relative trajectory as it moves through depth. Our results show that feedback from MSTd cells leads to stronger directional modulation in MT cells tuned to congruent disparities (Roy & Wurtz, 1990), which suggests a more complete recovery of world-relative object motion when the object is stereoscopically defined. We show how disparity and directionally tuned suppressive receptive field surrounds in MT- may provide a redundant local mechanism to complement the opponent motion antagonism included in the model of Layton & Fajen (2015, SfN).

Disclosures: O.W. Layton: None. **B.R. Fajen:** None.

Poster

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Title: Causal evidence of directional signals in macaque middle temporal area pooled for heading computation based on optic flow

Authors: *Y. GU[1], X. YU[2];
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Abstract: Accurate heading perception relies on visual information integrated across a wide field, i.e. optic flow, but how this spatial pooling mechanism is implemented by underlying

neural circuitry remains unclear. In the current study, we trained macaques to judge heading directions based on global optic flow, with or without local perturbation cues, while we simultaneously recorded from the middle temporal area (MT). MT neurons typically contained much smaller receptive fields that occupied only a restricted visual field compared to those in the downstream areas, e.g. MSTd, yet they were highly responsive to the global heading stimuli. Electrical microstimulation in MT significantly biased the animals' heading judgments for nearly half of the cases, and for majority of them, the biased directions were predictable from the tuning curves of the stimulated neurons. Interestingly, the microstimulation effects on the behavior were significantly larger when neurons with excitatory receptive field surrounds were artificially activated compared to those neurons with inhibitory surrounds. Hence our data provide direct neurophysiological evidence demonstrating that the local visual motion signals represented in MT are weighted pooled by downstream areas such as MSTd for global heading computation. Such an integration mechanism also accounts for the biased heading estimation effect in the situation when part of the visual field is distorted, e.g. by moving objects independent on ego-motion, as observed in many psychophysical studies.

Disclosures: **Y. Gu:** None. **X. Yu:** None.

Poster

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Title: Rats can process high level motion: a behavioral study using a discrimination task

Authors: ***R. BELLACOSA MAROTTI**, S. E. ROSSI, D. F. ZOCCOLAN;
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Abstract: Rats are emerging as interesting models of high-level visual processing, as they can perform complex visual tasks, such as invariant visual object recognition. Still, very little is known about how these animals process high-level motion signals, i.e., whether they can discriminate global motion of a complex pattern. Previous findings on humans and primates suggest that an integrative stage is required for correct discrimination of these patterns.
This study investigates how rats perform high-level motion discrimination, to assess whether this species can be a useful model of late stages of motion processing.
We tested 12 long-evans rats in a global motion discrimination task using plaid patterns. Plaids

are formed by two superimposed gratings moving towards different directions. Perceived direction is obtained by combining the directions of the grating components. Rats had to discriminate vertical (either upward or downward) from horizontal (either leftward or rightward) motion. We first trained rats using 100% coherently moving random dots at different display contrasts (0.3 to 1 Michelson) and dot sizes (0.5 to 8 deg). Dots were drifting at 40 deg/s. We then tested task generalization to drifting gratings and plaids (0.7 contrast, 0.25 cycles/deg spatial frequency, 10 and 1 Hz temporal frequency). In generalization trials, no feedback was provided to the animals about the correctness of their responses. This ensured spontaneous response to novel stimuli.
We found that rats were able to discriminate dot directions at most size and contrast combinations, with higher discriminability at intermediate values. Generalization was successful with plaids, especially at high temporal frequency (~77% correct responses on average). Performance with gratings was instead below chance (~37% correct responses on average) for both drifting and static patterns.
Results show that rats can successfully perceive the global direction of plaids, spontaneously generalizing to these patterns after having been trained with dots. By contrast, they seem to adopt a strategy based on orientation rather than direction when dealing with gratings. This indicates that rats are capable of processing high-level motion, but they likely rely on different perceptual strategies depending on the pattern they have to process.

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Poster

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Support: NIH Grant EY018613

Title: MT neurons have different tuning properties at contrast threshold and above

Authors: *A. PAWAR, S. GEPSHTEIN, T. D. ALBRIGHT;
Salk Inst. VCL-A, La Jolla, CA

Abstract: Background: Behavioral studies of visual perception often employ stimuli near the threshold of visibility (for example by measuring contrast sensitivity of humans and animals) and also well above the threshold. But the neural mechanisms underlying visual perception are most commonly studied using suprathreshold stimuli, under the assumption that many characteristics

of neuronal responses at the threshold and above the threshold are similar to one another. Here we investigate neuronal behavior across the full range of luminance contrasts and ask whether cell tuning at the threshold can be accurately predicted from suprathreshold contrast measurements. **Methods:** We measured responses of 80 cells in the middle temporal (MT) area of the visual cortex of one alert macaque monkey engaged in a fixation task. The stimuli were sinusoidal gratings at multiple contrasts (0.5-100%) at 5 spatial frequencies (SF) and 1-3 temporal frequencies (TF). For each cell, we obtained response functions and contrast sensitivity functions (CSF). We defined the response function as the firing rate measured at a fixed contrast for multiple SFs. We defined CSF as the reciprocal of the contrast at which the firing rate was one standard deviation above the resting firing rate for multiple SFs. We compared the SF peaks of response functions for multiple contrasts with the SF peaks of contrast sensitivity within cells. **Results:** A previous study concluded that response functions at 25 and 50% contrasts in anesthetized cats were an accurate predictor of the sensitivity function in MT cells (Movshon et al., 1978). We found that peaks of CSF and response functions matched only in 25% of all cases and mostly at lower contrasts (below 15%). At high contrasts, the peak of the response function tended to drift toward low or high SF. We modeled frequency tuning of the network using a canonic inhibition-stabilized circuit (arXiv:1410.4237). At low stimulus contrasts, in the linear regime, such a circuit behaved as a linear filter intrinsically tuned to stimulus frequency. At high contrasts, in the nonlinear regime, the intrinsic tuning of the circuit changed and its frequency tuning shifted similar to that of MT neurons. Compared to the contrast normalization models, in which visual cortical computation are represented by banks of frequency-tuned linear filters with a separate stage of response nonlinearity (Carandini & Heeger, 2012), this model dynamically changes its tuning properties as a function of contrast. **Conclusions:** MT neurons have different tuning properties at the threshold of luminance contrast and above the threshold. This behavior is predicted by a model of canonic cortical computation.

Disclosures: **A. Pawar:** None. **S. Gepshtein:** None. **T.D. Albright:** None.

Poster

329. Visual Motion

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 329.17/HH7

Topic: D.06. Vision

Support: NSF 1147440

NSF 1238599

NIH U01-HD-076595

Title: The appearance and disappearance of visual forms defined by differential motion evokes distinctive EEG responses in school-age children

Authors: *R. O. GILMORE, D. A. FOUAD, 16802, M. G. DEXHEIMER, 16802, A. R. SEISLER;
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Abstract: Differential motion patterns aid in the segmentation of visual figures from the background. Adults show evoked brain responses to time-varying motion-defined forms over posterior scalp regions (Fesi et al., 2011; 2014); in these participants, EEG amplitudes scale with the magnitude of direction differences between the figure and background. However, little is known about the development of brain responses to motion-defined forms in childhood. In this study, we measured steady-state visual evoked potential (SSVEP) responses in n=37 children (mean age: 6.4 years; 16 female). Participants passively viewed random dot kinematogram displays that depicted visual forms which differed in direction from uniform background motion by 5, 45, or 180 deg. Four 9x9 deg square-shaped figure regions emerged from and disappeared into the background at a rate of 1.2 Hz (F1). Figure and background regions were populated with white (39 cd/m^2) dots on a black (.065 cd/m^2) background at a density of 10%; dot positions were updated at 36 Hz (F2). Each condition was presented at two speeds (1.2 and 6.0 deg/s). EEG was collected at 432.43 Hz using a 128 channel EGI system and PowerDiva Video 3.4 software and submitted to a discrete Fourier transform. The complex domain (real and imaginary) components of each channel were analyzed using mixed-effects MANOVA, with direction difference and speed as fixed factors and participant as a random factor. We chose $p<.0005$ as our alpha level to reduce the likelihood of reporting false positives. Statistically significant effects for direction were found at 1F1, 2F1, and 3F1, and these showed a broad distribution across the scalp. No channels met criterion for the effect of speed at any harmonic. Many, but not all channels showed the scaling of amplitude by figure/background direction difference found in adults, an effect particularly pronounced at 3F1. Complex domain plots of the most reponsive channels at 2F1 and 3F1 showed consistent phase and amplitude profiles. These results show that the appearance and disappearance of visual forms defined by local motion differences engages a widespread network of brain regions in school-age children that is similar but not identical to adults.

Disclosures: R.O. Gilmore: None. **D.A. Fouad:** None. **M.G. Dexheimer:** None. **A.R. Seisler:** None.

Poster

329. Visual Motion

Location: Halls B-H

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Topic: D.08. Visual Sensory-motor Processing

Support: HHMI Funds

Title: Visual projection neurons link feature detection to distinct behavioral programs in *Drosophila*

Authors: *M. WU[1], A. NERN[2], W. WILLIAMSON[2], M. MORIMOTO[2], M. REISER[2], G. CARD[2], G. RUBIN[2];
[1]Dr. Gerald Rubin's lab, [2]HHMI/Janelia Res. Campus, Ashburn, VA

Abstract: How does a brain use visual information to guide behavior? To address this question, we study a class of *Drosophila* visual projection neurons called lobula columnar (LC) cells. LC neurons project from early visual processing areas to distinct central brain structures called optic glomeruli. We anatomically describe 22 different LC types and show that, for several types, optogenetic activation in freely moving flies evokes specific, highly penetrant behavioral phenotypes including jumping, backward walking and reaching. The takeoff jumping and backward walking phenotypes of two LC types closely resemble natural avoidance behaviors triggered by a visual loom. *In vivo* two-photon calcium imaging reveals that these LC types respond to looming stimuli, while another type does not, but instead responds to the motion of a small object. Furthermore, to emulate the naturalistic activation of these neurons, which would typically consist of asymmetrical visual stimulation - stronger on one eye than the other - we used a genetic approach to stochastically label and activate LC neurons on only one side of the brain. We found that such unilateral activation can result in either attractive or aversive turning behaviors depending on the cell type. Taken together, our results suggest that LC neurons convey information on the presence and location of behaviorally relevant visual features and that LC activation mimics the presence of such features in the environment, triggering specific behavioral responses. Our data provide a starting point to further study the processing of visual information in the brain for generating appropriate behavioral programs.

Disclosures: M. Wu: None. **A. Nern:** None. **W. Williamson:** None. **M. Morimoto:** None. **M. Reiser:** None. **G. Card:** None. **G. Rubin:** None.

Poster

329. Visual Motion

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Topic: D.08. Visual Sensory-motor Processing

Support: NIH EY017605

Title: Dynamic motion-tuning of macaque MST neurons before impending saccades

Authors: *J. DUIJNHOUWER, B. KREKELBERG;
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Abstract: Saccadic eye movements enhance vision by quickly directing the high acuity fovea at points of interest within the visual scene. However, they do this at the expense of adding spurious motion to the visual stream. We investigated how motion-tuning of neurons in the middle superior temporal (MST) cortex area of the macaque changes before an impending saccade. We performed single-unit recordings of the responses to random sequences of motion steps in eight different directions, while the macaque made cued saccades between two horizontally separated fixation points. We extracted motion tuning parameters using a generalized linear modeling approach and compared motion tuning parameters well before the saccade with those just prior to it. We found that the amplitude of motion tuning strongly reduced before a saccade, whereas the untuned component of the response increased. The preferred direction and tuning-width were not systematically affected. Both a decrease in amplitude and an increase in the untuned response are compatible with a reduced behavioral sensitivity to motion, hence we hypothesize that these tuning changes serve to reduce the disrupting effects of saccades.

Disclosures: J. Duijnhouwer: None. **B. Krekelberg:** None.

Poster

329. Visual Motion

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Program#/Poster#: 329.20/HH10

Topic: D.08. Visual Sensory-motor Processing

Support: NC3Rs NC/L000385/1

EC FP7 PVPITM

Title: Freeze or flight: vision guides choice of defence strategies in mice

Authors: G. DE FRANCESCHI, T. VIVATTANASARN, A. B. SALEEM, *S. G. SOLOMON;
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Abstract: In prey species like mice, avoidance of predators is key to survival and drives instinctual behaviours such as freeze and flight. Previous work has shown that mice generally flee to a refuge when presented with a dark looming visual stimulus (Yilmaz and Meister, 2013, Curr Biol 23, 2011-15). Freezing behaviour has instead mainly been investigated using real predators or their odours. Here we asked if variation in visual stimulus alone can determine if mice choose freeze or flight behaviour. We monitored acclimatised mice freely exploring an arena that was surmounted by a monitor. Visual stimuli were triggered when mice neared the centre of the arena, and we measured responses to one visual stimulus per day. The 'loom' stimulus was an expanding black disc (increasing to 50 degrees in 0.25s), simulating an approaching predator. The 'sweep' stimulus was a 5 degree black disc translating on the monitor at 21 degrees/s, simulating a gliding predator. We defined freezing as reduction of movement speed to less than 2 cm/s, for at least 0.5s. Confirming previous work, most mice showed rapid flight behaviour in response to a looming stimulus (88% of trials). By contrast, mice generally froze in response to the sweep stimulus (84% of trials), and showed flight behaviour in 22% of trials, usually after freezing. White sweep stimuli produced similar results. Further tests showed similar freeze responses to sweep stimuli at 5 and 42 degrees/s, with increased probability of subsequent flight at higher sweep speeds. At the highest speed tested (84 degrees/s), mice showed sub-second flight behaviour in response to sweep stimuli but this response was preceded by a brief freeze (ca. 0.2s). Flight responses to loom stimuli did not show this brief freeze. We have discovered that a sweeping visual stimulus elicits a strong defensive response: freezing. Using the same apparatus a different visual stimulus, loom, elicits an opposing defensive response: flight. Together these provide a simple, easily controlled behavioural paradigm, in which mice make behavioural choices based on vision alone.

Disclosures: G. De Franceschi: None. **T. Vivattanasarn:** None. **A.B. Saleem:** None. **S.G. Solomon:** None.

Poster

329. Visual Motion

Location: Halls B-H

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Program#/Poster#: 329.21/HH11

Topic: D.08. Visual Sensory-motor Processing

Support: DFG EXC307

Title: Sharper, stronger, faster upper visual field representation in primate superior colliculus: implications for afferent and efferent collicular topography

Authors: *Z. M. HAFED, C.-Y. CHEN;
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Abstract: The primate superior colliculus (SC) is critical for a variety of visual, cognitive, and motor functions. An overwhelming assumption about the SC's anatomical representation of space is that it magnifies foveal representations, but equally so for different elevations from horizontal. Here we demonstrate a dramatic and categorical difference in the SC's representation of upper (UVF) versus lower (LVF) visual fields. We recorded from 419 visual, visual-motor, and motor-related SC neurons in 2 monkeys. The monkeys performed a variety of standard behavioral tasks, including visually-guided, delayed-visually guided, and memory-guided saccades. Our neural database (covering fovea to ~20 deg eccentricities and -90 to 90 deg directions from horizontal), allowed us to functionally identify differences between UVF and LVF representations. We found that UVF response fields (RF's) are much smaller than LVF RF's. Moreover, this effect persisted for both visual as well as saccade-related RF's. UVF visual RF's additionally exhibited stronger and lower-latency visual responses, as well as higher spatial frequency preferences. Given the importance of SC visual responses in influencing saccadic reaction times (SRT's), these results provide a direct neural mechanism for behavioral observations of shorter UVF SRT's. Such shorter SRT's have eluded a clear neurobiological substrate for several decades. Moreover, motivated by UVF/LVF RF differences, we also searched for and found additional saccade effects. For example, UVF saccade endpoint accuracy was higher, even for memory-guided saccades in the absence of a visual stimulus. This may be a direct consequence of smaller UVF saccade-related RF's, independent of visual representations. Finally, we found that there are structural implications of smaller UVF RF's on SC topography. Specifically, ensuring coverage of the UVF by the SC would require an over-representation of this field in the SC's neural tissue. We confirmed this by analyzing preferred directions and eccentricities at SC surface for many electrode tracks, and we found a significant UVF over-representation. Because this observation is dramatically different from the universally accepted Ottes et al. (1986) model of SC retinotopic mapping, we developed a modified model of afferent/efferent topography, which is more accurate, and which motivates recasting of structure-

function relationships in the visual system in general. Despite its appearance as a continuous layered sheet of neural tissue, the SC contains a functional discontinuity between UVF and LVF representations, paralleling a physical discontinuity present in cortical visual areas.

Disclosures: Z.M. Hafed: None. **C. Chen:** None.

Poster

329. Visual Motion

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Topic: D.08. Visual Sensory-motor Processing

Support: CIHR MOP-115178

NSERC 341534-12

Title: Visual receptive fields and cortical oscillations during saccadic suppression in area V4

Authors: *T. P. ZANOS[1,2], P. J. MINEAULT[2], D. GUITTON[2], C. C. PACK[2];
[1]Ctr. for Bioelectronic Med., Feinstein Inst. For Med. Res., Manhasset, NY; [2]Neurol. and Neurosurg., Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: We explore our visual surroundings by moving our eyes to point our retinas at behaviorally relevant areas of our visual fields. These rapid displacements of our retinal image might be expected to produce a perception of sudden jumps of the visual image, but nevertheless our visual perception remains stable. One of the best known mechanisms for maintaining perceptual stability is *saccadic suppression*, a decrease in visual sensitivity that occurs with each eye movement. While saccadic suppression has been well documented both psychophysically and neurophysiologically, its underlying mechanisms are not fully understood. One plausible candidate is a saccade-triggered reduction in the gain of visual responses to specific stimuli. Alternatively, saccadic suppression might involve *additive* influences on visual responses, independent of any specific stimulus. Moreover, these influences might be identical across the visual field, as suggested by some recent psychophysics, or they might be stronger in the peripheral visual field, as suggested by older psychophysical studies. We have examined these issues in visual cortex area V4, where neurons are known to be strongly modulated by both visual stimuli and saccades. In order to study the dynamics of neural signals across this map, we chronically implanted 10x10 microelectrode arrays into dorsal V4 of two macaque monkeys. We examined the activity of single neurons and local field potentials (LFPs) during experiments in which the monkeys made saccades into and out of the area of the visual field accessible to the

arrays, while we simultaneously presented a sparse noise visual stimulus to map single-neuron receptive fields. A model-based approach allowed us to separate and track the multiplicative and additive parts of the responses of V4 neurons in space and time. We show that a significant number of V4 neurons exhibit neural correlates of saccadic suppression. This suppression transitions between two phases that are characterized by differences in both their retinotopic organization and their mechanistic aspects. The first phase, occurring before and during the execution of a saccade, is driven by a multiplicative suppression that is roughly the same across all eccentricities. This is followed by a second phase that involves an additive component limited only to peripheral neurons. The transition between these two phases is accompanied by a strong increase in the power of alpha-band (7-13Hz) LFP activity at peripheral V4 sites. Together these results may account for various psychophysical results, and suggest a new mechanistic interpretation of saccadic suppression.

Disclosures: **T.P. Zanos:** None. **P.J. Mineault:** None. **D. Guitton:** None. **C.C. Pack:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: NIH, RO1 NS-026143-29

Kavli Institute for Neuroscience

Title: Monitoring cortical state to explain variability in visual detection behavior and evoked responses in primary visual cortex

Authors: *G. T. NESKE, D. A. MCCORMICK;
Neurosci., Yale Univ., New Haven, CT

Abstract: Processing of sensory stimuli is strongly dependent on cortical state. While the most dramatic shift in state occurs during the transition from sleep to wakefulness, the waking period itself is associated with frequent state fluctuations. These waking state fluctuations significantly influence both sensory-evoked responses in primary sensory cortices and perceptual decision making.
The cortical state that promotes optimal sensory processing is unclear. Using pupillometry to track the full spectrum of waking states, our group recently showed that the highest gain and reliability of evoked responses in mouse primary auditory cortex and optimal performance on an

auditory detection task occur during a state characterized by intermediate arousal without locomotion (*Neuron*, 87: 179, 2015). Yet, many previous investigations of state-dependent sensory processing in mouse primary visual cortex (V1) have suggested that optimal sensory processing occurs during locomotion. Would an analysis of the full spectrum of waking states reveal that optimal visual detection and V1 gain modulation occur during intermediate arousal without locomotion, as in the auditory system, or would such an analysis confirm that optimal visual cortical processing in fact occurs during locomotion?
To answer this question, we have designed a visual stimulus-in-noise detection task for head-fixed mice free to walk on a cylindrical treadmill. We monitor cortical state by imaging the pupil of the non-stimulated eye and by monitoring treadmill speed. In the visual stimulus-in-noise detection task, each trial begins with a *foreperiod* consisting of sequences of 1.5-s-long Gaussian white noise movies (the number of sequences ranging from 1 to 6, exponentially distributed) during which time the mouse can respond by licking (false alarm) or withhold licking (correct rejection). False alarms are followed by a time-out, while correct rejections are followed by a 1.5-s-long *target period* consisting of a drifting grating of variable contrast embedded in one of the white noise movies during which time the mouse can respond by licking (hit) or withhold licking (miss). We quantify performance on this task as a function of cortical state by comparing values for discriminability (d'), bias, and reaction time across the range of pupil diameters extracted from videos of the non-stimulated eye. To investigate state-dependent gain modulation of evoked V1 responses, we consider multi-unit V1 responses to individual white noise movies and estimate multiplicative gain and additive offset factors that transform peri-stimulus time bins sorted by spike count into time bins sorted according to pupil diameter.

Disclosures: **G.T. Neske:** None. **D.A. McCormick:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Wellcome Trust 095669

Wellcome Trust 095668

Simons Foundation 325512

Title: Voltage imaging in mouse primary visual cortex reveals late correlates of perceptual decisions

Authors: *D. SHIMAOKA, N. A. STEINMETZ, K. D. HARRIS, M. CARANDINI;
Univ. Col. London, London, United Kingdom

Abstract: [**Introduction**] When detecting visual contrast, mice rely on the signals present in primary visual cortex (V1). Indeed, unilateral inactivation of V1 strongly decreases contrast sensitivity in the corresponding visual field (Glickfeld et al. *J Neurosci*, 2013, Burgess et al. *bioRxiv* 2016). However, it is not known if signals in mouse V1 are purely sensory or whether they also correlate with perceptual decisions.
[**Methods**] To study how neural responses correlate with perceptual decisions, we used widefield voltage imaging in mice performing a two-alternative forced choice task (Burgess et al., *bioRxiv*, 2016). In the task, head-fixed mice received water reward for correctly reporting the location of a grating stimulus by turning a wheel. The mice expressed a voltage-sensitive fluorescent protein, VSFP-B1.2, in all excitatory neurons via a triple transgenic approach (Madisen et al, *Neuron*, 2015). We imaged fluorescence of the two chromophores in VSFP-B1.2 using two cameras, and combined their signals to estimate membrane potential.
[**Results**] The voltage response of V1 to the stimuli consisted of two phases. During an early phase (100-200 ms), the voltage signal depended on stimulus contrast. During a later phase period (300-400 ms), there was a second depolarization, whose latency depended on stimulus contrast. On a session-to-session basis, however, the mean amplitude of the late phase correlated with the percentage of correct trials in the session.
[**Conclusions**] Consistent with results obtained in primates (Roelfsema, *Neuron*, 2007) our results indicate a differential involvement of early and late V1 responses in sensation and perception. The early response appears to be purely sensory and does not seem to play a role in perceptual decisions. Later aspects of the response, however, might be modulated differentially by variations in cortical state, or might either be in involved in or reflect the decision-making process.

Disclosures: D. Shimaoka: None. **N.A. Steinmetz:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Jonas Salk Fellowship

The Gatsby Charitable Foundation

The Fiona and Sanjay Jha Chair in Neuroscience

Title: The effects of uncertainty on change detection in the marmoset

Authors: *M. AVERY, J. REYNOLDS;
Salk Inst., San Diego, CA

Abstract: The common marmoset (Callithrix Jacchus) has become an increasingly important nonhuman primate model due to recent successes in germline transmission of modified genes in the marmoset, their short reproductive cycle, and their lissencephalic cortex. A great deal of work has been done to map out the visual system of the marmoset and study it under anesthesia. To examine mechanisms of active vision we have trained a marmoset to perform a cued detection task. In this task, the animal was trained to detect a change in orientation in one of six equally spaced Gabor patches surrounding a fixation point. In addition, we varied the uncertainty regarding the location that the target would be presented. In high uncertainty conditions, the target appeared at any of the six possible locations. In medium uncertainty conditions, the target appeared in only the left or right three locations, and in low uncertainty conditions, the target only appeared at a single location. The size of the orientation change varied from 3 to 45 degrees. Performance improved with the size of orientation, increasing from just above chance performance for trials on which the orientation change was 3 degrees to 90% for changes of 45 degrees. Analysis of preliminary behavioral data suggests that the cue in the low uncertainty condition yields faster and more accurate detection of targets appearing at the cued location, as compared targets in the low uncertainty condition.

Disclosures: **M. Avery:** None. **J. Reynolds:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: NIH R01EY13962

Title: Dissociation of confidence from performance in the monkey.

Authors: *S. CHO, P. GRIMALDI, H. LAU, M. A. BASSO;
UCLA, Los Angeles, CA

Abstract: Confidence is a belief about the validity of our own thoughts, knowledge or performance and relies on a subjective feeling (Luttrell et al., 2013). Measuring confidence in monkeys commonly relies on tasks that involve two choice discriminations and either the possibility of opting out through a third choice when not confident or post decision wagering. However, in opt out tasks commonly used in monkeys, confidence and performance are correlated since when the choice is easy the animal has good performance and high confidence. Therefore, here, we aim to dissociate confidence from performance by designing a novel task for use in monkeys based on work performed in humans that dissociated confidence and performance. We trained a rhesus macaque monkey to discriminate random moving dots and report their decision by making an eye movement to indicate the perceived direction, either leftward or rightward. Rewards (0.33ml) were provided for correct choices and no rewards were provided for incorrect choices. To assess confidence we used an opt-out task: in half of the trials, a third target, the sure choice (S) appeared in addition to the two directional choice targets that always yielded a smaller reward (80-90% of the correct reward). The monkey reported confidence accurately, choosing S more frequently when the motion coherence decreased and the decision was more difficult. Correct target choices increased when motion coherence increased. To dissociate confidence from performance we independently manipulated the proportion of dots moving toward the main direction, called positive evidence (PE) from the proportion of the dots moving in the opposite direction (negative evidence, NE). This manipulation maintained a fixed d', while generating a systematic confidence difference between the conditions, because performance was mainly affected by the ratio of PE/NE, whereas confidence was mainly influenced by PE alone, consistent with observations in humans (Koizumi et. al., 2015). The monkey chose S with less frequency in high PE trials than in low PE trials ($p = 0.021$), but the performance across coherence ratios remained consistent ($p > 0.05$). Our results establish a task that can dissociate performance from confidence for future neurophysiological studies using a monkey model.

Disclosures: **S. Cho:** None. **P. Grimaldi:** None. **H. Lau:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **M.A. Basso:** A. Employment/Salary (full or part-time): University of California, Los Angeles.

Poster

330. Visual Cognition: Decision Making

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Program#/Poster#: 330.05/HH17

Topic: D.06. Vision

Support: NIH Grant EY11378

Howard Hughes Medical Institute

Title: Effects of optogenetic inactivation in macaque areas MT and MST on choice and confidence during a direction discrimination task

Authors: *C. R. FETSCH[1], Y. EL-SHAMAYLEH[2], N. N. ODEAN[1], G. D. HORWITZ[2], M. N. SHADLEN[1];
[1]Dept. of Neurosci., HHMI & Columbia Univ., New York, NY; [2]Physiol. & Biophysics, Univ. of Washington, Seattle, WA

Abstract: Inactivation of specific neural populations has the potential to reveal a causal influence on perceptual and cognitive functions. However, traditional methods—including lesions, pharmacological inactivation and cooling—lack the temporal resolution needed to probe the role of activity on the time scale of individual decisions. These methods may also induce plasticity or compensation by other structures, complicating the interpretation of negative results. Hyperpolarizing opsins can overcome these limitations, yet have not been used extensively in nonhuman primates for which robust psychophysical paradigms exist for studying higher vision and cognition.

The goal of this study was to test the effect of transient suppression of neural activity in a well-studied primate model of decision making. We expressed the cruxhalorhodopsin Jaws under the control of the CaMKII promoter in cortical areas MT and MST of one rhesus monkey, via injection of a recombinant viral vector (AAV8-CaMKII-Jaws-KGC-GFP-ER2). Immunohistochemistry in a second monkey confirmed robust expression in supragranular and infragranular layers, and a strong tropism for excitatory pyramidal neurons. We advanced a custom optrode targeting clusters of MT/MST neurons with consistent selectivity for direction and speed of motion. Once a suitable cluster was identified, the monkey began a direction discrimination task with post-decision wagering (PDW) in which the random-dot motion stimulus was tailored to the receptive field and tuning properties of the neurons. The PDW task allows the monkey to indicate a lack of confidence in the direction decision by opting out of the primary report and receiving a guaranteed but smaller reward.

Multi-unit visual responses were suppressed an average of 34% during simultaneous illumination with red light (633 nm, 5-30 mW/mm^2), which occurred on a random half of trials. The laser remained on throughout the stimulus viewing period, which was of variable duration under experimenter control. Optogenetic suppression caused significant effects on both choice and confidence—consisting mainly of a bias against the preferred direction of the neurons—for short duration trials, especially early in the recording session. These effects became weaker or reversed direction on long-duration trials and those later in the session, despite a similar degree of light-induced suppression of the recorded neurons. The results raise the possibility of compensation and/or changes in readout at sub-second and minute time scales, reinforcing the notion that precise temporal control can reveal patterns that may be obscured by traditional inactivation methods.

Disclosures: **C.R. Fetsch:** None. **Y. El-Shamayleh:** None. **N.N. Odean:** None. **G.D. Horwitz:** None. **M.N. Shadlen:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: NIH Grant EY022411

Title: Monkeys use different strategies to achieve near-optimal performance on a visual motion discrimination task with unequal rewards.

Authors: *Y. FAN[1], J. I. GOLD[2], L. DING[2];
[1]Neurosci. Grad. Group, Univ. of Pennsylvania, [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Humans and monkeys show remarkable flexibility in adapting to different reward contexts on perceptual decision tasks (Simen et al., 2009; Mulder et al., 2011; Summerfield et al., 2010; Gao et al., 2011, Feng et al., 2009; Nomoto et al., 2010; Teichert et al., 2012). For example, we trained three monkeys to discriminate the direction of motion of a noisy visual stimulus, using a version of the task in which correct choices for the two alternatives were associated with different reward magnitudes. All three monkeys had choices and reaction times (RTs) that reflected both the strength and direction of the motion stimulus and the reward asymmetry. However, these reward-dependent effects on choice and RT differed considerably across sessions and across monkeys, suggesting different computational strategies. The goal of this study was to analyze these differences with respect to the optimization of reward rate, and whether they reflected: 1) inefficiencies in the monkeys' abilities to achieve optimal behavior; or 2) flexibility in the monkeys' abilities to achieve near-optimal behavior. We accounted for their behavior using a sequential-sampling framework with two distinct reward-modulated computational parameters. One parameter controls the interpretation of evidence received at each moment (ΔME). The second parameter controls the total amount of evidence required to commit to a decision (ΔSV). Although all three monkeys based their decisions on both motion evidence and reward context, they exhibited different modulation patterns of ΔME and ΔSV across sessions and across monkeys. In this study, we numerically obtained expected reward rate per unit time and per trial as a function of ΔME and ΔSV. Consistent with previous theoretical studies, we found that the optimal strategy typically corresponds to specific values of each parameter(Bogacz et al., 2006; Simen et al., 2009). However, nearly optimal outcomes (e.g, >95% maximum expected reward rate) can be achieved using a much broader range of parameter values. We found that, despite the individual variability in reward modulation of ΔME and ΔSV, all three monkeys tended to use combinations of values that achieved near-optimal performance. These results underscore the importance of considering the shape of the function relating

computational components to desired outcomes (the "optimality surface") near its peak. This surface may prescribe a specific computational strategy to achieve truly optimal performance, but a wide range of strategies to achieve near-optimal performance. Taking into account if and how that range is used is critical when assessing the underlying neural mechanisms.

Disclosures: Y. Fan: None. **J.I. Gold:** None. **L. Ding:** None.

Poster

330. Visual Cognition: Decision Making

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 330.07/II2

Topic: D.06. Vision

Support: RO1 EY11749

P30 EY01319 to the Center for Visual Science

Title: Inactivation of the lateral prefrontal cortex increases neuronal activity in the ipsilateral area MT during memory-guided comparisons of visual motion

Authors: C. CHU, P. M. SPINELLI, *T. PASTERNAK;
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Abstract: The prearcuate region of the lateral prefrontal cortex (LPFC) is reciprocally interconnected with the motion-processing area MT in the same hemisphere. Recordings during memory-guided motion comparison tasks revealed that the activity of neurons in both areas is indicative of strong functional links between them (Zaksas & Pasternak, 2006; Hussar & Pasternak, 2012). The functional significance of these links is also supported by similar deficits produced by permanent unilateral lesions of either regions during comparisons of contralateral motion (Bisley & Pasternak, 2000; Pasternak et al, 2015).
We examined the contribution of the LPFC to neuronal activity in the ipsilateral MT by temporarily inactivating LPFC with muscimol and recording from MT while monkeys compared the directions of two stimuli, S1 and S2, separated by a brief delay. S1 and S2 either appeared in the MT receptive field (RF) or were separated, with S2 appearing in the RF and S1 appearing at a remote ipsilateral location. In the absence of inactivation, MT neurons showed direction selective (DS) responses during S1 and S2, consistent with their role in motion processing but also displayed activity of more cognitive nature reflecting the influences arriving from the LPFC. Thus, many MT neurons showed anticipatory modulation of activity during the delay and their responses during S2 reflected comparisons between current and remembered stimuli. In addition,

the analysis revealed brief periods of transient stimulus-selective activity during the memory delay occurring in different neurons at different times. Many MT neurons also showed weak responses to the S1 at ipsilateral locations distant from MT RFs.
LPFC inactivation resulted in a number of changes in activity in the ipsilateral MT. Perhaps the most striking effect was a dramatic increase in firing rates throughout the task, suggesting a shift in the operating range of neuronal circuits within MT. This increase was accompanied by the reduction of anticipatory activity during the delay and an apparent enhancement of responses to the ipsilateral stimuli, the two types of signals attributable to the top-down inputs from the LPFC. In contrast, DS of responses during S1 and S2 appeared to be unaffected.
The dramatic increase in the overall activity in MT resulting from the removal of top-down prefrontal influences points to the LPFC as an important source of gain control of MT neurons processing motion information. The more selective effects of inactivation on signals of cognitive nature recorded in MT further highlight the role of continuous interactions between the LPFC and MT during memory-guided motion comparison tasks.

Disclosures: **C. Chu:** None. **P.M. Spinelli:** None. **T. Pasternak:** None.

Poster

330. Visual Cognition: Decision Making

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Support: NIH Intramural program

Title: Noise correlations of macaque MT neurons for bistable stimuli are task-dependent

Authors: I. KANG[1], *B. G. CUMMING[2];
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Abstract: The orthographic projection of dots on the surface of a rotating cylinder produces a compelling three-dimensional percept that is bi-stable, undergoing spontaneous reversals. Appropriate horizontal disparities specify the rotation direction and render the stimulus unambiguous. Many neurons in monkey Middle Temporal (MT) area selective for direction and for disparity signal the rotation direction of unambiguous (disparity-defined) cylinders. This stimulus leads to large Choice Probabilities (CP, a correlation between trial-by-trial fluctuations of the neuronal response and the subject's perceptual choice). In pooling models, a larger CP implies larger noise correlations between neurons supporting the same decision. One way in which larger noise correlations might arise is if the cylinder task engages mechanisms that

modulate activity in specific groups of MT neurons. We therefore examined noise correlations in area MT of one monkey trained to report the direction of cylinder rotation, recording with 24 contact linear arrays. On each trial the stimulus rotated about a fixed axis for 2 seconds while the monkey fixated, then rotation direction was reported with an eye movement. Four different cylinder orientations were used (-45, 0, 45 and 90°). To quantify the tuning of the neuronal responses to the disparity signal we calculated d' values for each stimulus relative to a stimulus with zero disparity, then estimated the slope with respect to disparity. This tuning slope was estimated separately for each stimulus orientation. For each pair or neurons, we computed the product of these tuning slopes. Pairwise noise correlations measured in responses to zero-disparity stimuli strongly depended on both the magnitude and the sign of this product. For 241 pairs of neurons in which the responses of both members shared the same sign and were highly tuned (absolute tuning slope above 75th centile), the mean noise correlation measured with a zero disparity cylinder (at the same orientation) was 0.36. This correlation was strongly dependent on the task - the same pairs of neurons when the animal reported the rotation of an orthogonal zero-disparity cylinder had a mean correlation of 0.16 (difference $p<10^{-9}$, t-test). For 113 pairs of neurons with equally strong tuning but opposite preferences, the mean noise correlation was significantly negative (-0.09, $p < 0.01$) and became close to zero (0.02, difference $p < 0.05$) when measured with orthogonal stimuli. Thus, the noise correlation of pairs of MT neurons can be profoundly altered by task. This is difficult to explain in current feedforward accounts of noise correlation.

Disclosures: I. Kang: None. **B.G. Cumming:** None.

Poster

330. Visual Cognition: Decision Making

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Support: NEI EY016178

DC014678

Title: Perception of object motion during self-motion: Neural computations for flexible reference frame transformations in macaque areas VIP and MSTl

Authors: *R. SASAKI[1], D. E. ANGELAKI[2], G. C. DEANGELIS[1,3];
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Abstract: When a moving observer views a moving object, the retinal image velocity of the object is the vector sum of object motion in the world and self-motion. To estimate object motion in the world, one has to compensate for the effect of self-motion on the retinal image. Other times, we may need to judge how an object moves relative to our head/body, which does not require compensating for self-motion. Therefore, the brain must flexibly incorporate self-motion signals into computations of object motion.
We trained monkeys to report whether an object moves up-right or up-left during lateral self-motion (SFN 2015). In the world coordinate task, the monkey judges whether the object moved to the left or right of vertical in the world; in the head coordinate task, the animal reports left or right relative to the head. The two tasks are randomly interleaved, as cued by the fixation point. Self-motion information was provided by optic flow or by a combination of optic flow and translation of a motion platform. Monkeys successfully switched between performing the two tasks, and performance in the world coordinate task was significantly better when both visual and vestibular self-motion signals were available. We recorded responses of macaque VIP and MSTl neurons during each task. Single-unit tuning curves show diverse results, with greater effects of task in VIP than MSTl. Given this heterogeneity, we tested whether population responses could account for the flexible reference frame transformations exhibited in behavior. We trained a linear decoder to classify object motion as right or left relative to vertical. For each task, the decoder is trained to report object direction across all stimulus conditions. For the head coordinate task, decoding either MSTl or VIP activity produces good performance, which is not surprising since self-motion signals are not required for this task. Results for the world coordinate task, which requires self-motion signals, reveal clear differences between areas. For MSTl, decoder performance shows biases due to self-motion that are substantially larger than behavioral biases. In contrast, decoding of VIP responses shows a pattern of results similar to behavior, including a benefit of combining visual and vestibular self-motion signals.
Our results suggest that MSTl only partially integrates self-motion signals to represent object motion in world coordinates. In contrast, the representation of object motion in VIP appears to account for the ability to flexibly represent object motion in different reference frames. Thus, our results provide evidence for a novel role of VIP in constructing flexible, task-dependent representations of moving objects.

Disclosures: **R. Sasaki:** None. **D.E. Angelaki:** None. **G.C. DeAngelis:** None.

Poster

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Title: Choice-driven and stimulus-related activity is confounded in parietal neurons: implications for choice probabilities

Authors: *A. ZAIDEL[1,2], G. C. DEANGELIS[3], D. E. ANGELAKI[2];
[1]Bar Ilan Univ., Ramat Gan, Israel; [2]Baylor Col. of Med., Houston, TX; [3]Univ. of Rochester, Rochester, NY

Abstract: Trial by trial correlations between neural responses and choices (choice-probabilities, CPs) are often interpreted to reflect a causal contribution of neurons to task performance. However, CPs may arise from top-down, rather than bottom-up, signals. An important difference between bottom-up and top-down origins of CPs involves the expected directionality of their influence on the firing rates of sensory neurons. Specifically, bottom-up neuronal fluctuations should influence choices in a manner predictable from the neuron's tuning. By contrast, if CPs arise through top-down influences, choice-related modulations may be unrelated to a cell's tuning preference. Using a novel approach, we isolated distinct sensory and decision contributions to single-unit activity recorded from the dorsal medial superior temporal (MSTd) and ventral intraparietal (VIP) areas of monkeys during perception of self-motion. Superficially, neurons in both areas show similar tuning curves during task performance. However, tuning in MSTd primarily reflects sensory inputs, whereas choice-related signals dominate tuning in VIP. Importantly, the unique choice-related contribution to a VIP neuron's activity was not predictable from its task-relevant stimulus tuning. For example, a neuron may show a clear preference for rightward headings when responses are conditioned on choice, but the cell may respond more strongly when the animal makes leftward choices. Importantly, sensory- and choice-related signals may be confounded in CP measurements, which can create an artificial predisposition for CPs > 0.5. This occurs when choice-related signals are strong enough to dictate the tuning preference of the cell, which happens frequently in VIP. In such cases, that apparent stimulus preference of the neuron is really a choice preference, thus ensuring that CP>0.5 even when further analysis reveals that heading and choice signals are opposite in sign. Dimensionality reduction analysis further exposed unique stimulus- and choice-related signal components at the population level. Our results demonstrate a predominance of choice signals in VIP (unlike MSTd) with specificity not generally predictable from a neuron's heading tuning. Rather, choice signals make a distinct contribution to VIP responses. Therefore, these choice signals likely reflect the action of top-down feedback, as opposed to bottom-up effects of random fluctuations in response. Also, these choice signals sometimes overwhelm the stimulus-related component of neuronal activity, exposing a major potential pitfall of traditional CP analyses that may call for reappraisal of some previous conclusions.

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Poster

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Title: Modality-dependent evidence accumulation in multisensory decision-making

Authors: ***H. HOU**, Y. ZHAO, Q. K. ZHENG, Y. GU;
Inst. of Neuroscience, CAS, Shanghai, China

Abstract: To form unified percepts and make sensible decisions, the brain often needs to interpret and synthesize evidence over both sensory modality and time. Although several sensory areas in the brain have been found to encode multisensory evidence, little is known about how these pieces of evidence are decoded and accumulated in downstream sensorimotor transformation areas during multisensory decision-making. Here we recorded from well-isolated single neurons in macaque lateral intraparietal area (LIP) while animals were performing a visual-vestibular heading discrimination task. We found that ramping activities of LIP neurons depended on sensory modality in two ways, with respect to both temporal dynamics and task-difficulty dependence. First, the rising phases of ramping activities under the vestibular and combined conditions were substantially earlier than that under the visual condition; when we changed the peak time of acceleration while keeping the peak time of velocity unchanged by varying the half-width of the velocity profile, the vestibular ramping shifted accordingly, but the visual ramping did not. These results suggest that the brain may integrate over time sensory information from different modalities in terms of distinct physical quantities: visual velocity, but vestibular acceleration. Consistent with this idea, when we artificially aligned the peak times of visual velocity and vestibular acceleration in the combined condition by shifting the visual motion ~250 ms ahead of the vestibular motion, the monkey's performance was, surprisingly, even better than that under the natural scenario (alignment of visual and vestibular velocity). Second, we found that task-difficulty dependence of LIP's ramping activity was also modality-dependent. The conventional easier-trial-steeper-ramping relationship was only evident under the visual and combined conditions, but not the vestibular condition, indicating that LIP selectively accumulates visual evidence *per se*, whereas for the vestibular cue, it might receive on-line categorical choice signals delivered from elsewhere. Taken together, our observations point to a novel "late convergence" hypothesis on multisensory decision-making: in contrast to the

prevailing view that sensory inputs from different modalities converge early in multisensory areas before sensorimotor transformation, the convergence may happen at a later stage and via communication between more than one decision-related area. Our results may thus place important constraints on theoretical considerations for neural basis of multisensory integration and multisensory decision-making.

Disclosures: **H. Hou:** None. **Y. Zhao:** None. **Q.K. Zheng:** None. **Y. Gu:** None.

Poster

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NSF CAREER award 0955640

Title: Topographical organization, local cortical connectivity, and feature encoding in frontoparietal cortices

Authors: ***N. Y. MASSE**, A. SARMA, J. M. HODNEFIELD, S. SWAMINATHAN, D. J. FREEDMAN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Despite much progress, we still do not fully understand the relative roles of excitatory and inhibitory neurons in encoding stimulus information. Here, we hypothesize that the cortical organization of visual inputs into prefrontal (PFC) and posterior parietal (PPC) cortices affects the encoding capacity of inhibitory neurons, possibly accounting for some of the discrepancy reported in the literature on the relative roles of these two neuron classes. We combined data from six monkeys involved in three similar experiments to compare how the PFC and PPC encode spatial location and motion direction. We find: 1) both areas encode spatial location more accurately than motion direction, 2) during tasks which require simply remembering the motion direction, the PFC encodes this information more accurately compared to the PPC, particularly during task periods requiring short-term memory and 3) inhibitory PFC neurons encode spatial location more accurately than excitatory neurons, whereas there was no significant difference between these two neuron classes for motion direction. These differences can be explained by a simple feedforward model in which 1) inputs into both areas are retinotopically mapped, but less well organized for motion direction than location in PFC, and even less in PPC, and 2) inhibitory

neurons non-selectively and densely integrate inputs from nearby neurons. In support of this model, we find that the low-pass filtered LFP signal, believed to primarily reflect the synaptic activity within the local volume, is spatially selective in both areas, but only weakly direction selective in PFC, and weaker still in PPC. Furthermore, the preferred spatial location of the LFP signal is more correlated with the preferred spatial location of inhibitory neurons recorded on the same electrode, compared to excitatory neurons. In summary, this work suggests that organized cortical inputs increase the encoding capacity of inhibitory neurons. Future work will examine how this cortical organization can be dynamically modulated based on the behavioral context.

Disclosures: **N.Y. Masse:** None. **A. Sarma:** None. **J.M. Hodnefield:** None. **S. Swaminathan:** None. **D.J. Freedman:** None.

Poster

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Support: McKnight Endowment Fund for Neuroscience

NIH/NEI EY019041

Title: Contributions of parietal and prefrontal cortices to categorical match vs. non-match decisions

Authors: *Y. ZHOU, S. SWAMINATHAN, D. FREEDMAN;
The department of neurobiology, The Univ. of Chicago, Chicago, IL

Abstract: The prefrontal cortex (PFC) and posterior parietal cortex are known to play important roles in mediating decision-making tasks. In previous work in our lab, we trained monkeys a delay match to category (DMC) task, in which they must release a bar when the categories of sequentially presented sample and test stimuli matched, or hold the bar when the sample and test categories did not match. Neuronal activity in PFC and lateral and medial intra-parietal (LIP and MIP) areas all reflect the learned categories, especially during the sample and delay periods, while LIP appears more closely involved in the decision about sample-category than PFC and MIP. Here we examine the roles of PFC, LIP, and MIP in the match/non-match decision during the test period of the DMC task. We find that neuronal activity in all three cortical regions reflected monkeys' match/non-match choices, but appear to play different roles in the match/non-match decision-making process. First, match/non-match encoding arose with a

shorter latency in PFC than in LIP and MIP, and it was weaker in LIP than in PFC and MIP. Second, LIP and MIP responded preferentially to match than non-match choice, while PFC showed relatively balanced preference for match and non-match. Third, match/non-match signals in both PFC and MIP correlated with monkeys' reaction time (RT) but showed different patterns: in PFC, it started with a similar latency in both shorter and longer RT trials but increased with a steeper slope in the shorter RT trials; while in MIP, it started with a significantly shorter latency in the shorter RT trials than in the longer RT trials, but increased with similar slope in both types of trials. Fourth, match/non-match signal in LIP was more robust (earlier and stronger) in task conditions with higher behavioral performance compared to lower performance conditions. This pattern of results suggests that PFC is more closely involved in the match/non-match decision, while LIP activity more closely tracks the monkeys' confidence in their decisions. MIP appears to be more directly involved in the motor responses, i.e., the act of releasing the lever. Furthermore, additional analysis examines the mixed selectivity of PFC and LIP neurons, indicating that the match/non-match decision in our DMC task may be mediated by mixed encoding of the four possible combinations of sample and test categories, rather than by directly compare the similarity of sample and test stimulus features, and that these combinations may then be mapped onto planned movements. Together, our results give insight into the relative functions of parietal and frontal cortices in the category-based match/non-match decisions.

Disclosures: **Y. Zhou:** None. **S. Swaminathan:** None. **D. Freedman:** None.

Poster

330. Visual Cognition: Decision Making

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NSF CAREER award 0955640

Title: Interaction between spatial and feature attention in posterior parietal cortex

Authors: ***G. IBOS**, D. J. FREEDMAN;
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Abstract: Both space-based attention (SBA) and feature-based attention (FBA) modulate representation of task-relevant information in the Lateral Intraparietal area (LIP), an area closely involved in attentional control and decision-making. However, the joint impact of SBA and FBA

on spatial and non-spatial selectivity in LIP remains unexplored. Moreover, the extent to which LIP generates attentional modulations, or receives attentional control signals from other brain areas remains unclear. On one hand, time course of SBA modulations in LIP and area MT places LIP as one of the main sources for SBA. On the other hand, FBA-dependent feature-tuning shifts in LIP reflect the bottom-up integration of FBA modulations of upstream visual areas, in order to enhance the representation of task-relevant features and to facilitate decision-making. The degree to which LIP is an "emitter" or "receiver" of SBA and FBA signals remains an unanswered question, which needs to be addressed to understand LIP's precise role in attention and decision making.
We recorded from 74 LIP neurons from two macaque monkeys performing a spatial delayed conjunction matching task. Successions of visual stimuli (each composed of a conjunction of one color and one motion-direction) were presented simultaneously at two positions. One position was within the recorded neuron's receptive field (RF), while the other position was in the opposite quadrant of the display. At the beginning of each trial, one of two sample stimuli cued the monkeys about which spatial location and which conjunction of color and direction were behaviorally relevant. Monkeys had to release a manual lever when a test stimulus matched the position, color and motion-direction of the cued sample stimulus.
We show that FBA affects LIP neurons in a spatially global manner as feature tuning shifts toward the relevant features (typical of FBA in LIP) were qualitatively independent of the spatial position of attention. However, SBA modulated FBA effects as the amplitudes of feature-tuning shifts were larger when monkeys attended stimuli located inside neurons' RFs. Interestingly, the amplitude of SBA modulations depended on the feature tuning of LIP neurons, with larger modulations when monkeys attended neurons' preferred feature conjunctions. These results show that both types of attention potentiate each other's effects in LIP. Finally, we tested a feed-forward two-layer integrative model in which the modulations of both spatial and feature selectivity observed in LIP arise via integration of SBA and FBA-modulations of neurons from upstream visual areas such as MT and V4, consistent with LIP acting as a receiver of both SBA and FBA modulations.

Disclosures: **G. Ibos:** None. **D.J. Freedman:** None.

Poster

330. Visual Cognition: Decision Making

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Title: Looking where we want to look: Relating neuronal and behavioral correlates of image familiarity

Authors: *W. J. JOHNSTON, K. MOHAN, D. J. FREEDMAN;
Univ. of Chicago, Chicago, IL

Abstract: The primate visual system is composed of two parallel processing streams: the ventral, "what," or "vision for perception" stream and the dorsal, "where," or "vision for action" stream. These two streams are often studied independently, although behaviors that require coordinated shape and spatial processing are expected to require close coordination of both. One such behavior is the innate preference of primates and other animals for viewing novel compared to familiar visual stimuli. This requires integration of both stimulus position (encoded primarily in the dorsal stream) and familiarity (encoded primarily in the ventral stream).

We trained monkeys to perform a preferential looking-based task (PLT) to probe how familiarity guides viewing behavior and impacts neural signals in the inferotemporal cortex (ITC). In the task, eye position was tracked as the monkey freely viewed two natural images for five seconds following a brief fixation period. We consider the number of previous viewings of a particular image to be that image's familiarity and partition familiarity into three classes: trial-unique (no previous viewings), novel (1 - 50 viewings), and familiar (>5000 viewings). A preference for viewing trial-unique images emerges after a single viewing of the opposing image (6% more viewing of trial-unique than single-view in the first 2 seconds, $p < .05$) and plateaus by 50 viewings (21% more viewing of trial-unique than 50-views in the first 2 seconds, $p < .05$). Within a trial, the novelty bias peaks in magnitude shortly following stimulus presentation, achieving significance only in the first two seconds of viewing. During PLT performance, we recorded 70 visually selective neurons in ITC. 50/70 show significantly higher responses to novel images than to familiar images. Further, we show that familiarity encoding is evident prior to the first free viewing saccade (100ms before, $p < .05$), indicating that ITC could be a source of the familiarity signals causal to the PLT novelty bias.

How can spatially coarse familiarity signals in ITC be used to guide spatially precise eye movements? Using a rate-neuron network model, we identify a conjunctive coding strategy that exploits nonlinear transfer functions to amplify the spatially diffuse familiarity signals only when they coincide with the stimulus position. This model predicts that the novelty bias in the PLT will decrease as the two images are moved closer together and that there will be a corresponding decrease in the difference between stimulus representations in regions involved in saccade planning. These predictions will be tested in upcoming behavioral and neurophysiological experiments.

Disclosures: W.J. Johnston: None. **K. Mohan:** None. **D.J. Freedman:** None.

Poster

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McKnight Scholar award

Title: Task-specific vs. generalized category encoding in parietal cortex during task switching

Authors: *K. MOHAN, O. ZHU, S. K. SWAMINATHAN, D. J. FREEDMAN;
Dept. of Neurobio., The Univ. of Chicago, Chicago, IL

Abstract: Neuronal activity in the lateral intraparietal area (LIP) can reflect the learned category membership of visual stimuli. Neuronal category encoding in LIP has almost always been studied during a delayed match-to-category (DMC) task, which requires monkeys to maintain category information in short-term memory and decide whether sequentially presented (sample and test) stimuli are categorical matches or non-matches. However, it is not known whether LIP is engaged by the DMC task because it plays a direct role in the categorization process per se or because of its short-term memory or matching requirements. To explore whether LIP plays a generalized role in category-based decision making, we examined neuronal activity in LIP as monkeys alternated between two motion categorization tasks in which monkeys had to group 360° of motion directions into two categories according to a learned category boundary. Although both tasks used the same visual stimuli and category boundary, the tasks differed in several important ways. The first task was a DMC task, in which monkeys had to release a lever to indicate whether a test stimulus was in the same category as a previously presented sample stimulus. The second task was a one-interval categorization (OIC) task in which monkeys had to identify the category of a single sample stimulus and rapidly report its category membership via a saccade to either a red or green target, to indicate “category 1” and “category 2” respectively. We recorded from 97 LIP neurons from two monkeys as they alternated between the DMC and OIC tasks in blocks of 30 trials, allowing us to assess direction, category, and decision related encoding during each task for each LIP neuron. During sample presentation, many LIP neurons were direction selective in the DMC (63/97) and OIC (72/97) tasks, with 54 neurons selective in both tasks (one-way ANOVA, $P<0.01$). At the population level, category selectivity was evident with a short latency (~160 ms) following sample onset in both tasks, with many examples of individual neurons that showed qualitatively similar patterns of category selectivity in both tasks—suggesting that the two tasks tap into common directional and category representations.

We also observed task-dependent encoding in LIP among a substantial fraction (70%) of LIP neurons (unpaired t-test, $P<0.01$), which showed stronger activity in the OIC task potentially due to the requirement of rapid decisions as opposed to the delayed decisions in the DMC task. Our results suggest that LIP category signals in different task paradigms are mediated by shared neuronal mechanisms and thus, evidence that LIP plays a generalized role in the visual categorization process.

Disclosures: K. Mohan: None. **O. Zhu:** None. **S.K. Swaminathan:** None. **D.J. Freedman:** None.

Poster

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Support: KAKENHI

JST PRESTO

Title: NMDAR antagonist ketamine affects sensitivity to irrelevant information and onset of build-up activity in the parietal cortex

Authors: *Y. SUDA[1,2], T. UKA[2,1];
[1]Brain Sci. Inst., Tamagawa Univ., Tokyo, Japan; [2]Neurophysiol., Grad. Sch. of Medicine, Juntendo Univ., Tokyo, Japan

Abstract: Adaptive decision making depending on context is a fundamental ability for humans. Although the process of evidence accumulation for perceptual decision making can be explained with a recurrent neural network model implementing NMDA receptors, the role of NMDA receptors in integrating relevant rather than irrelevant information still remains unclear. To elucidate this question, we investigated the effect of NMDAR antagonist ketamine on behavior and neural activity while monkeys performed a switching task. Two macaque monkeys were trained to switch between discriminating motion direction (up or down) and depth (near vs far) in a random dot stereogram. The cue indicating which task they should perform changed randomly from trial to trial. We varied task difficulty by manipulating the motion coherence and the binocular correlation of the visual stimulus. While the monkeys performed this task, we systemically manipulated NMDA receptor function by intramuscular injection of ketamine and compared it with saline injection. We recorded local field potential (LFP) and single unit activity

from isolated LIP neurons that responded during the delay period while the monkeys performed a memory-guided saccade task. To evaluate behavioral performance, we examined switch ratio (SR) and reaction time. Although the SR for depth discrimination was not affected by ketamine, that for direction discrimination decreased from 0.89 to 0.79 with the sensitivity to the irrelevant feature rising two folds. Consistent with this behavioral performance, only the slope of build-up activity for the irrelevant feature during direction discrimination increased with ketamine administration, indicating that ketamine induced the degradation of switching ability through increased sensitivity to the irrelevant feature. Furthermore, ketamine administration increased the reaction time for the visual stimulus in both tasks irrespective of task difficulty. The onset of build-up activity was delayed with ketamine but not with saline administration. These results suggest that the NMDA receptor is engaged in the process of discarding irrelevant information, and the control of starting the accumulation process for perceptual decision making.

Disclosures: **Y. Suda:** None. **T. Uka:** None.

Poster

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Pew Scholars Program Award

Title: Choice certainty reveals equivalence of POMDP and drift-diffusion model

Authors: K. KHALVATI[1], R. KIANI[2], *R. P. RAO[1];
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Abstract: Most perceptual decision making tasks can be reduced to the problem of finding a hidden state in the environment based on noisy sensory inputs in order to obtain reward. For

example, in the well-known random dots motion discrimination task, the animal is rewarded only if it correctly guesses the net direction of motion of a group of randomly moving dots. Difficulty of each decision is controlled by the fraction of dots that move coherently in the same direction—the higher the coherence the easier the decision. While previous models have shed light on the dependency of the subject's behavior on duration and difficulty of the stimulus on each trial, less is known about the evolution of the subject's belief about stimulus difficulty during the course of a trial. The uncertainty about the stimulus difficulty significantly affects the subject's behavior, especially in tasks involving self-assessment. Here we propose a normative model that explains how a subject's belief about difficulty evolves during perceptual decision making and how it shapes the subject's expected accuracy. We demonstrate our model on a 2-choice random dots motion discrimination task with post-decision wagering, where wagering is based on the decision makers' expected accuracy of a direction choice. Our model is based on a partially observable Markov process (POMDP) with a continuous state space that jointly codes for motion direction and stimulus difficulty. We show that this model explains both performance and belief about stimulus difficulty in nonhuman primate subjects. By fitting model parameters to the performance of the subject, we are able to predict the subject's behavior in the post-decision wagering task. Finally, we show that belief updating in the continuous POMDP model has a one-to-one mapping to the classic drift-diffusion multiple-race model previously proposed as a neural mechanism for perceptual decision making. Specifically, belief updating in the continuous POMDP model reduces to a Kalman filter, while the drift-diffusion model reduces to the information filter for the same observation model. Our results demonstrate that the two frameworks are equivalent, with one providing a descriptive model of how the brain makes decisions and the other showing why it is an optimal strategy.

Disclosures: **K. Khalvati:** None. **R. Kiani:** None. **R.P. Rao:** None.

Poster

330. Visual Cognition: Decision Making

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Sloan Fellowship

Title: Face discrimination under uncertainty depends on linear integration of visual features over space and time

Authors: *G. OKAZAWA, L. SHA, R. KIANI;
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Abstract: To make decisions, we often gather discrete pieces of evidence and combine them. Our current understanding of the neural mechanisms of this integrative process for sensory decisions is based largely on simple stimuli that vary along a single dimension (e.g., direction of stochastic moving dots or orientation of gratings). Little is known about decisions on more naturalistic, complex stimuli such as visual objects consisting of multiple features. Here, we develop a new task for studying the neural mechanisms that underlie spatiotemporal processing of visual features for discrimination of faces. Human subjects (n=7) engaged in a two-alternative forced choice task where they reported the identity of a face as soon as ready. The stimuli were drawn from morph continuums between photographs of faces. Two critical aspects of the task allowed us to directly measure integration of visual features over space and time. First, we developed a powerful, custom algorithm to independently manipulate facial features (eyes, nose, and mouth). This algorithm enabled us to study spatial integration by creating ambiguous stimuli in which different features could support different choices. Second, on each trial, facial features underwent a sequence of subliminal changes along the morph lines, enabling us to study temporal integration by creating stimuli that could vacillate from one identity to another over time. We show that subjects integrated visual information over space and time for their decisions. This integration was largely linear, different facial features were weighed differently, and the weights were largely constant over time. Further, the weights were flexible and changed depending on the task: eyes were weighted more than mouth in an identification task but mouth weighed more for discrimination of emotions. These weights could not be attributed to mere differences in discriminability of features. A multi-feature extension of bounded accumulation model—where the evidence conferred by visual features is integrated over space and time toward a decision bound—quantitatively explained choices and reaction times. We ruled out alternative models in which subjects did not integrate over space (e.g., attention to only one feature), did not integrate over time (e.g., decision based on a snapshot), or separately identified individual features and then chose the option with a majority support. Our results shed light on the neural mechanisms that underlie decisions about complex visual stimuli and suggest that a realistic extension of existing decision-making models based on linear spatiotemporal integration of visual evidence explains choices in more natural settings.

Disclosures: **G. Okazawa:** None. **L. Sha:** None. **R. Kiani:** None.

Poster

330. Visual Cognition: Decision Making

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Title: Evidence for a predictive coding account of bistable perception

Authors: *K. SCHMACK, V. WEILNHAMMER, H. STUKE, G. HESSELMANN, P. STERZER;
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Abstract: In bistable vision, subjective perception wavers between two interpretations of a constant ambiguous stimulus. This dissociation between conscious perception and sensory stimulation has motivated various empirical studies on the neural correlates of bistable perception, but the neurocomputational mechanism behind endogenous perceptual transitions has remained elusive. Here, we recurred to a generic Bayesian framework of predictive coding and devised a model that casts endogenous perceptual transitions as a consequence of prediction errors emerging from residual evidence for the suppressed percept. Data simulations revealed close similarities between the models predictions and key temporal characteristics of perceptual bistability, indicating that the model was able to reproduce bistable perception. Fitting the predictive coding model to behavioral data from an fMRI experiment on bistable perception in 20 human observers, we found a correlation across participants between the model parameter encoding perceptual stabilization and the behaviorally measured frequency of perceptual transitions, further corroborating that the model successfully accounted for participants perception. Most importantly, model-based analyses of the fMRI data revealed that prediction error time courses correlated with neural signal time courses in bilateral inferior frontal gyri and anterior insulae. Voxel-wise model selection indicated a superiority of the predictive coding model over conventional analysis approaches in explaining neural activity in these frontal areas, suggesting that frontal cortex encodes prediction errors that mediate endogenous perceptual transitions in bistable perception. Taken together, our current work provides theoretical, behavioral and neural evidence for a predictive coding account of bistable perception that posits a crucial role of prediction error signaling in the resolution of perceptual ambiguities.

Disclosures: **K. Schmack:** None. **V. Weilnhammer:** None. **H. Stuke:** None. **G. Hesselmann:** None. **P. Sterzer:** None.

Poster

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NRF-2015R1A5A7037676

Title: Cortical dynamics of 'surprise' and 'entropy' during stochastic perceptual transition

Authors: *J. LEE, S.-H. LEE;
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Abstract: Multi-stable perception—incessant fluctuation over time in perceptual appearance despite unchanging physical stimulation—has been a popular tool for exploring the neural mechanisms substantiating stochastic perceptual transition. To access an observer's ongoing percepts, experimenters typically make the observer actively report perception. By doing so, the observer is inevitably engaged in perceptual decision-making, which invites the neural activities that are not directly associated with perceptual transition per se, but rather reflect the observer's cognitive effort of predicting her own ever-changing, dynamic percepts. We reasoned that this cognitive process of '*perceptual prediction'* engenders two different quanta of uncertainty, *'expected (EU)'* and *'unexpected uncertainty (UU)'*, information quantities crucial for adaptation to volatile environments. Using the Dynamic Belief Model (DBM) [1], Bayesian formalism for updating the expected probability of stochastic events based on a past stream of events, the current study explored the cortical mechanisms substantiating the EU and UU while the brain underwent bistable perceptual transitions. Specifically, we acquired functional Magnetic Resonance Imaging (fMRI) measurements while human observers viewed a 'structure-from-motion (SfM)' display, in which ambiguous 2D motion of coherently moving dots gives perceptual alternations in 3D motion perception between bistable states—clockwise vs counterclockwise rotational motion, and constantly reported their moment-to-moment perceptual states. By slowing down the alternation rate of SfM using the intermittent stimulation technique [2], we could identify a set of cortical regions where the time series of activity were well accounted for by the DBM-predicted time series of either EU, or UU, or both. In support of the previously proposed linkage of the uncertainty representations with the neuromodulatory systems [3], the fMRI dynamics in the cortical regions associated with norepinephrine (NE) and acetylcholine (ACh) were predicted by the UU and EE predictors, respectively. The tight linkage of the quantum of UU with the LC(locus coeruleus)-NE system was further confirmed by the

temporal dynamics of observers' pupil size, which dovetailed with the predicted time course of the UU. Our results suggest that the cortical activities previously claimed as being responsible for triggering perceptual transitions during bistable perception are likely to reflect the quantal fluctuations of uncertainty engendered by the cognitive process of predicting stochastic perceptual events.

Disclosures: J. Lee: None. **S. Lee:** None.

Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Gatsby Charitable Trust GAT3414

Title: Sparse neural coding at the limits of visual performance

Authors: *B. SRIRAM[1], L. LI[1], A. CRUZ-MARTÍN[1,2], A. GHOSH[1,3];
[1]Div. of Biol., UCSD Div. of Biol., La Jolla, CA; [2]Boston Univ., Boston, MA; [3]Neurosci. Discovery and Biomarkers, F. Hoffmann-La Roche, Basel, Switzerland

Abstract: What features of cortical responses does the brain use to interpret the world? Are highly reliable responses necessary for correct interpretation, or can cortical networks manage with sparse, distributed, unreliable responses? And how big should this population be to perform tasks? We find that typical stimuli used to probe visual performance (high contrast gratings with long durations ~1s) drive reliable responses in mouse V1 Layer 2/3 neurons, but this reliability falls steeply as stimulus durations or contrast decrease. This reduction in reliability is true for all neurons and leads to a sparse, unreliable representation of these low contrast, short duration stimuli, leading to greater than 90% failure rates in stimulus-evoked responses in individual neurons. Surprisingly, mice are capable of discriminating the visual stimuli even at these short durations/low contrast. Thus, at the limits of visual discrimination, sparse, unreliable responses are sufficient for adequate performance.
We use models (spike count vs. evidence accumulation) to probe potential decoding schemes for mice performing these visual discrimination tasks. These models along with known physiological response properties and behavioral performance allows us to probe the mechanism by which mice perform a visual discrimination task. For a simple spike-counting model, very few neurons (~300) are needed to perform as well as mice perform during behavior. Thus mice are able to use information from sparse, unreliable activity in a small population of available

neurons to mediate visually-guided tasks. This may explain the exceptional performance of the visual system under naturalistic conditions that involve very brief dwell times on objects.

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Poster

330. Visual Cognition: Decision Making

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Topic: D.06. Vision

Support: Dana Foundation

NIH EY13692

Title: Impaired use of priors in patients with Parkinson's disease is independent of dopaminergic medications

Authors: *A. PERUGINI[1], M. A. BASSO[2];
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disease that includes both motor and non-motor dysfunction. Degeneration of the dopaminergic system is responsible for the motor symptoms of PD and impairs value-based decision-making in patients in a way that is predicted from reinforcement learning models; on medications they are unimpaired in learning from positive feedback whereas off medications they are impaired (Frank et al., 2004). Our recent work shows that in perceptual decision-making, patients on medications are impaired in using priors, in contrast to predictions based on reinforcement learning ideas. Therefore, we sought to determine whether the impaired use of prior information in perceptual decision making in patients with PD depended upon dopamine tone. Patients with PD and heathy control (HC) participants performed a two-alternative forced choice task in which they discriminated the orientation of Glass patterns. We manipulated the quality of the sensory information by varying the Glass pattern coherence. We also manipulated the statistics of the stimulus by making one orientation more frequent than the other (prior information). Patients performed this task in two sessions; one while on their regular medication regime and one after withdrawing from their medications for at least 12 hours before the experiment. The order of the sessions was counterbalanced across participants. As we found previously, patients with PD on dopaminergic

medications were impaired compared to HC in combining prior information with sensory information to make choices in conditions of uncertainty but remained unimpaired when sensory information was clear (Perugini et al., and Basso et al., SFN 2015). Patients off medications remained impaired in combining prior and sensory information to make accurate decisions (N = 10 equal prior vs unequal prior: $p > .05$). Our preliminary data suggest that neurochemical circuits other than dopamine (e.g. serotonergic or cholinergic) may be involved in integrating prior information and sensory information to guide perceptual decision-making under conditions of uncertainty. Our results also suggest that different mechanisms are involved in value-based and perceptual decision-making. *Supported by Dana Foundation and NIH EY13692 to MAB.*

Disclosures: A. Perugini: None. **M.A. Basso:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NIDCD grant RO1DC006914 to PMD

Title: Multisensory convergence in brainstem structures: transfer of taste and odor information between the NTS and the PbN in awake, freely licking rats

Authors: *O. D. ESCANILLA, P. M. DI LORENZO;
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Abstract: Integration of chemosensory stimuli such as taste and odor is an integral part of flavor formation, but its underlying mechanisms are not very well understood. While multisensory integration (MSI) was originally discovered in a subcortical structure, the superior colliculus, the majority of the studies in MSI have focused on the roles of higher cortical and primary 'unisensory' cortical areas. Here, we investigate the role of subcortical structures in the brainstem in chemosensory integration. Previous work in our lab has shown that taste and odor convergence is present in the nucleus of the solitary tract (NTS) (Escanilla et al., 2015) and the parabrachial nucleus (PbN) (Di Lorenzo and Garcia, 1985), the first and second central nuclei of the gustatory system, respectively. To better understand the roles of these structures in flavor construction, we examined how convergent chemosensory information is transferred from the NTS to the PbN in the awake, behaving rat by simultaneously recording responses to tastants, retronasal odorants, and paired taste-odor stimuli from both structures. To do this, rats were implanted with an 8-tungsten wire electrode in both the NTS (AP: -15.3, ML: 1.8, with a 4 mm

head tilt) and the PbN (AP: -12.5, ML: 1.6, with a 4 mm head tilt) and allowed to recover. Rats were then moderately water deprived and placed in the experimental chamber containing a lick spout with access to presentations of taste only (0.1 M Sucrose, 0.1 M NaCl, 0.01 M Citric Acid, 0.0001 M Quinine, artificial saliva (AS)), retronasal odor only (0.01% octanoic acid or 0.01% phenylethyl alcohol, diluted in AS), and paired taste-odor stimuli (each odorant diluted in each tastant). Each taste, odor, or taste-odor stimulus was presented for 5 consecutive licks separated by 5 AS rinses on a variable ratio 5 schedule. Similar to our previous findings in the NTS, results show that in an awake behaving rat, taste responses in the PbN were modulated when tastants were paired with odorants. Additional analyses of coherence and cross correlation functions of joint firing patterns from cells in both NTS and PbN might reveal properties that could underlie the functional connectivity between the two structures. These findings will help us elucidate the neural circuitry between these two brainstem structures and how they are correlated to flavor formation.

Disclosures: O.D. Escanilla: None. **P.M. Di Lorenzo:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Program#/Poster#: 331.02/JJ3

Topic: D.09. Multisensory Integration

Title: Temporal ensemble coding in subsecond sensory events: an MEG study

Authors: *L. CHEN[1,2], H. XU[3];
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Abstract: Modality temporal precision hypothesis states precisions of temporal processing differ across visual and auditory modalities, with the former higher and latter lower. The present study examined the ensemble coding towards a sequence of sensory events containing multiple sub-second inter-intervals. Participants either heard a sequence of sound beeps (6 sounds, with mean inter-interval of 600 ms) or viewed a sequence of six visual flashes with the same mean inter-intervals of 600 ms. They were asked to produce half, sharp or double 'mean' interval with respect to the preceding mean interval (600 ms) , immediately after the last beep or flash. The results have shown the trend of general over-estimation of the mean interval for multiple sensory events. Importantly, variances of estimation (hence the 'encoding') for the sharp mean intervals were larger than those in 'half' and 'double' conditions, although our brain embraces remarkable

ability of temporal ensemble coding. Corresponding to the behavioral tasks, we recorded neuromagnetic signals. In prefrontal areas, the sharp-cycle sampling leads to largest M100 response amplitude than the half-cycle and double-cycle samplings do. Moreover, with the unfolding of the stimuli sequence, time-frequency analysis showed that the mean alpha oscillatory activities were shifting to the earlier latencies and were reduced in power, with reference to the onset of element stimulus (beep or flash), indicating enhanced efficiency of temporal averaging when observers encode sufficient intervals before producing target interval.

Disclosures: L. Chen: None. **H. Xu:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: Natural Sciences and Engineering Research Council of Canada

Canadian Chiropractic Research Foundation

Title: Sensorimotor delays and the vestibular control of standing balance

Authors: *B. G. RASMAN[1], R. M. PETERS[1], R. CHUA[1], J. T. INGLIS[1,2,3], J.-S. BLOUIN[1,2,4]; [1]Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Djavad Mowafaghian Ctr. for Brain Health, Univ. of British Columbia, Vancouver, BC, Canada; [3]Intl. Collaboration on Repair Discoveries, Univ. of British Columbia, Vancouver, BC, Canada; [4]Inst. for Computing, Information and Cognitive Systems, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: To keep the standing body upright, the CNS monitors postural orientation and modulates muscle activity accordingly. The vestibular control of standing balance represents one process in which this is achieved (Fitzpatrick & Day, 2004). This control is task-dependent, such that vestibular-evoked balance responses are only present in appendicular muscles that are actively engaged in keeping the body upright (Britton et al. 1993; Fitzpatrick et al. 1994; Luu et al. 2012). Specifically, motor commands and sensory signals of whole-body sway must match for the CNS to transmit vestibular signals for balance (Luu et al. 2012). How does the CNS gate this vestibular control of standing balance when sensorimotor signals are spatially congruent, but temporally incongruent? Here, we varied an additional delay between ankle-produced torques and whole-body sway in an effort to determine the temporal coupling needed to engage the

vestibular control of standing. Human participants performed standing trials on a robotic balance simulator while being exposed to electrical vestibular stimulation (consisting of frequencies up to 25 Hz). Surface EMG was recorded from the soleus muscle. The robotic balance simulator was programmed with the dynamics of an inverted pendulum (Luu et al. 2011, 2012), mimicking antero-posterior whole-body sway about the ankle joints in response to the torques produced at the ankles (measured by a force plate). Adjustable delays (20-500 ms) between the ankle torques produced by the subjects and the resulting whole-body motion were implemented in the balance simulation loop to assess the level of sensorimotor temporal coupling required to activate the vestibular control of balance. A baseline delay (20 ms) and five experimental delays (100, 200, 300, 400 & 500 ms) were presented in a random order for brief periods of time (10-60 s). The presence and magnitude of vestibular-evoked balance responses were estimated using the cross-covariance between the electrical vestibular stimuli and soleus muscle activity. Vestibular-evoked muscle responses for delays up to 200 ms exhibited similar magnitudes to the no delay (20 ms) condition. For longer delays, the vestibular-evoked responses were reduced substantially, with response estimates for the 400 and 500 ms delays often not surpassing 95% confidence intervals. We therefore suggest that the temporal coupling between motor commands and whole-body sway must be within ~200 ms to engage the vestibular control of balance.

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Poster

331. Temporal Factors of Crossmodal Integration

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Title: Characteristic of visual feedback delay detection in apraxia following stroke

Authors: *S. MORIOKA[1], S. NOBUSAKO[1], R. ISHIBASHI[2], M. OSUMI[1], T. ZAMA[3], S. SHIMADA[3];
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Abstract: Introduction: Apraxia is a motor disorder caused by damage to the left parietal cortex. The Test of Upper-Limb Apraxia (TURIA) is often used in international studies. Although this assessment is useful for determining the severity of apraxia, an evaluation of body consciousness is not included. Furthermore, this is not objective and quantitative evaluation, with regard to disownership and disagency. The purpose of this study was to objectively and quantitatively evaluate apraxia by using a visual feedback delay system. Methods: Twenty stroke patients participated in the study. None of the patients had strong cognitive impairment or asomatgnosia. We also excluded a limb with the motor and sensory paralysis to analyze the pathophysiology of apraxia alone. Informed consent to participate in the study was obtained from all patients. Patients experienced tactile stimulation, passive movement and active movement under conditions of delayed visual feedback (7 delay conditions: from 33 to 594ms) and judged whether observed hand image were delayed with respect to the true felt. To examine the differences in judgment curve shape between tactile stimulation and passive or active movement, logistic curves were fitted to the patient's responses in the incongruent judgment task. We calculated the delay detection threshold (DDT) and the steepness in the determination curve shapes of the three conditions from psychophysics experiments. DDT represents the delay length where congruent and incongruent judgment probabilities are equal (50%). Steepness indicates the slope of the judgment curve. Evaluation of apraxia was performed by the apraxia screen of TULIA (AST). The limbs of patients were classified into 3 groups based on the apraxia evaluation by AST. Normal limbs free of apraxia with perfect scores of 12 points in the AST were defined as apraxia-, limbs with an AST cut-off score of ≥9 points - ≤11 points that were free of apraxia, but exhibited apraxia symptoms, were defined as apraxia+, and limbs with an AST cut-off score of <9 points were judged as apraxia and defined as apraxia++. Results and Discussion: There is a significant difference, in that DDT at active movement in the apraxia++ group is higher than that in apraxia- or apraxia+ groups ($p<0.01$). The steepness data at active movement in the apraxia++ group was lower than that in apraxia- or apraxia+ groups. The severity of apraxia was only significantly correlated with the steepness under active movement conditions ($r=0.43$, $p<0.05$). This suggests a specific dysfunction of the efferent copy in apraxia. By using a visual feedback delay system, we investigated a characteristic of visual feedback delay detection in apraxia.

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Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Title: Attractive and repulsive multisensory interactions in time perception

Authors: *L. LAI[1], J. M. YAU[2];
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Abstract: The ability to perceive the timing of events influences many daily functions, such as motor coordination and speech perception. Though several models have been proposed, the neural mechanisms by which the brain processes sub-second timing information remain unclear. However, what is known is that the perception of time involves the contribution of multiple senses. In the present study, we investigate the temporal interactions between the auditory and tactile sensory systems to better understand multisensory time perception. Using human psychophysics, we explore the influence of auditory distractors on tactile judgments of duration. We find that the duration of sounds, which subjects are instructed to ignore, systematically influences the perceived duration of a tactile stimulus. Specifically, short duration auditory distractors have an attractive influence on tactile duration: a vibration is perceived as shorter when paired with a short duration sound. In contrast, long duration auditory distractors have a repulsive influence on tactile duration: a vibration is also perceived as shorter when paired with a long duration sound. Thus, auditory distractors had opposing effects on tactile duration judgments depending on their duration relative to the tactile stimulus. We tested the influence of auditory distractors on tactile judgments across multiple timing ranges and found consistent patterns. These patterns reveal interactions between audition and touch in the time domain that are surprisingly specific. Preliminary modeling suggests that these interactions can be understood within a causal inference framework.

Disclosures: **L. Lai:** None. **J.M. Yau:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NSERC Discovery Grant

Title: Using a novel prepulse inhibition paradigm and electrophysiology to assess audiovisual temporal integration

Authors: K. SCOTT, A. SCHORMANS, S. SCHMID, *B. L. ALLMAN;
Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: Our brains are capable of naturally integrating information from our different senses. The precise timing of this multisensory information affects whether we perceive the stimuli as occurring synchronously or asynchronously, and subsequently, our reaction to it. Like humans, when rats are presented coincident auditory and visual stimuli, the response is more robust than either stimulus presented alone, indicating the increased saliency of an audiovisual stimulus. However, behavioural paradigms that rely on conditioned responses can require exhaustive training, which is problematic for models of compromised cognitive function. Therefore, we sought to design a behavioural task capable of quickly and reliably assessing audiovisual temporal processing in Sprague-Dawley rats, as such a model could then allow for the study of the mechanisms underlying impaired audiovisual temporal integration. Our model utilizes the acoustic startle response (ASR) and its modulation by a prepulse stimulus to assess a rat's ability to integrate audiovisual stimuli. To that end, we developed an audiovisual prepulse paradigm in which a 10-ms auditory (70 dB) and/or visual (LED flash; 310 lux) stimulus was presented at varying timing offsets (0, 20, 40 or 60 ms) prior to a startle-eliciting stimulus (105 dB, 20 ms). The level of prepulse inhibition (PPI) was used to determine the rat's ability to integrate audiovisual stimuli. Consistent with our predictions, preliminary results show that the level of PPI is enhanced when auditory and visual prepulses are presented synchronously (0 ms offset) compared to when either prepulse stimulus is presented alone. Furthermore, the level of PPI was influenced by the timing of the paired stimuli (e.g., PPI is greater when the auditory stimulus precedes the visual stimulus by 40 ms, but not 60 ms); findings which are consistent with other behavioural paradigms showing the level of integration depends on the precise timing of the paired stimuli. Given these results, we are conducting experiments to investigate the neural basis for the integration of audiovisual stimuli found at the level of PPI by performing extracellular electrophysiological recordings in the pedunculopontine tegmental nucleus (PPT), a brain region crucial for mediating PPI. Similar to other well-established multisensory brain areas (e.g., superior colliculus and cortex), our preliminary experiments have revealed neurons in the PPT capable of integrating auditory and visual stimuli, and these responses are sensitive to the timing of the paired stimuli. Future studies will use this behavior as a method of assessing audiovisual integration in the circuit mediating PPI.

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Poster

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Support: NSERC Discovery Grant

Title: Investigating audiovisual temporal processing in rats using electrophysiology and novel operant conditioning-based behavioral tasks

Authors: *A. L. SCHORMANS[1], K. SCOTT[1], D. STOLZBERG[2], B. L. ALLMAN[1];
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Abstract: To assess audiovisual temporal processing in rats, we developed two behavioral paradigms which are similar to those used in humans: (1) a simultaneity judgment task, in which rats responded according to whether they perceived the auditory and visual stimuli to be presented synchronously (0 ms offset) or asynchronously (200 ms offset); and (2) a temporal order judgement (TOJ) task, in which rats had to report whether the auditory or visual stimulus was presented first when the stimuli were offset by 200 ms. For both tasks, Sprague-Dawley rats were trained with appetitive operant conditioning in a 2-alternative forced-choice paradigm to accurately discriminate between various audiovisual stimulus offsets. Once the rats reached the performance criterion (i.e., >75% correct on the trained stimuli combinations), they were tested at additional stimuli offsets (simultaneity: visual before auditory at 10, 40,100 ms offsets; TOJ: auditory or visual being presented first at 0, 40, 100 ms offsets), as this allowed us to discern the rat's perception of the timing of the audiovisual stimuli. Largely consistent with studies on humans, a psychophysical relationship between stimuli offsets and performance was observed in both tasks. In the simultaneity task, 80% of trials at 10 ms and 100 ms offsets were correctly perceived as being synchronous and asynchronous, respectively. However, when the visual stimulus preceded the auditory stimulus by 40 ms, the rats performed near chance (i.e., ~50% of the trials were correctly identified as being asynchronous). In the TOJ task, when the auditory and visual stimuli were presented at the same time (0 ms offset), the rats did not perform at chance levels; rather, the rats perceived the synchronously-presented stimuli to be similar to the trained condition where the visual stimulus preceded the auditory stimulus (i.e., they favored the "visual first" choice). These findings are in accordance with human studies of temporal order judgment, in which subjects more often report "visual first" when auditory and visual stimuli are actually presented simultaneously. In an additional series of experiments, electrophysiological recordings were made in the audiovisual cortex of anesthetized rats, and the neuronal firing rates in response to the various behavioral stimulus offsets were compared, so as to provide insight into the neural basis of audiovisual temporal processing. Future studies will use our novel behavioral tasks to examine the mechanisms of audiovisual temporal processing.

Disclosures: **A.L. Schormans:** None. **K. Scott:** None. **D. Stolzberg:** None. **B.L. Allman:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.08/JJ9

Topic: D.09. Multisensory Integration

Support: NSERC RGPIN-05435-2014

Network in aging research: Emerging scholar seed grant

Title: Perceived timing of a postural perturbation with and without visual feedback

Authors: *R. E. MCILROY, M. BARNETT-COWAN;
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Abstract: Balance control is a complex task that requires the central nervous system to continually process and integrate multiple sensory cues from the somatosensory, visual and vestibular systems. Failure to rapidly detect and respond to postural perturbations can lead to increased fall risk. Previous research in our lab using a lean-and-release paradigm has shown that the perceived onset of a postural perturbation is slow compared to an auditory comparison stimulus. This indicates that postural perturbation onset must precede an auditory cue by approximately 44ms in order for the two events to be perceived as simultaneous. However, this previous research was preformed with participants' eyes closed, removing visual cues from assisting with the detection of the instability. The aim of this study is to determine if providing visual cues to participants will assist in the detection of the onset of a postural perturbation. Participants wore noise cancelling headphones, listened to white noise, were positioned into a standardized lean angle of 10% body mass using a harness and release cable, and assumed a standardized foot position. On a given trial, the cable would release participants from their set lean angle and an auditory cue was presented at various stimulus onset asynchronies relative to postural perturbation onset and repeated multiple times using the method of constant stimuli. Participants either kept their eyes closed under a blind fold or kept their eyes open and fixated on an earth-fixed target 1m in front of them prior to postural perturbation onset. Participants were to indicate via button press whether the perturbation onset or the auditory cue onset came first in a temporal order judgment task. Our preliminary results suggest that the perceived timing of a postural perturbation changes with and without visual feedback. Future work is planned to manipulate the type and characteristics of visual feedback so to better understand the role of visual input in the perceived timing of a postural perturbation.

Disclosures: R.E. McIlroy: None. **M. Barnett-Cowan:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NIH/NINDS grant R21 NS086356 to JH

Title: Using visual-haptic synchrony to facilitate and manipulate cross-modal integration

Authors: *J. HEGDE[1,2];
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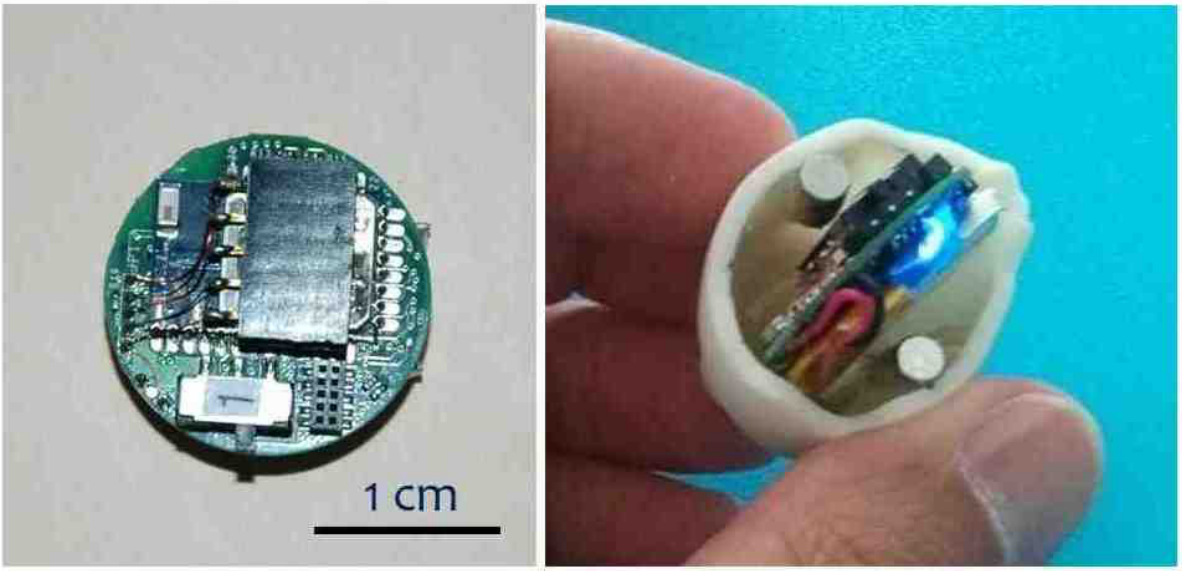
Abstract: In the real world, visual and haptic sensations are always synchronized in real time. Evidence from other sensory modalities indicates that spatio-temporal congruity among the senses plays key role in cross-modal integration, and that manipulating this congruity can help 'gate' or 'drive' the perceptual outcome. Tools did not previously exist to bring about, and/or manipulate, visual-haptic synchrony. Therefore, it has not been possible heretofore to systematically study whether and how the brain utilizes visual-haptic synchrony in cross-modal integration, let alone manipulate this synchrony to drive the integrative process in laboratory or clinical settings. To help overcome this barrier, we have developed a novel toolkit, which we refer to as the 'Hermes' toolkit, that allows the user to systematically manipulate visual-haptic synchrony in real time. Our approach is to implant a small, custom-designed wireless sensor chip inside the haptic object (see figure), read the object's 3-D coordinates in real time, and use the coordinates to render the given visual object with the given temporal synchrony and 3-D viewpoint. The chip can reliably measure the real-time 3-D coordinates of any object it is mounted on, implanted in or, more generally, any object whose 3-D coordinates are related to those of the chip by a known affine transformation. To operate the toolkit, a fully charged Hermes chip is inserted in the custom-built hollow of haptic object (right panel). The real time 3-D data from the haptic object is used to concurrently render the desired 3-D visual object/s on a computer monitor, so that when the observer manipulates the haptic object, the corresponding visual object moves accordingly on the monitor, with the user-specified spatiotemporal transformations. Thus, this toolkit will allow the user to manipulate all aspects of visual-haptic multisensory integration in laboratory studies as well as in clinical settings, including visual-haptic rehabilitation.



Disclosures: **J. Hegde:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NWO VENI grant (MaGW 451-12-040)

STW 12160

Title: The magnitude of the size-weight illusion depends on when size information is provided

Authors: ***I. A. KULING**, M. A. PLAISIER, E. BRENNER, J. B. J. SMEETS;
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Abstract: To judge an object's weight, we typically lift the object and use the resulting haptic information to estimate the weight. However, the percept can be influenced by visual information, as has been shown in the size-weight illusion: the effect that small objects feel heavier than larger ones of the same weight. It has been suggested that this illusion is caused by a mismatch between the expected and actual weights of the objects. The expected weight is presumably based on (visual) size information, while the actual weight is perceived through the

haptic modality during lifting. If so, one might predict that visual information about an object's size will only influence its judged weight if it is presented before lifting the object.
We tested this prediction in an experiment in which size could only be perceived through vision. In each trial, we made vision available for a 200 ms interval. The interval started at various times from lift onset: between 200 ms prior to lift onset until when the maximum lifting height was reached. As predicted, the magnitude of the size-weight illusion depended on when visual information was available, but the magnitude was only reduced when vision was available about 300 ms after lift-off. Thus, although the relative timing of visual size and haptic weight information does indeed determine the multisensory weight percept, size information does not need to be available prior to the onset of the lifting action to influence the perceived weight.

Disclosures: I.A. Kuling: None. **M.A. Plaisier:** None. **E. Brenner:** None. **J.B.J. Smeets:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NSERC RGPIN-05435-2014

Emerging scholar seed grant

Title: Audiovisual simultaneity and temporal order in the young and elderly: an erp study

Authors: *A. BASHARAT, G. BEDARD, A. WISE, M. ADAMS, W. R. STAINES, M. BARNETT-COWAN;
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Abstract: Auditory and visual information from an audiovisual event can arrive at the brain at different times. To compensate for physical and neural delays between auditory and visual information, the brain seems to maintain a temporal binding window (TBW) within which two multisensory events separated by a short period of time will be perceived as occurring simultaneously. Simultaneity Judgment (SJ) and Temporal Order Judgment (TOJ) tasks can be utilized to investigate multisensory integration in time and TBW width. Here, participants are asked to assess whether an audiovisual pair of stimuli occurred simultaneously or successively (SJ) or alternatively which stimulus came first (TOJ). Previous research has suggested that different neural mechanisms may subserve SJs and TOJs. Here we assessed the amplitudes and

latencies of the P1 and N1 event related potentials (ERPs) as they have been linked to perceptual processing and modulation of perceptual processing. Additionally, they have been found to differ between the young and elderly population. We also assessed later components in order to gain a better understanding of the neural mechanisms involved in SJ and TOJ at later points of time. Our preliminary results suggest that although the TBW as measured by the SJ and TOJ tasks, are moderately correlated, there are differences across tasks and between populations such as in the young and elderly indicative of separate neural mechanisms that differently change with age.

Disclosures: **A. Basharat:** None. **G. Bedard:** None. **A. Wise:** None. **M. Adams:** None. **W.R. Staines:** None. **M. Barnett-Cowan:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Program#/Poster#: 331.12/JJ13

Topic: D.09. Multisensory Integration

Title: A common parieto-frontal network for impact prediction to the face and peripersonal space encoding: an non-human primate fMRI study

Authors: ***J. CLÉRY**, O. GUIPPONI, S. ODOUARD, C. WARDAK, S. BEN HAMED; Ctr. De Neurosci. Cognitive, Bron, France

Abstract: In the jungle, survival is highly correlated with the ability to detect and distinguish between a predator and a putative prey. From an ecological perspective, a predator approaching its prey is a stronger cue for flight than a sleeping predator. Experimentally, such a situation can be modeled by a looming stimulus approaching the subject of the experiment. In a recent study (Cléry et al., J. Neurosci., 2015), we show that looming stimuli towards the face enhance tactile sensitivity at the predicted time and location of the impact, suggesting the involvement of multisensory integration areas in the prediction of impact to the body. In order to test this hypothesis and identify the neural bases of the prediction of impact to the face by a looming stimulus, we use functional magnetic resonance imaging (fMRI) in monkeys. Specifically, very weak airpuffs (modeling the impact to the body) were delivered either after a degraded looming visual stimulus, at the predicted time of impact (temporal predictive condition) and at the predicted location of the impact or at the opposite, or during the visual sequence (simultaneous visuo-tactile presentation). We show that maximal cortical activations are observed when the looming stimulus spatially and temporally predicts the tactile stimulus. These predictive processes activate, a parieto-frontal network composed of the ventral intraparietal area VIP and premotor area F4, as well as striate and extrastriate visual areas. Thus, the prediction of the

heteromodal consequences of a looming visual stimulus onto the tactile modality recruits a network previously described for its contribution to multisensory convergence (Guipponi et al., J. Neurosci. 2013, NeuroImage, 2015) and multisensory integration. This network has also been suggested to play a key role in the representation of peripersonal space that is the space that directly surrounds us and which we can act upon (Cléry et al., Neuropsychologia, 2015, for review). We use fMRI in a naturalistic 3D environment to describe the non-human primate near (peripersonal) and far space cortical networks. Overall, we describe two extended functional networks respectively encoding near and far space processing. These two networks are highly overlapping, indicating that several areas involved in visual processing are activated by both near and far visual stimuli. Importantly, the cortical network selectively involved in the processing of near space highly overlaps with the network predicting impact to the face. These results are discussed in the context of peripersonal space and body margin of safety representation.

Disclosures: J. Cléry: None. **O. Guipponi:** None. **S. Odouard:** None. **C. Wardak:** None. **S. Ben hamed:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Support: NIH grant R21NS091752

UTHSC College of Medicine iRISE Pilot Program

UTHSC Dept. of Anatomy and Neurobiology

UTHSC Neuroscience Institute

Title: Respiration modulates neuronal activity in visual cortex

Authors: *S. S. MCAFEE, Y. LIU, D. H. HECK;
Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: We previously reported that respiration modulates neuronal activity in the somatosensory whisker cortex of awake mice, and that respiration-driven olfactory bulb (OB) activity was the main cause of this modulation. We hypothesized that this rhythmic OB input to the whisker cortex might facilitate tactile sensory processing by creating high-excitability cortical states during whisks, via synaptic input. We have since conducted experiments showing

that respiration modulates neuronal activity in several additional cortical areas and here report on results obtained in the primary visual cortex (V1) of awake, head-fixed mice. Visual acquisition does not occur in a rhythmic manner like whisking, and there is no known link between visual perception and respiration. Here we report that respiration rhythmically modulates neuronal spike and local field potential (LFP) activity in V1. Using circular statistics and cross-correlational analysis, we were able to determine the degree of modulation at baseline, and again while blocking OB activity. OB inhibition did not eliminate respiration-locked modulation of V1 neuronal activity, as it did for the somatosensory whisker barrel cortex; Instead we observed an increase in the depth of respiration-locked modulation of neuronal activity. Next we asked whether respiration also modulated visually evoked responses using 100ms presentations of checkerboard stimuli to the contralateral visual field. Visual evoked potentials recorded in layer 4 of V1 were significantly altered following inhibition of olfactory bulb activity using both lidocaine and inhibitory DREADDs activation. These results show that olfactory bulb activity modulates visual processing at the level of V1 neuronal responses. Whether this modulation affects perception at a behavioral level remains to be shown. Lastly, we investigated whether altered visual-evoked responses in V1 coincide with disrupted oscillatory synchrony between structures in the visual system. Simultaneous LFP recordings from V1 and the lateral geniculate nucleus (LGN) during the presentation of checkerboard stimuli revealed decreased beta (15-30Hz) and gamma (30-100Hz) coherence following OB inhibition, suggesting that OB output is required for normal temporal coordination of oscillatory activity between the LGN and V1. These findings confirm that respiration-locked, rhythmic neuronal activity reaches V1 via more than one pathway and significantly modulates V1 activity. We identified the OB and LGN as two independent pathways. Altered responses to visual stimulation furthermore suggest a role for OB output in the processing of visual information.

Disclosures: **S.S. McAfee:** A. Employment/Salary (full or part-time): University of Tennessee Health Science Center. **Y. Liu:** A. Employment/Salary (full or part-time): UniVersity of Tennessee Health Science Center. **D.H. Heck:** A. Employment/Salary (full or part-time): University of Tennessee Health Science Center.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

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Program#/Poster#: 331.14/JJ15

Topic: D.09. Multisensory Integration

Title: Beta oscillations reflect supramodal information during perceptual judgment

Authors: *S. HAEGENS[1,2], J. VERGARA[3], L. LEMUS[3], R. ROMO[3,4];
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Abstract: Here, we explored oscillatory dynamics in the medial premotor cortex (MPC) involved in supramodal perceptual decision-making. We recorded local field potentials (LFPs) and spikes in two monkeys (Macaca mulatta) trained to perform a tactile/acoustic discrimination task.
In this task, the animal had to discriminate the difference in frequency between two sequentially delivered stimuli, which could be somatosensory (mechanical vibrations) and/or auditory (acoustic pulse trains). Trials could be unimodal or crossmodal. The monkey's task was to indicate whether the second stimulus (f2) was of lower or higher frequency than the first (f1), by means of a button press.
We studied the role of oscillatory activity as a function of stimulus properties (frequency and sensory modality) and decision outcome (f2>f1 or f2<f1). During the working memory delay, there was an increase of beta band activity (approx. 20-30 Hz range), accompanied by an alpha/low beta band decrease (approx. 10-15 Hz). We found that beta band power was reflective of relevant stimulus properties: there was a significant modulation by stimulus frequency during the retention interval, as well as a modulation by stimulus modality during stimulus presentation that extended into retention only in the case of a purely unimodal task, where modality information was relevant to prepare for the upcoming second stimulus.
We then asked whether the oscillatory modulation was predictive of decision outcome. Here, we contrasted trials with f2>f1 vs. f2<f1 outcome, and found a significant modulation of beta power starting during f2 presentation. Additionally, we separately analyzed the trials with the lowest and highest f1, for which there was no lower or higher f2 presented, respectively. In this case the animal could make the decision based on f1 alone, i.e., effectively performing a categorization task. Taking these "categorization" trials only, we found the same beta modulation as before, now starting right after f1 offset, further confirming that this particular modulation reflects the decision outcome.
Finally, we computed spike-field coherence (SFC) in order to assess how the observed population beta oscillations interact with the single-unit spikes, and found significant SFC matching our LFP observations.
In conclusion, we demonstrate that beta power in MPC is reflective of stimulus features in a context dependent manner, and additionally reflects the decision outcome. This information is coded in a supramodal manner – modality information is only retained when relevant for the task at hand.

Disclosures: **S. Haegens:** None. **J. Vergara:** None. **L. Lemus:** None. **R. Romo:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Network in aging research: Emerging scholar seed grant

Title: Perceived timing of active head movement with and without visual feedback

Authors: *W. CHUNG[1], M. BARNETT-COWAN[2];
[1]Applied Hlth. Sci., [2]Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Knowing when the head moves is crucial information for the central nervous system in order to maintain a veridical representation of the self in the world for perception and action. Our head is constantly in motion during everyday activities, and thus the central nervous system is challenged with determining the relative timing of multisensory events that arise from active movement of the head. The vestibular system plays an important role in the detection of head motion as well as compensatory reflexive behaviours geared to stabilizing the self and the representation of the world. Although the transduction of vestibular signals is very fast, previous studies have found that the perceived onset of an active head movement is delayed when compared to other sensory stimuli such as sound, meaning that head movement onset has to precede a sound by approximately 80ms in order to be perceived as simultaneous. However, this past research has been conducted with participants' eyes closed. Given that most natural head movements occur with input from the visual system, could perceptual delays in head movement onset be the result of removing visual input? In the current study, we set out to examine whether the inclusion of visual information affects the perceived timing of vestibular-auditory stimulus pairs. Participants performed a series of temporal order judgment tasks between their active head movement and an auditory tone presented at various stimulus onset asynchronies. Visual information was either absent (eyes-closed) or present while either maintaining fixation on an earth or head-fixed LED target in the dark or in the light. Our results to date confirm that head movement onset has to precede a sound with eyes-closed. The results also suggest that head movement onset must still precede a sound when fixating targets in the dark. Fixating targets in the light, however, suggests that head movement onset and sound onset do not have to precede each other in order to be perceived as simultaneous. Further results suggest that participants are less precise in their responses when fixating targets in the light. Together, these results suggest perception of head movement onset is persistently delayed in the dark, but may be resolved with full field visual input.

Disclosures: **W. Chung:** None. **M. Barnett-Cowan:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Program#/Poster#: 331.16/JJ17

Topic: D.09. Multisensory Integration

Support: Grant-in-Aid for JSPS Fellows (26・1927)

MEXT Grant-in-Aid for Scientific Research on Innovative Areas "The Science of Mental Time" (25119003)

Title: Inference of multimodal duration information from unimodal subjective durations

Authors: *K. YUASA[1,2], Y. YOTSUMOTO[3];
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Abstract: While visual information is dominant in the integration of multimodal spatial information, auditory information is known to be dominant in multimodal temporal processing. These crossmodal interactions reflect the precision difference in sensory information, indicating that sensory information is optimally integrated in a Bayesian framework to obtain information that is more precise (Ernst, 2012; Kording et al., 2007). However, van Wassenhove et al. (2008) reported the exceptive effect of visual dominance in multimodal duration perception. Recently, we reported that perceived duration for 10.9Hz visual flickers are dilated while the perceived duration for 10.9Hz auditory flutters are compressed (Yuasa and Yotsumoto, 2015). These time distortions indicated that participants perceived different durations for each sensory stimulus. When the flickers and flutters were presented simultaneously, participants perceived the veridical durations, indicating that the distortions in the two modalities were cancelled out. The result seemed to be an exception to the traditional prediction of auditory dominance as well as the study results of van Wassenhove et al. (2008). This type of integration suggests that the duration information is processed after the integration of sensory information. In the present study, we used Bayesian inference and investigated how the multimodal subjective durations can be predicted using the unimodal subjective durations. Analyses of perceived duration of unimodal stimulus revealed that the reliabilities were similar across modalities. Maximum likelihood estimation indicated similar contributions of each modality in the integration of duration information, which means neither visual nor auditory modality was dominant in the perception of simultaneously presented visual flickers and auditory flutters (multimodal subjective durations).

The results exhibited similar trends with the behavioral observations, but the estimated multimodal durations did not match the observed multimodal subjective durations. Next, we introduced weights of prior knowledge into each modality contribution. Prior knowledge biases the multimodal perception toward unisensory information, which is generally more accurate. As a result, a large contribution of the auditory modality was estimated, which explained all our experimental results with great accuracy (MAPE = 0.70%). These results suggest that the duration information is processed in each modality before multimodal integration occurs and that even distorted subjective duration information is integrated in a statistically optimal fashion.

Disclosures: K. Yuasa: None. **Y. Yotsumoto:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NSF IGERT DGE-1250104

Alfred P. Sloan Research Fellowship

Title: Multisensory interactions in frequency sweep perception

Authors: *L. E. CROMMETT[1], D. MADALA[2], J. M. YAU[1];
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Abstract: Naturally occurring signals in audition and touch are complex. Frequency modulations, or changes in frequency, are one aspect of this complexity. Frequency sweep processing has been extensively studied in the auditory domain, but much less is known about sweep processing in the tactile domain. Because audition and touch can interact in the frequency domain for simple sinusoids, here we assessed whether these senses also interact in higher-order frequency processing. In a series of psychophysical experiments, we tested the hypothesis that touch and audition interact in processing frequency sweeps. Participants performed a sweep direction discrimination task. On every trial, they judged whether a tactile frequency sweep was increasing or decreasing in frequency. On some trials, auditory sweeps co-occurred with the tactile sweeps, and subjects were instructed to ignore the distractor sounds. We manipulated the relationship between the sweep directions of the sounds and vibrations. We found that auditory sweeps systematically biased the tactile perception of sweep direction. Specifically, the presence of an auditory up sweep increases the likelihood that a concurrent vibration is perceived as

sweeping upward. Similarly, the presence of an auditory down sweep increases the likelihood that a concurrent vibration is perceived as sweeping downward. We implemented a simple model that represents frequency sweep magnitude and direction with likelihood functions computed from a population of sensory neurons. By granting the sensory neurons access to both auditory and tactile sweep signals, our model reproduced the perceptual biases induced by the auditory distractors. Our preliminary results support the hypothesis that auditory and tactile signals interact in frequency sweep processing and suggest a mechanism based on shared neural circuits.

Disclosures: L.E. Crommett: None. **D. Madala:** None. **J.M. Yau:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Network in aging research: Emerging scholar seed grant

Propel Center for Population Health Impact: CDPI seed grant

Title: Genetic determinants of multisensory integration

Authors: *A. WISE[1], M. BARNETT-COWAN[2], R. DUNCAN[2];
[1]Kinesiology, Univ. of Waterloo, Clinton, ON, Canada; [2]Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: To form a coherent representation of the self and the world, the central nervous system determines whether and how to bind multisensory information in space and time. For over a hundred years, research has been investigating how humans integrate multisensory information, and models that have been developed to characterize the neural mechanisms that determine how multisensory information is integrated are becoming mature. However it has also been shown that individuals perceive space and time differently from one another, often challenging the generalizability of such neural models. For example, our lab and others have shown that audio and visual stimuli are likely to be judged as occurring simultaneously when they occur within 300ms of each other, but the width of this "temporal binding window" varies across individuals and groups (e.g., young vs. older adults). We have also shown that visual, vestibular and somatosensory information is differently weighted across individuals and groups (e.g., males vs. females) when judging preferred lighting to extract shape from shading

information, as well as the perceptual upright - the orientation at which humans optimally recognize objects. The purpose of this experiment was to determine whether or not these differences are the result of a genetic component whereby genetic variation in genes related to cognition and perception could be responsible for this variation. Four multisensory psychophysical tasks were completed: i) audiovisual simultaneity judgment, ii) audiovisual temporal order judgment, iii) shape-from-shading, and iv) perceptual upright. Candidate genes included sex-linked genes as well as BDNF, APOE, COMT and KIBRA, among others, which have been found to be polymorphic in the general population and to be related to performance on neurological tasks, were analyzed. Here we report our preliminary findings suggesting that some individual differences in multisensory integration can be partially explained by genetic heterogeneity.

Disclosures: **A. Wise:** None. **M. Barnett-Cowan:** None. **R. Duncan:** None.

Poster

331. Temporal Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: VICTR VR12837

Title: Utilizing multisensory integration to improve auditory alarm design in the intensive care unit

Authors: *J. SCHLESINGER[1], M. WALLACE[2];
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Abstract: Sound exposure in the hospital can have deleterious effects on patients and practitioners. Clinicians perform worse on tasks involving patient monitoring in noisy and highly attentionally demanding environments. Research on the signal-to-noise ratio of alarms can decrease the overall sound exposure by decreasing the alarm fraction contribution of total sound. Alarms in the ICU sound frequently and 85-99% of cases do not require clinical intervention. As alarm frequency increases, clinicians develop ‘alarm fatigue’ resulting in desensitization, missed alarms, and delayed responses. This is dangerous for the patient when an alarm-provoking event requires clinical intervention but is inadvertently missed. Alarm fatigue can also cause clinicians to: set alarm parameters outside effective ranges to decrease alarm occurrence, decrease alarm volumes to an inaudible level; silence frequently insignificant alarms; and be unable to distinguish alarm urgency. Since false alarm and clinically insignificant alarm rates reach 80-

99%, practitioners distrust alarms, lose confidence in their significance, and manifest alarm fatigue. Yet, failure to respond to the infrequent clinically significant alarm may lead to poor patient outcomes. Fatigue from alarm amplitude and nonspecific alarms from uniform uninformative alarms is the post-monitor problem that can be addressed by understanding the acoustic features of alarms and the aural perception of clinicians.
Our experimental paradigm determines near-threshold auditory perception of alarms, and then uses clinical scenarios to determine the stimulus-response relationships for changes in auditory alarm intensity, spanning negative to positive signal-to-noise ratios (SNRs), when performing an audiovisual secondary task designed to tax attentional and decisional resources. The result is a stimulus-response curve in dB above ambient noise.
Results show near-threshold auditory perception of alarms is around -27 decibels (dB) from background noise at 60 dB. Additionally, with visual offset of a patient monitor, there is preserved performance measured by response time and accuracy to a clinical task at -11 dB as compared with +4dB with worsening at more negative SNRs. Thus, clinician performance is maintained with alarms that are *softer* than background noise. These results can inform future work on alarm fatigue to address the music perception and cognition components of novel psychoacoustic alarm presentations in concordance with existing standards (IEC 60601-1-8).

Disclosures: J. Schlesinger: None. **M. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.20/KK4

Topic: D.09. Multisensory Integration

Title: Neural correlates of rapid audiovisual temporal recalibration.

Authors: *J.-P. NOEL, D. M. SIMON, M. M. WALLACE;
Vanderbilt Univ., Nashville, TN

Abstract: Audiovisual temporal asynchrony is ubiquitous in the natural environment due to differences in the propagation time of light and sound. Rapid adaptation to the statistical asynchronies present in the world is thus crucial for appropriate realignment and integration of these multisensory signals. Recently, multisensory temporal recalibration has been shown to occur at the single trial level in psychophysical experiments, yet the mechanistic basis of this rapid recalibration process has not previously been elucidated using physiological techniques. Here we investigated the neural basis of rapid recalibration to audiovisual temporal asynchrony in human participants using psychophysical and high-density electroencephalography methods.

Consistent with previous reports of rapid recalibration effects, participant's perception of audiovisual temporal synchrony was found to be strongly influenced by the temporal relationship of the previous trial. Neural activity was similarly modulated by the temporal relationship of the previous trial, manifesting as differences in central and parietal positivity on trials with large stimulus onset asynchronies. Critically, at the individual subject level, the magnitude and direction to which the evoked neural responses were modulated by the temporal relationship of the previous trial predicted the magnitude of psychophysical recalibration. Our results indicate that single trial adaptation to audiovisual temporal asynchrony results in asymmetric modulation of late evoked components, and that the asymmetry of neural responses likely contributes to individual perceptual plasticity.

Disclosures: J. Noel: None. **D.M. Simon:** None. **M.M. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.21/KK5

Topic: D.09. Multisensory Integration

Support: SNSF P300PB_164754

Title: Weighting perception of ambiguous motion stimuli: The curious case of audition trumping vision

Authors: *A. THELEN[1], M. CHADHA[2], A. R. NIDIFFER[2], R. RAMACHANDRAN[2,1], M. T. WALLACE[1,2];
[1]Vanderbilt Brain Inst., Nashville, TN; [2]Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN

Abstract: The ability to combine and integrate information from the different senses into a single coherent percept is an inherent ability of our nervous system. Moreover, this ability is crucial when available sensory information is degraded and/or ambiguous, and can confer powerful behavioral and perceptual benefits. While a large body of work has focused on static multisensory stimuli, less is known about the principles underlying the integration of dynamic (i.e. ethologically valid) stimuli. To address this question at both the behavioral and neuronal levels, we employed dynamic motion stimuli (i.e. random dot kinematograms and auditory motion embedded in noise) in a two alternative forced choice task in which subjects had to judge the direction of motion. We manipulated both stimulus efficacy (i.e. motion coherence) and congruency between auditory and visual motion (i.e. leftward or rightward) stimuli on a trial-by-

trial basis. Subjects performed the task while high-density EEG data were concurrently acquired. Preliminary findings revealed behavioral benefits under congruent multisensory presentation conditions as compared to either unisensory condition alone. These behavioral benefits were further increased as a function of motion coherence. Intriguingly, under incongruent pairing conditions, we found that subjects more heavily weighted auditory information. Moreover, auditory weights were further increased for pairings with high (60%), as opposed to low (6%) visual motion coherence. These findings seem to be inconsistent with prior findings, that suggest that subjects attribute higher perceptual weights to visual information in spatial tasks (i.e. Modality Appropriateness Hypothesis). Analyses of the scalp evoked responses focus on revealing the neuronal correlates in terms of response strength (Global Field Power) and neuronal generators (Topographic Dissimilarity) underlying the attribution of perceptual weights, and ultimately sensory-motor transformation. Moreover, we seek to identify the neuronal loci that are differentially recruited as a function of stimulus efficiency and behavioral choice. Some of the most informative analyses focus on trials in which the stimuli are identical but it which the behavioral responses differ, thus providing insight into the network differences attributable to sensory statistics or perceptual choice. We expect to observe increased neuronal responses (e.g. GFP) in congruent trials underlying behavioral benefits. However, incongruent pairings, would result in the recruitment of differential neuronal networks, as a function of the perceptual weights attributed to either unisensory cue.

Disclosures: **A. Thelen:** None. **M. Chadha:** None. **A.R. Nidiffer:** None. **R. Ramachandran:** None. **M.T. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.22/KK6

Topic: D.09. Multisensory Integration

Support: SNSF P300PB_164754

Title: Integration of ambiguous auditory-visual motion stimuli to form perceptual judgements

Authors: ***M. CHADHA**[1], A. THELEN[2], A. R. NIDIFFER[1], R. RAMACHANDRAN[1], M. T. WALLACE[2];
[1]Hearing & Speech Sci., [2]Vanderbilt Brain Inst., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Faced with ambiguous or weak sensory inputs, the nervous system combines information from different sensory modalities in a statistically optimal manner to form a robust

and coherent percept of our surroundings and to facilitate behavioral performance. A number of past studies employing static multisensory stimuli have repeatedly reported, that in audio-visual localization tasks, the perceived location of an auditory stimulus is biased toward the location of visual cue(s). Such evidence has been taken as support for the Modality Appropriateness Hypothesis, which posits that vision will dominate in spatial tasks. Whether the same principle applies, as well as the respective weighting of the individual sensory cues, has yet to be determined for dynamic multisensory stimuli. To examine this question, we presented human subjects with moving audio-visual stimuli (random dot kinematograms and auditory motion embedded in noise) and had them perform a two alternative forced choice task judging the direction of motion. We manipulated both stimulus efficacy (i.e. motion coherence) and the directional congruency between auditory and visual motion (i.e. leftward or rightward) stimuli on a trial-by-trial basis. Signal detection theoretic methods were used to analyze the data. Preliminary results show multisensory facilitation under conditions of congruent audio-visual motion, both in terms of reporting the direction of motion and in a reduction in reaction times compared to unisensory conditions. Under incongruent audio-visual stimulus conditions however, subjects appeared to more heavily weight the auditory motion, a finding inconsistent with previous results. The sensitivity to visual motion stimuli (slope of psychometric function) predicted the influence of auditory motion (auditory weights) as a function of auditory coherence on multisensory trials, and was consistent with the principle of inverse effectiveness. These results can be described by a model using weighted linear combination of unimodal cues under conditions of incongruent auditory and visual motion. We show that weights assigned to the individual cues depend on their reliability, rather than the modality's appropriateness.

Disclosures: **M. Chadha:** None. **A. Thelen:** None. **A.R. Nidiffer:** None. **R. Ramachandran:** None. **M.T. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.23/KK7

Topic: D.09. Multisensory Integration

Support: Conte Center Grant P50 MH096972

Title: Cortical multisensory circuits: implications for autism spectrum disorder

Authors: *G. E. DICARLO, M. T. WALLACE;
Vanderbilt Univ., Nashville, TN

Abstract: Autism spectrum disorder (ASD) is a developmental disorder characterized by impairments in social communication and restricted, repetitive behaviors, interests, or activities. While there are no clinical biomarkers of ASD, nearly 30% of patients with ASD have elevated whole blood serotonin, implicating serotonergic dysregulation in the pathogenesis of this condition. Serotonin has been shown to be critical in key cortical developmental processes and in the formation of sensory processing networks. Alterations in serotonergic signaling have been shown to disrupt the formation and function of primary sensory cortices (somatosensory, auditory, and visual). It is not therefore surprising that the Diagnostic and Statistical Manual of Mental Disorders includes sensory processing abnormalities as a core symptom of ASD. Since an essential function of the nervous system is to integrate sensory information into a coherent percept, it is likely that disruptions in sensory processing that extend beyond unisensory processing alone may underlie the core deficits associated with ASD. This is supported by evidence indicating differences in connectivity between distant brain regions in children with ASD, as communication across cortical regions is necessarily required for the processing of information across sensory domains. Here we demonstrate the impact of a serotonin transporter mutation associated with ASD, SERT Gly56Ala (Ala56), on the pattern of thalamocortical and intercortical projections from and between primary visual and auditory cortices in the mouse using neuroanatomical tract tracing. Specifically, 10kDa biotinylated dextran amine (BDA) and fluoro-ruby (FR) was injected into primary auditory and visual cortex (respectively) to label anterograde projections from these regions in both wild type and Ala56 animals. We used immunocytochemical techniques to label the distribution of serotonin and the serotonin receptors 5-HT 1B and 5-HT 2A, both of which have been implicated in the formation of sensory maps, throughout the cortex in wild type and Ala56 animals at key postnatal developmental time points. Previous work has demonstrated projections from primary auditory and visual cortices to the lateral region of V2 in wild type animals, indicating a possible role for this region in multisensory integration. As serotonergic innervation is critical to the formation of primary sensory maps and their function, we anticipate a reduction in the number and density of intercortical projections in Ala56 animals, as well as a decrease in the serotonergic innervation of primary visual and auditory cortices throughout development.

Disclosures: **G.E. Dicarlo:** None. **M.T. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.24/KK8

Topic: D.09. Multisensory Integration

Support: The Basic Science Research Program (2015R1D1A3A01019256) through the National Research Foundation of Korea (NRF)

The Basic Research Laboratory (BRL) Program (2013R1A4A1069507) through the National Research Foundation of Korea (NRF)

Title: Magnetic fields modulate horizontal movements of the fruit fly, *Drosophila melanogaster*

Authors: *K.-S. CHAE, S.-H. LEE, I.-T. OH;
Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: The geomagnetic field (GMF) acts as a sensory cue for magnetoreceptive animals such as birds, butterflies, and sea turtles in long-distance migrations and flies, ants, and cockroach in short-distance movements. Those animals have been known to receive the information of total intensity or inclination of the GMF for the magnetoresponsive behaviors. However, declination of GMF has never convincingly been claimed to be used as a behavioral cue in any organism. Using the fruit fly *Drosophila melanogaster* as a model organism, we show that GMF modulates horizontal directional movements of fruit fly in a declination-dependent manner. In an appetite-associative learning assay, flies showed significantly oriented horizontal movements for food under the ambient GMF and the 90°clockwise-turned GMF conditions, suggesting that flies remember the direction of GMF and exploit the GMF as a directional reference. Moreover, change in *X* (North-South axis) or *Y* (East-West axis) value of GMF, maintaining total intensity and inclination as the same, produced significant directional movements of flies for food, indicating that flies can discriminate and exploit difference in those horizontal parameters of GMF. These results demonstrate for the first time that flies use declination of GMF in at least short-distance horizontal directional movements, and suggest a more complex paradigm for GMF usage in magnetoreceptive organisms.

Disclosures: K. Chae: None. **S. Lee:** None. **I. Oh:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.25/KK9

Topic: D.09. Multisensory Integration

Title: Audiovisual integration in cochlear implant users

Authors: *I. M. BUTERA[1], R. A. STEVENSON[3], R. H. GIFFORD[2], M. T. WALLACE[1]; [1]Vanderbilt Brain Inst., [2]Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN; [3]Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Cochlear implants (CIs) allow those with profound hearing loss to experience sound, some of them for the first time. This highly successful neuroprosthetic device can drastically improve speech comprehension for some individuals; however, postoperative speech proficiency remains highly variable and difficult to predict. Although visual orofacial articulations play a crucial role in verbal communication both before and after cochlear implantation, clinical measures assessing implant candidacy and monitoring postoperative performance are currently limited to auditory-only speech measures. As a result, current assessments may be providing a partial picture of aural rehabilitation with a CI. The aim of this study is to characterize audiovisual integration in a cohort of pre- and post-lingually deafened CI users. This characterization includes unisensory and multisensory speech perception at the phoneme and word level. Doing so enables us to relate an illusory multisensory task (i.e. the McGurk effect) to variable proficiency in clinical auditory only speech measures. Accordingly, we recruited 33 CI users (6-81 years old) and 33 normal-hearing controls (7-76 years old) who completed clinical speech testing and a McGurk task. When presented with conflicting audiovisual information (i.e. viseme “ga” with the phoneme “ba”), CI users reported a fused token “da” or “tha” on an average of 42% of trials compared to 69% in the NH group. These initial descriptive statistics indicate a considerably greater range of these illusory reports in the CI group (median = 40%) compared to NH controls (median = 85%). Within the CI group, ongoing analyses aim to relate these behavioral measures to clinical variables including onset and duration of severe-to-profound hearing loss, pre- or post-lingual onset of hearing loss, and auditory-only speech testing. The goal of this work is to better understand audiovisual integration and how it relates to variability in speech comprehension of cochlear implant (CI) users. This knowledge is essential for our understanding of proficiency with a CI and, most importantly, for how users can best utilize all sensory information to enhance intelligibility and improve quality of life.

Disclosures: I.M. Butera: None. **R.A. Stevenson:** None. **R.H. Gifford:** Other; Dr. Gifford is a member of the Audiology Advisory Board for Advanced Bionics and Cochlear Americas. **M.T. Wallace:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

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Program#/Poster#: 331.26/KK10

Topic: D.09. Multisensory Integration

Support: NIH U54 HD083211

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CA183492

HD83211

MH102272

Marino Autism Research Institute

Wallace Foundation

Title: Neurophysiological substrates and developmental sequelae of sensory seeking in infants at high risk for autism spectrum disorder

Authors: *T. G. WOYNAROSKI[1], C. DAMIANO[3], D. SIMON[5], L. IBANEZ[6], M. MURIAS[4], M. WALLACE[2], W. L. STONE[6], C. CASCIO[5];
[1]Hearing and Speech Sci., Vanderbilt Univ. Med. Ctr., Thompsons Station, TN; [2]Vanderbilt Univ. Med. Ctr., Nashville, TN; [3]Inst. for Brain Sci., [4]Duke Univ., Durham, NC; [5]Vanderbilt Univ., Nashville, TN; [6]Univ. of Washington, Seattle, WA

Abstract: Children with autism spectrum disorder (ASD) show a broad range of unusual responses to sensory stimuli and experiences. It has been proposed that early differences in sensory responsiveness may arise from atypical neural function and produce "cascading effects" on development across a number of domains. A primary challenge to confirming these hypotheses is that ASD cannot always be definitely diagnosed in the earliest stages of development (i.e., infancy). A potential solution is to prospectively follow infants at heightened risk for ASD based on their status as infant siblings of children who are diagnosed. The present study examined the developmental sequelae and possible neurophysiological substrates of one specific pattern of responding to sensory stimuli known as "sensory seeking" in this high risk group (HR) in comparison to a control group of infants at relatively lower risk for ASD (LR; siblings of children with typical developmental histories). Research questions included: a) Do HR infants differ from LR infants in sensory seeking behavior?, b) Does sensory seeking predict concurrent social orienting and future socialization?, and c) Is sensory seeking predicted by early frontal alpha asymmetry? To answer these research questions, we carried out a longitudinal correlational investigation in which 23 HR infants and 20 LR controls were followed over 6 months. At entry to the study, sensory seeking and social orienting were measured in the context of the Sensory Processing Assessment, and alpha asymmetry was measured via resting state EEG. Six months later, parents reported infants' socialization on the Vineland Adaptive Behavior Scales. HR infants showed elevated sensory seeking relative to LR controls (p = .017), and increased sensory seeking predicted reduced social orienting across groups, concurrently (r = .43). Seeking behavior additionally predicted future socialization and was predicted by frontal alpha asymmetry, according to risk group status (ps for the seeking inventory x risk group interaction on socialization and for the alpha asymmetry x risk group interaction on sensory

seeking = .025 and .008, respectively). Specifically, sensory seeking was *positively* associated with frontal alpha asymmetry and future socialization in LR infants, but *negatively* correlated with frontal alpha asymmetry and future socialization in HR infants, who showed an extended range of sensory seeking behaviors. Findings suggest that sensory seeking may produce cascading effects on social development in infants at heightened risk for ASD. Atypical frontal alpha asymmetry may underlie this atypical behavioral pattern of sensory responsiveness.

Disclosures: T.G. Woynaroski: None. **C. Damiano:** None. **D. Simon:** None. **L. Ibanez:** None. **M. Murias:** None. **M. Wallace:** None. **W.L. Stone:** None. **C. Cascio:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

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Program#/Poster#: 331.27/KK11

Topic: D.09. Multisensory Integration

Support: Hessian initiative for the development of scientific and economic excellence (LOEWE) Neuronal Coordination Research Focus Frankfurt (NeFF)

Title: Neural basis of the expanded temporal binding window in autism spectrum disorder: An MEG study

Authors: *J. CHAN[1,2], M. NAUMER[2], A. LANGER[2], C. FREITAG[3], J. KAISER[2]; [1]Sch. of Applied Psychology, Univ. Col. Cork, Cork, Ireland; [2]Inst. for Med. Psychology, [3]Dept. of Child and Adolescent Psychiatry, Goethe-University, Frankfurt, Germany

Abstract: The sound-induced flash illusion (SiFi) is an audio-visual illusion whereby two beeps are presented along with a single flash. Participants typically perceive two flashes if the auditory beeps are presented in rapid succession. This illusion has been used to demonstrate that specific populations exhibit multisensory deficits (e.g., in people with autism spectrum disorder (ASD), older adults, older adults prone to falling, and people with mild cognitive impairments). In these populations, the behavioural outcome is the same; that is, they perceive illusions across a wider range of stimulus-onset asynchronies (SOAs) compared to their healthy controls (HC). However, the cortical processes underlying this common outcome can be completely different. Previously, using magnetoencephalography (MEG) and a novel combination of transfer entropy and dynamic causal modelling, we demonstrated that older adults perceive more illusions than younger adults. This effect was associated with increased pre-stimulus beta-band activity. This supports the theory of predictive coding, whereby increased template information is reflected by beta-band activity. In the current study, we tested a total of 52 males; 22 ASDs (mean age = 18

years) and 30 HCs (mean age = 20 years). Outside the MEG, they were presented the SiFi with a wide range of SOAs (50-500 ms). ASDs perceived significantly more illusions, across a wider range of SOAs, compared to HC. In the MEG, participants performed a similar task with one SOA. A time-frequency analyses was conducted and we performed a 2x2 mixed-design non-parametric ANOVA with Group and Perceived Illusion as factors. We corrected for multiple comparisons using a cluster-based permutation method. We found that contrary to the previous studies, the ASD group showed decreased alpha band activity (4 -8 Hz) in the frontal regions between 400-300 ms prior to stimulus onset for the perceived illusion trials compared to no-illusion trials. This pattern of activity was not apparent in the control group. However, there was increased alpha-band activity in in the ASD group, compared to HC, following the perception of the illusory flash in temporo-occipital sensors 150-350 ms post stimulus onset. These data suggest the perception of the SiFi for ASDs is related to a different mechanism compared to populations tested in previous studies.

Disclosures: **J. Chan:** None. **M. Naumer:** None. **A. Langer:** None. **C. Freitag:** None. **J. Kaiser:** None.

Poster

331. Temporal Factors of Crossmodal Integration

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 331.28/KK12

Topic: D.09. Multisensory Integration

Title: Neuromodulation of primary somatosensory cortex alters auditory perception

Authors: ***S. CONVENTO**, M. RAHMAN, J. M. YAU;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Audition and touch interact in the frequency domain. Auditory and tactile interactions may be supported by neural circuits that process frequency information regardless of sensory modality. In fact, recent evidence from neuroimaging and neurophysiology indicates that multiple brain regions respond to both audition and touch. If a cortical region supports processing for audition and touch, what is its functional relationship with somatosensory cortex? Here, we directly assess whether primary somatosensory cortex (S1) is functionally coupled to cortical circuits that also support auditory frequency perception by combining human psychophysics with transcranial magnetic stimulation (TMS). We hypothesized that the connectivity between S1 and a hypothetical supramodal frequency processor depends on the deployment of attention to vibration frequency. Accordingly, we predicted that auditory frequency perception would only be affected by S1-TMS when subjects also attended to

vibration frequency. To test this prediction, we asked healthy participants to perform auditory and tactile frequency discrimination tasks during blocks in which attention was directed to either sensory modality alone (unimodal blocks) or over both (mixed blocks). We applied TMS over S1 on every trial. Our preliminary results show that S1-TMS impaired auditory performance only during the mixed blocks. In contrast, S1-TMS impaired tactile performance during unimodal and mixed blocks. TMS application over a control site (i.e., the occipital pole) did not impair tactile or auditory performance in any block. Our results provide a clear demonstration that primary somatosensory cortex can be functionally coupled to cortical systems supporting auditory frequency perception. Thus, somatosensory cortex may communicate with a cortical network that supports frequency processing regardless of sensory modality. Importantly, the connectivity within this supramodal network appears to depend on the specific deployment of attention.

Disclosures: S. Convento: None. **M. Rahman:** None. **J.M. Yau:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.01/KK13

Topic: E.04. Voluntary Movements

Title: The influence of visual feedback on perturbed reaches

Authors: *F. ZAHED[1], M. BERNIKER[2];
[1]Mechanical Engin., [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Reaching movements in the laboratory setting are straight under a wide range of conditions. This is true even when curved reaches are more efficient than straight ones, such as when moving in a force field. Could the visual feedback used in these experimental settings be the cause of these straight reaches? To answer this question we are investigating the influence of visual feedback on adapted hand paths. In a series of experiments we explore force field adaptation while systematically removing visual feedback. All groups made center out reaches to four targets (up, down, left and right) ten centimeters from the home position, while adapting to a clock-wise curl field (20Ns/m). In one group, subjects were shown the target location, but adapted while observing only their hand distance to the target. A bar whose length was proportional to target error was displayed on the monitor. In another group, subjects adapted without any visual feedback of the target or their hand location, but instead relied on haptic feedback. Each target was labeled with a unique number, and a virtual “dimple” was rendered to help subjects “feel” the target location. The results from both groups were compared against the results obtained when using the standard visual feedback: a small round cursor depicting hand

location. The differences across these three groups were used to understand how visual feedback changes hand path curvature. These results may help to explain how visual feedback can bias reaching behaviors, perhaps masking those natural features seen outside the laboratory setting.

Disclosures: F. Zahed: None. **M. Berniker:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.02/KK14

Topic: E.04. Voluntary Movements

Support: Wellcome Trust

Royal Society

EPSRC

Title: Separate motor memories are engaged when controlling different points on the same tool

Authors: *J. HEALD[1], J. N. INGRAM[1], J. R. FLANAGAN[2], D. M. WOLPERT[1]; [1]Engin., The Univ. of Cambridge, Cambridge, United Kingdom; [2]Ctr. for Neurosci. Studies and Dept. of Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: Effective tool-use often involves controlling the kinematics of the location, on the tool, used to contact and interact with objects in the environment. The location of such control points may change depending on use. For example, a baseball player will control different points on the bat depending on whether they are hitting for power or percentage. Here, we examine how we learn novel dynamics for different control points on an object and whether separate motor memories can be formed for different control points, even when the movement of the tool is similar. Using a planar robotic interface and virtual reality system we linked the movement of a rectangular virtual tool to the motion of the hand. Both the hand and the tool were visible at all times. The hand grasped the tool in the middle. There were two control points, one to the left and one to the right of the grasp point. On each trial one of two possible targets was chosen, either directly above the left or right control point. The task required subjects to move the tool so that the corresponding control point reached the target. Critically, although different points on the tool were controlled, the movement made by the hand and tool was identical for both targets. We linked each control point with different dynamics (clockwise and counterclockwise velocity-dependent force fields) and showed despite the identical movement there was strong learning of these opposing force fields. On channel trials used to examine generalization we either translated

the hand, the tool, or both. Results were consistent with a tool-based representation, which depended on the control point and not on the target or spatial location of the tool. Our results suggest that control points are important for partitioning motor memories and that skillful tool-use can involve learning separate dynamics for tools which have more than one control point.

Disclosures: J. Heald: None. **J.N. Ingram:** None. **J.R. Flanagan:** None. **D.M. Wolpert:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.03/KK15

Topic: E.04. Voluntary Movements

Support: DFG HA 6861/2-1

NSERC

Title: Proprioceptive and predicted consequences of action disentangled

Authors: *B. M. 'T HART, A. A. MOSTAFA, D. Y. P. HENRIQUES;
Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Motor learning should change predictions about the sensory outcome of actions, and hence affect state estimates of the position of an unseen limb. Several studies have used localization of the invisible, trained hand with the visible, untrained hand to assess changes in predicted sensory consequences of actions after motor learning (Izawa et al., 2012; Synofzik et al., 2008). Our lab has measured changes in proprioception after motor learning, by assessing changes in state estimates with hand localization. Thus it is unclear how much proprioception and prediction each contribute to state estimates. Here we disentangle the roles of proprioception and prediction in hand localization. Participants trained hand movements with aligned visual feedback and with a gradually introduced visuomotor rotation of 30°. The training movements were either active, generated by the participant, or passive, controlled by a robot. Passive training prevented visuo-motor errors and updating efference-based predictions, but did expose participants to a discrepancy between proprioception and vision, to induce proprioceptive recalibration. Before localization of the trained hand, participants actively placed their hand, or it was passively placed, which prevented efference-based predictions of hand position - whether or not these were updated through training. Since active and passive movements both reflected changes in perceived hand position, we could determine its relative contribution to hand

localization. Hand localization changed after both types of training for both types of localization movements. As expected, passive training did not lead to a difference between active and passive localization, suggesting both shifts purely reflect proprioceptive recalibration. With active training, the change after passive placement is equal to the change found in passive placement after passive training, and accounted for two thirds of the change after active placement. This leaves about one-third of the shift to changes in prediction. A maximum likelihood integration account should lead to a decrease in variance after active hand placement as compared to passive, but we didn't find evidence for this. Our results show that the contribution of felt limb position - or proprioception - to state estimates used in motor performance is much larger than previously thought.

Disclosures: B.M. 't Hart: None. **A.A. Mostafa:** None. **D.Y.P. Henriques:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.04/KK16

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Time course of reach adaptation and proprioceptive recalibration: During volitional and exposure training with a rotated cursor

Authors: *J. E. RUTTLE[1], D. Y. P. HENRIQUES[2];
[1]York Univ., Bolton, ON, Canada; [2]York Univ., Toronto, ON, Canada

Abstract: Reaching with altered visual feedback leads to adaptation of internal motor plans, which results in aftereffects (deviated reaching without visual feedback) and proprioceptive recalibration, a shift in perceived hand location (Cressman & Henriques, 2010). The rate at which these motor and sensory changes arise is still being elucidated. Zbib et al., 2016 found motor changes arise more quickly than proprioceptive changes, which required prolonged training to become significantly shifted. But their methodology may not have captured the finer incremental changes in aftereffects and proprioception. Our lab also investigated the time course of these changes by using a much quicker method of proprioceptive assessment so that we were able to measure aftereffects and proprioception every 6 rotated cursor training trials. Participants returned 7 days later to learn to reach with the opposite rotation, to test for interference and retention. Results suggest that both motor and proprioceptive recalibration occurred in as few as 6 rotated-cursor training trials (7.6° & 3.9° respectively), with no retention or interference

present one week after training. Our second study focused on the specific contribution of cross-sensory error signals throughout training on reach aftereffects and proprioceptive recalibration. Participants moved their hand to a remembered target while they were constrained to a force channel. The cursor always moved straight to the target site, while the hand was either abruptly deviated 30° or was gradually shifted 1° per trial until the hand was guided 30° CCW of the intended target (making the cursor rotation CW as per the previous study). This passive training resulted in significant aftereffects within 6 training trials (abrupt = 5°, gradual =2.7°) as well as a change in felt hand position (abrupt =7.5°, gradual =1.2°). Reach aftereffects consistently deviated across rotation introduction types by the end of passive-training (abrupt = 10.6°, gradual = 10.2°), which was expectedly somewhat smaller those produced during volitional reaches with a CW cursor (15.7°). In addition, all participants recalibrated their sense of felt hand position equally (abrupt =11.3 °, gradual =7.5°), which is also similar to the shift seen with volitional reaching (4.8°). The time course of these sensory and motor changes differed slightly across experiments but more across the different measures (motor vs sensory). But these changes did not correlate with each other which suggest that these processes are separate, with even the mere discrepancy between felt and seen hand location being enough to drive robust motor adaptation.

Disclosures: J.E. Ruttle: None. **D.Y.P. Henriques:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.05/KK17

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Dual adaptation to opposing visuomotor rotations by skewing movement trajectories

Authors: *M. N. AYALA[1], D. Y. P. HENRIQUES[2];
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Abstract: When planning movement, the human central nervous system (CNS) can actively compensate and adapt to two or more distinct perturbations simultaneously (“dual adaptation”) though this process only occurs when each visuomotor map is associated with a valid contextual cue. Because not all contextual cues are effective and only more “intrinsic” or motor-based cues tend to be useful to the CNS, we sought to investigate whether movement skewedness allows for dual adaptation of opposing visuomotor rotations. Here, we associated a reach path obstacle which effectively skews the reach trajectory thereby acting as a contextual cue preceding target

acquisition. Using a virtual reality paradigm, participants manipulated a projected hand-cursor using a digitizing tablet in a semi-dark room with an opaque board occluding visual feedback of the hand. Cursor rotations of 30° clockwise and 30° counter-clockwise were each associated with a left and right visual obstacle partially obstructing the direct path to some targets but not all. Participants completed pre-training where they were instructed to reach towards visual targets with aligned feedback, training (misaligned feedback), and post-training where they reached without visual feedback. In the training condition, participants either completed CW trials only, CCW trials only, or both interleaved within the same block ("dual group"). We found significant consistent adaptation and a faster rate of learning to unobstructed targets across all measures for both single and dual distortion groups suggesting that learning was less likely to generalize to obstructed targets. Reach errors significantly decreased over time for the dual group suggesting that movement skew is a sufficient cue for recalling a previous visuomotor map. The single group was able to return to baseline levels while the dual group only partially dual-adapted although this magnitude may be masked by the forced curved path to acquire the most obstructed target. This adaptation was further reflected in the presence of significant aftereffects following training for both single and dual distortion groups independent of the level of target obstruction. Our results suggest that movement skew, an intrinsic cue, is effective at facilitating dual adaptation.

Disclosures: M.N. Ayala: None. **D.Y.P. Henriques:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

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Program#/Poster#: 332.06/KK18

Topic: E.04. Voluntary Movements

Support: Indiana University Faculty Research Support Program (FRSP)

Title: Multisensory effects of force field adaptation

Authors: *B. M. SEXTON[1,2], Y. LIU[1,2], A. K. LYNCH[1], D. J. OSTRY[3,4], H. J. BLOCK[1,2]; [1]Dept. of Kinesiology, Indiana Universtiy, Bloomington, IN; [2]Program in Neurosci., Indiana Univ., Bloomington, IN; [3]McGill Univ., Montreal, QC, Canada; [4]Haskins Labs., Yale Univ., New Haven, CT

Abstract: The human brain processes multisensory information to control voluntary movements. Visual and proprioceptive estimates of hand position are weighted and combined to form an integrated estimate, which is used to plan hand movements. Adapting to a force

perturbation has been shown to shift, or realign, an individual's proprioceptive estimate of their hand; specifically, a rightward field results in a small leftward shift in the somatosensory perceptual boundary, and vice versa (Ostry et al. 2010). But what happens to multisensory processing? The brain generally keeps vision and proprioception aligned with each other to some degree, and shifting proprioception without also shifting vision would create a misalignment between the two. Here we ask whether force field adaptation results in visual as well as proprioceptive realignment. In a pilot experiment, subjects performed a force adaptation task, making right handed straight-ahead movements with a Kinarm robotic manipulandum (BKIN) in either a rightward (9 subjects) or leftward (8 subjects) velocity-dependent force field. Before and after force adaptation, subjects completed a perceptual estimation task we have used previously to assess alignment of visual and proprioceptive estimates of right hand position. While seated at a reflected rear projection touchscreen apparatus, subjects pointed with their left (indicator) finger at a series of three target types: a visuo-proprioceptive target (white square projected to appear directly above right hand grasping replica Kinarm handle), a visual-only target, and a proprioceptive-only target. Importantly, subjects receive no performance feedback on the sensory test. While task development is ongoing, proprioceptive realignment in this sample does appear consistent with previous literature: compared to a null field session, proprioceptive alignment shifted leftward 5.4±7.6mm after subjects adapted to the rightward field, and rightward 3.6±4.4mm after subjects adapted to the leftward field. We expect our most recent paradigm to reduce inter-subject variability, making it possible to identify changes in both vision and proprioception in an estimated 20 subjects per group. We hypothesize that vision will realign in the same direction as proprioception, which would minimize the spatial misalignment between these two modalities. This would indicate that force adaptation may have more widespread multisensory effects than previously known. On the other hand, if visual realignment does not occur, it would suggest that force adaptation selectively causes proprioceptive realignment, creating a misalignment with vision that the brain does not correct.

Disclosures: B.M. Sexton: None. **Y. Liu:** None. **A.K. Lynch:** None. **D.J. Ostry:** None. **H.J. Block:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS078311

ONR N00014-15-1-2312

NIH Grant F31NS095706

Title: Estimating properties of the fast and slow adaptive processes during sensorimotor learning

Authors: *S. T. ALBERT, R. SHADMEHR;
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Abstract: Experiments across various paradigms have revealed two consistent properties of motor memory: spontaneous recovery and savings. Spontaneous recovery refers to the observation that following adaptation and then washout, passage of time causes behavior to return to the previously adapted state. Savings refers to the observation that despite washout, adaptation is faster when a perturbation is revisited. Current models (Smith et al. 2006) suggest that motor memory is supported by two parallel learning processes: a fast process that learns a lot from error but forgets rapidly, and a slow process that learns a little from error but exhibits robust retention. Estimating these hidden processes from measured behavioral data remains a fundamental problem. To solve this problem, earlier works have used least-squares to mathematically isolate the hidden states of the learner. We found that this approach was often unreliable, due to its assumption that the sensorimotor system is noise-free. Other works have instead applied a statistical algorithm called Expectation Maximization (EM) to uncover the hidden states. This approach has the advantage of using a probabilistic representation of behavior. However, application of EM to modern sensorimotor experiments is limited because of changes in experimental conditions (e.g. error-clamp vs. perturbation trials) which make generative models of the data time variable. To address this limitation, we utilized Generalized EM (GEM), in which probabilistic learning problems with hidden processes can be considered in the framework of modern sensorimotor experiments. GEM outperformed least-squares in identifying the fast and slow adaptive processes. We next considered a visuomotor adaptation experiment (n=20 subjects) involving an adaptation block, error-clamp block, washout, set break, and then re-adaptation. We observed the two basic characteristics of learning: spontaneous recovery following the set break, and savings despite washout. GEM revealed that spontaneous recovery arose from the differing dynamics of the fast and slow timescales of memory: during washout, the slow and fast states were in competition. During the set-break, the fast state decayed, allowing the slow state to be expressed through spontaneous recovery. We found that the increased rate of re-adaptation, i.e., savings, was due to up-regulation of the error-sensitivity in the fast process, with little or no change in the slow process. In summary, spontaneous recovery appears to be caused by the differing rates at which the fast and slow processes decay in time, and savings appears to be due to an increase in the error-sensitivity of the fast process.

Disclosures: S.T. Albert: None. **R. Shadmehr:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.08/LL2

Topic: E.04. Voluntary Movements

Title: Using a real-world chopping task to study interference in a motor learning task

Authors: *A. H. NEPOTIUK, L. E. BROWN;
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Abstract: Typically task interference is studied using reaching adaptation tasks (visuomotor rotation and/or force-field learning). Participants in these experiments are already experts at the base task (point-to-point, planar reaching) and their ability to adapt reaching to the imposed perturbation is studied. The pattern of data induced by the perturbation is used to make inferences about the nature and neural correlates of our learning and memory for reaching perturbations, specifically, and motor performance in general. These studies have revealed that memory for the perturbation is formed, that it is fairly general (it generalizes somewhat to other locations in the workspace or to other hands, for example). We want to see if it is possible to demonstrate this same interference pattern using a novel vegetable-chopping task, where we can easily recreate natural performance settings using a task for which we can easily identify non-experts. Participants come into the lab, where motion trackers are fixed to their shoulder, elbow, wrist, and hand. Subjects perform a chopping task in which they are asked to chop a sweet potato into 5 mm-wide slices, matching the beat of a metronome (120 bpm). Following this initial learning, participants are exposed to an interference condition in which either force (i.e. chopping a different vegetable), or frequency are manipulated. A control group simply rests during this time. Participants then perform trials of the original task again. Measures of movement time, slice width and variability are taken. Interference will be inferred if the second performance of the original task is impaired, compared to initial performance and that of controls. Our initial results indicate that manipulating chop frequency is more likely to induce interference. This novel paradigm can be used as a way to study interference in real-world tasks across populations with a wide range of skill levels. Supported by NSERC (to LEB).

Disclosures: A.H. Nepotiuk: None. **L.E. Brown:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: NIH-R01-HD045639

NIH-R01-HD081346

NIH-R01-HD087089

NSF-EAGER 1548514

Title: Structure of solution space in a redundant motor task determines learning

Authors: *Z. ZHANG, M. HUBER, S.-W. PARK, D. STERNAD;
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Abstract: Motor variability is an intrinsic feature of human movement and can serve as a window into the determinants of skill acquisition and control. Variability is especially informative when the task is redundant, i.e. the same result can be obtained in a multitude of ways, and may reveal how humans learn novel motor skills. Previous research by Sternad and colleagues on a virtual throwing task revealed that small variations of the task can alter the structure of the solution space. This study compared performance in four task variations, each with a distinct topology in solution space, to assess how variances in task structure result in different degrees of task difficulty. Analysis of the distributional structure of variability revealed how learning and control strategies are influenced by task difficulty.Subjects threw a ball tethered to a post to hit a target. Subjects controlled the virtual ball by rotating an instrumented lever arm and opening the hand from a ball fixed to the lever arm triggered the release of the virtual ball. The ball's trajectory in the virtual workspace were fully determined by the angle and velocity at release; error was defined as the distance between trajectory and target. Throws with errors smaller than 1.1cm were defined as a successful hit, forming the solution manifold. Small changes in the target location altered the topology of the solution manifold. 4 groups of 8 subjects practiced 4 different target locations for 3 days each (240 throws per day). As expected, subjects increased their success rate and decreased error with practice. The error magnitudes differed significantly for the 4 tasks, both at the beginning and end of practice. To examine differences in difficulty between the tasks, variability was parsed into 3 components: Tolerance (exploration of error-tolerant strategies), Covariation (alignment with solution manifold), and Noise (reduction of random variability). Results showed that in tasks with higher errors, subjects could not align their variability along the solution manifold, i.e. could not take advantage of

Covariation, even though Tolerance dropped quickly on Day 1; Noise remained high throughout practice. For tasks with smaller errors, all components similarly dropped on Day 1 without showing any further change, suggesting that all possibilities to improve further were exhausted. These findings highlight that small changes in a redundant task can alter the challenge and afford different learning strategies to improve performance. The TNC-analysis presented informed how the difficulty level a redundant motor task is determined by task structure and how learning strategies are influenced by this difficulty.

Disclosures: Z. Zhang: None. **M. Huber:** None. **S. Park:** None. **D. Sternad:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.10/LL4

Topic: E.04. Voluntary Movements

Support: NIH Grant 5 RO1 HD073147

Title: Use-dependent learning reduces movement initiation latency

Authors: *F. MAWASE[1], A. HAITH[2], P. CELNIK[1];
[1]Physical Med. and Rehabil., [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Consistently repeating movement in a particular reaching direction leads future movement to be biased towards that direction - a phenomena that has been termed use-dependent plasticity or learning (UDL). It is unclear, however, why the motor system develops these biases and what benefit they may confer. Here, we show that directional bias induced by consistent repetition of movement in a particular direction is accompanied by a selective reduction in the reaction time when moving in that direction in future movements.
Right-handed subjects (n=13) performed three blocks of 7cm reaching movements. In the first block, subjects made 50 trials of reaching movements toward the center of an arc-shaped target. This block was designed to assess any default movement direction bias present at baseline. In the second block, subjects were instructed to reach a circular target as quickly as possible after hearing the fourth tone of a sequence of four tones (spaced 500 ms apart). In each trial, one of six potential targets (at 15°, 45°, 75°, 105°, 135° and 165°) appeared synchronously with the fourth tone. This predictable sequence of the auditory tones served to minimize the ambiguity about the time of target presentation. Targets appeared in a pseudorandom order, with each target appearing 24 times. For each reach we measured the reaction time at baseline and before inducing any directional biases. In the third block, subjects were instructed to make 476

repetitions toward the diagonal (either directed to 45° or 135°) of the arch target. To maintain the consistency of the repetitions, subjects received score-based feedback based on their distance from the desired direction (+3 points for |distance|<10°, +2 points for 10°≤|distance|<15°, +1 points for 15°≤|distance|<20°, 0 else). On a subset of trials (14%, ratio of 1/6), we introduced reaction time and bias probe trials.
We found that consistent repetitions induced significant directional bias toward the repeated target (p=0.034). Interestingly, there was a specific reduction of reaction time for the repeated directions compared to the non-repeated direction and relative to baseline (p=0.016). Furthermore, those individuals with larger movement direction bias showed bigger reductions in reaction time (ρ =-0.6, p=0.032). Our findings demonstrate that, in addition to biasing behavior toward a previously repeated movement, UDL accelerates how quickly we can initiate movements in that direction. We suggest that use-dependent learning might be one of the mechanisms individuals rely on to overcome warm-up decrements commonly observed during skill training.

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Poster

332. Reaching: Human Motor-Learning and Adaptation

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.11/LL5

Topic: E.04. Voluntary Movements

Title: Augmenting motor generalization by inducing instance-reliant plasticity

Authors: ***S. BAO**[1], Y. LEI[3], J. WANG[2];
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Abstract: Generalizability is a key to motor learning. Generalization of motor learning is consistent with the notion of an internal model because generalization would not occur without an internal model. However, the notion of an internal model alone cannot explain clearly why motor learning generalizes largely across limb configurations and workspaces, but generalizes only partly across movement directions and effectors. Here, we demonstrate that generalization of motor learning across movement directions can be augmented by inducing instance-reliant plasticity, that is, by allowing direction- and effector-specific motor instances to be accrued during passive reaching movements. In Experiment 1, participants were trained to adapt to a visuomotor rotation while concurrently experiencing a large number of passive reaching movements, which allowed additional motor instances to be accrued. This manipulation

increased the extent of motor generalization across movement directions and effectors, indicating a crucial contribution of direction- and effector-specific motor instances to motor learning. In Experiment 2, we show that the extent of generalization within the same arm was limited when we decreased the amount of motor instances provided during initial training, which suggests that motor instances can compete with each other within the same arm when retrieved later. Overall, our results demonstrate the role of instance-reliant plasticity in the generalization of motor learning across different movement directions and motor effectors. Support: No Special Requests: No

Disclosures: **S. Bao:** None. **Y. Lei:** None. **J. Wang:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.12/LL6

Topic: E.04. Voluntary Movements

Title: Retrieval of a motor memory triggered by a previously unseen error

Authors: ***N. J. POPP**[1], M. HARPER[2], A. M. HAITH[3];
[1]Neurosci., Univ. of Western Ontario, London, ON, Canada; [3]Neurol., [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Adaptation to a visuomotor perturbation leads to a long-term memory that is apparent in the form of savings: faster adaptation to a perturbation when it is experienced for a second time. Theories of savings have proposed that this phenomenon might either be due to an increase in sensitivity to the errors experienced during initial learning (*1*) or to retrieval of actions that were successful during initial learning (*2*). Multiple studies have demonstrated retrieval of a motor memory triggered by errors that were incongruent with those experienced during initial learning (*3-5*), suggesting that retrieval may indeed play an important role in savings. We sought to better isolate and characterize this retrieval effect by testing whether orthogonal errors, which subjects could not have seen previously, could trigger a similar retrieval effect. In an out-and-back reaching task in which only endpoint feedback was provided, experimental subjects adapted to a 40° visuomotor rotation. This adaptation was then washed out by a period of null trials. After this washout, we imposed a series of error-clamp trials in which horizontal reaching errors were clamped. To help mask the presence of the clamp, instead of seeing their true horizontal errors, subjects were provided with jittered horizontal feedback based on their individual baseline variability. In order to induce retrieval of prior learning, we introduced a novel perturbation that was orthogonal to the previously experienced rotation - a decrease in the gain of

the cursor such that for each 1 cm the hand moved from the start position, the cursor moved only 0.6 cm. We hypothesized that, in addition to compensating for the change in gain, subjects would partially retrieve their prior learning and change their reach direction, even though the errors experienced under the gain perturbation were orthogonal to the previously encountered errors. Following onset of the gain perturbation, subjects showed a clear increase in horizontal deviation in the same direction as the initial rotational perturbation. This retrieval of prior actions did not occur for a control group who never experienced a gain perturbation. These results reveal the existence of a memory for actions whose retrieval can be triggered by non-specific errors.

1. D. J. Herzfeld, et al., *Science*. **345**, 1349-1353 (2014).
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5. J. R. Morehead, et al., *J. Neurosci.* **35**, 14386-14396 (2015).

Disclosures: **N.J. Popp:** None. **M. Harper:** None. **A.M. Haith:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.13/LL7

Topic: E.04. Voluntary Movements

Support: NIH Grant K01AG047926

Title: Selective retroactive interference between two different functional motor tasks: Effect of training order

Authors: *T. K. LUMBRERAS[1], C. S. WALTER[2], S. Y. SCHAEFER[3];
[1]Biol., Utah State Univ., Lewiston, UT; [2]Physical Therapy, Univ. of Utah, Salt Lake City, UT;
[3]Arizona State Univ., Tempe, AZ

Abstract: Motor adaptation studies have provided substantial evidence that training under one condition can transfer to different conditions of the same task. Much less is known, however, if transfer can occur between different motor tasks altogether during skill learning. Our recent proof-of-concept studies have demonstrated in both neurologically-intact and –impaired adults that training on one functional motor task can transfer to a second functional motor task with different spatiotemporal characteristics. In an effort to better understand the underlying mechanism of transfer, the purpose of this follow-up study was to test whether training on the second motor task would transfer to the first. Fifteen neurologically-intact adults (mean±SD age:

21.4±1.9 yrs) were tested in this study involving a randomized crossover design. Eight subjects completed 50 consecutive trials of a motor task involving object manipulation (fastening buttons), then 50 more trials of a motor task involving reaching and tool use (spooning and transporting beans). Seven subjects completed the same amount of training but in the opposite task order. All subjects were also tested before and after training on both tasks to establish their baseline and post-test performances, measured as the number of repetitions per trial. We expected that both tasks would show improvement from baseline to post-test, regardless of which order they were practiced. In contrast, however, we found that post-test performance on the reaching task was significantly better than baseline only when it was practiced after the crossover (p<0.0001). Interestingly, the object manipulation task appeared to retroactively interfere with the reaching task, given that the group who practiced it after the crossover did not retain any reaching task improvements at post-test (p=0.34). Such interference was likely not due to any fatigue effects, as both groups improved on the object manipulation task at post-test relative to baseline (both p<0.0001), regardless of whether it was practiced before or after the crossover. These findings suggest that despite equal amounts of practice on both motor tasks, the order of practice can influence whether transfer or interference will occur. Nevertheless, this study replicated our previous findings indicating that transfer can occur from the reaching task to the object manipulation task. Future work is needed to understand *what* is transferring, given the marked differences in the tasks' spatiotemporal characteristics, and how the transfer process can be optimized.

Disclosures: **T.K. Lumbreras:** None. **C.S. Walter:** None. **S.Y. Schaefer:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.14/LL8

Topic: E.04. Voluntary Movements

Support: K01 AG047926/AG/NIA NIH

Title: Motor skill transfer of functional tasks: Is task similarity important?

Authors: ***C. WALTER**[1], G. N. OLIVIER[1], L. G. RICHARDS[1], S. Y. SCHAEFER[1,2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Utah State Univ., Logan, UT

Abstract: Motor skill transfer is the improvement on one motor task due to training on another. Current research is equivocal, with some suggesting that maximizing transfer of a skill requires that the trained task and the transfer task share characteristics, while other studies indicate that

task similarity is unnecessary. Our previous studies demonstrated that training on one functional motor task can transfer to a second functional task despite the two tasks having different spatiotemporal characteristics. The purposes of this study were: 1) to determine if training on an established reaching task transfers to a functional task with similar spatiotemporal characteristics, and 2) to determine if training on the reaching task transfers more to a task with similar spatiotemporal characteristics than it does to a task with dissimilar spatiotemporal characteristics. Eleven healthy subjects (Mean±SD age: 26.1±2.9 yrs) completed 50 consecutive training trials of a motor task involving reaching and tool use (spooning and transporting beans). This task and two transfer tasks were tested at baseline and again at a delayed posttest seven days after training. One transfer task was an object placement task (sorting cards) that shared similar spatiotemporal characteristics to the trained task, while the other transfer task was an object manipulation task (fastening buttons) which had dissimilar spatiotemporal characteristics. Trial time (in seconds) was the measure of performance for each task, with faster times indicating better performance. Paired-samples t-tests were conducted to compare baseline to delayed posttest performance on both transfer tasks. Training on the reaching task transferred to the object placement task ($p<.0001$). Training also transferred to the object manipulation task ($p<.0001$), replicating our previous findings. These findings suggest that training on a functional reaching task transfers to a functional object placement task with similar spatiotemporal characteristics. However, interestingly, the object placement task (spatiotemporally similar) demonstrated less transfer than did the object manipulation task (spatiotemporally dissimilar) as evident by a 9.9% and 24.5% improvement, respectively, from baseline to delayed posttest. This indicates that motor skill transfer is not fully reliant on degree of similarity between tasks, as more transfer occurred in the motor task with dissimilar spatiotemporal characteristics to the trained task compared to the transfer task with similar characteristics to the trained task. Future research is needed to identify motor task characteristics that modulate transfer of a motor skill.

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Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.15/MM1

Topic: E.04. Voluntary Movements

Title: Motor adaptation in head-mounted virtual reality versus conventional training

Authors: *J. M. ANGLIN, T. SUGIYAMA, S.-L. LIEW;
USC, Los Angeles, CA

Abstract: Recent technological advances have made immersive, head-mounted virtual reality (VR) an accessible tool for research and clinical use. VR provides a unique opportunity to understand how sensory environments affect motor learning. Evidence suggests that some forms of VR in rehabilitation can produce similar therapeutic gains as compared to conventional rehabilitation. However, motor adaptation in head mounted VR and its comparison to conventional training (e.g., real reality (RR)) has not been extensively explored. Here, we investigated whether adaptation on a visuomotor rotation task in immersive VR results in similar adaptation effects to adaptation in RR and whether these effects are achieved through similar mechanisms. More specifically, recent work has shown that motor adaptation may occur via both an implicit, error-based internal model and a more cognitive, explicit strategic component. We sought to measure both overall adaptation and the balance between implicit and explicit mechanisms. Twenty-four healthy individuals were placed in either a VR or RR environment and trained on a visuomotor adaptation task, modified from Taylor et al., (2014). In both VR and RR groups, participants were asked to reach towards a target flanked by numbers. Participants were only shown visual feedback when their hand crossed the target area (endpoint feedback). In addition, participants were instructed to explicitly say the number they were aiming at prior to reaching towards the target. In this way, their explicit aim was recorded, and the difference between their hand position and explicit aim was measured as the implicit component. After training on the task, a 45-degree perturbation was introduced as participants continued to reach for the targets, measuring explicit (aiming strategy) and implicit learning. Our results showed that the overall adaption time course was similar in both VR and RR groups ($t(22)=1.38$, $p=0.18$). However, VR participants utilized a greater cognitive strategy than RR participants ($t(22)=4.00$, $p<0.001$), while RR participants engaged in greater implicit learning than VR participants ($t(22)=3.67$, $p=0.001$). These results suggest that while both VR and RR conditions produce similar results in overall adaptation, the mechanisms by which motor adaption occurs in VR appear to be more reliant on cognitive strategies. This finding has implications for how VR is used in both motor learning studies and for clinical rehabilitation.

Disclosures: J.M. Anglin: None. **T. Sugiyama:** None. **S. Liew:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: NRCTR-IN15002

Title: Motor adaptation during a planar reaching using robotic device

Authors: ***J.-H. SHIN**[1], G.-L. PARK[2], H.-Y. KIM[1];
[2]Translational research center for rehabilitation robots, [1]Natl. Rehabil. Ctr., Seoul, Korea, Republic of

Abstract: Background: Motor adaptation is the change of performance depending on the mechanical environment. Upper extremity movements require more robustness to follow and maintain a desired performance against various situation, and this robustness is achieved via adaptation. The aim of this study is to explore adaptation according to perturbation and number of repetition during planar reaching task using robotic devices in healthy participants.Methods: A total of 13 healthy subjects (13 males, mean age: 24.6 ± 4.1) with no history of neurological or physical impairment participated in this study. Participants received robotic training on their non-dominant upper extremity using two dimension end-effector type robot, InMotion 2, which was specifically designed for clinical rehabilitation applications. They were presented 4 conditions of training with random orders; 640 repetition without perturbation, 1280 repetition without perturbation, 640 repetition with perturbation, 1280 repetition with perturbation. Robotic evaluations were administered at baseline before training, and after training with 5 times, with 1 minute of resting interval. The effects of adaptation on motor performance (smoothness, mean velocity, reach error and path error) were analyzed using within-subject 4 conditions x 6 evaluations repeated measures analysis of variance.Results: There was a trend of interaction between conditions and evaluations on smoothness ($p= 0.092$). There was significant interaction between conditions and evaluations of mean velocity ($p<0.001$) and significant main effect of evaluations ($P < 0.001$). Path error and reach error did not show any significant main effect or interaction. Conclusion: The motor performance changes indicate that adaptation was dependent on training and evaluation time and was the changing pattern was different among motor performance. In addition, training with perturbation seems to induce no significant adaptation.

Disclosures: **J. Shin:** None. **G. Park:** None. **H. Kim:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.17/MM3

Topic: E.04. Voluntary Movements

Support: USC Viterbi fellowship

Title: Dissociating the role of sensory prediction error from performance errors in strategy based motor adaptation

Authors: *K. LEE[1], Y. OH[1], J. IZAWA[2], N. SCHWEIGHOFER[1];
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Abstract: In theory, no goal is needed for motor adaptation: the sensory prediction error (SPE), i.e., the error between actual and predicted hand location, is sufficient to update the motor command. However, performance errors (PE) and rewards, which are known to play significant roles in motor adaptation, are typically present in most studies. Is there any effective strategy to dissociate the roles of SPE from PE? In the visuomotor adaptation studies by Mazzoni and Krakauer (2006) and Taylor and Ivry (2011), subjects were instructed to aim explicitly to a neighboring target of the main target to cancel the initial PE with respect to the main target due to the perturbation. In subsequent trials, subjects exhibited gradual over-compensation, which has been suggested to be the signature of internal forward model update by SPE. Here, we hypothesize that adaptation in this paradigm is due to three different types of errors: PE1, PE2, and SPE, from the visual cursor to the main target, the neighboring target, and visual prediction of the hand, respectively. To test this idea, we performed a visuomotor adaptation experiment with 45 degrees perturbation for 200 trials that extends that of Taylor and Ivry (2011) with 4 conditions. . After the second trial following the perturbation, subjects were instructed to shoot to the neighboring targets located at 45 degrees from the main targets. A localization test of 10 trials was then given, followed by 80 washout trials. Final cursor feedback was always available except during localization trials. In condition 1, the two targets remained on the screen during the trial (PE1+PE2+SPE). In condition 2, the main target disappeared as soon as the movement was initiated (PE2+SPE), while the neighboring target disappeared in condition 3 (PE1+SPE). In condition 4, both targets disappeared (SPE). Preliminary results indicate that the overcompensation following strategy was largest in condition 2, then in condition 1 and in condition 4, and least in condition 3, in which the initial overcompensation returned to near zero (as found in Taylor and Ivry, 2011). A large overcompensation similar to that observed in Mazzoni and Krakauer (2006) was only observed in condition 2. In addition, increase in localization errors were observed in all four groups. These results complement our previous results (Oh et al, NCM 2014) that no target is needed for adaptation. In summary, our study suggests that 1) PE2 plays a role in producing the over-compensation observed in Mazzoni and Krakauer (2006), 2) PEs and SPE influence motor adaptation by generating adaptive responses with different time courses, and 3) SPE is always present and is sufficient for motor adaptation.

Disclosures: K. Lee: None. **Y. Oh:** None. **J. Izawa:** None. **N. Schweighofer:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.18/MM4

Topic: E.04. Voluntary Movements

Title: Learning mechanism of nondominant single-joint elbow extension movements.

Authors: *J. SONG[1], K. LEE[1], S. Y. SCHAEFER[3], N. SCHWEIGHOFER[2];
[1]Biomed. Engin., [2]Biokinesiology and Physical Therapy, USC, Los Angeles, CA; [3]Dept. of Health, Physical Educ. and Recreation, Utah State Univ., Logan, UT

Abstract: Single joint elbow movement has been characterized through "pulse-height control" for the right-dominant arm and "pulse-width control" for the left-non-dominant arm (Sainburg and Schaefer 2004). Theoretically, in pulse-height control the feedforward motor command is appropriately scaled in amplitude to reach targets at different distances, yet in pulse-width control a constant amplitude, target-independent feedforward command is modified by feedback that allows the target to be reached. It is unclear however whether such variation in control strategies is due to innate differences between the dominant and non-dominant hemispheres, or whether it is acquired over time by the frequency of each arm's use in activities of daily living. Here, we perform an experiment in which we extensively trained subjects over five days to perform single-joint elbow extension movements with their non-dominant arm. Four targets at 10, 20, 30, and 40 degrees were presented in pseudorandom order. During days 1 and 5, right-handed subjects performed 150 movements with their non-dominant and dominant arms. During days 2, 3, and 4, subjects performed 500 movements per day with only their non-dominant left arm. Results showed that on day 1, subjects exhibited acceleration profiles similar to those of (Sainburg and Schaefer 2004), with a clear "pulse-height" strategy for the dominant arm and "pulse-width" strategy for the non-dominant arm. However, by day 5, the non-dominant arm had adopted a more "pulse-height" strategy, similar to that exhibited by the right arm. We simulated these results via a feedback error-learning algorithm, and found that the dominant arm's response was modeled by a well-learned inverse controller. In contrast, the non-dominant arm's response was better modeled by an inverse controller characterized by a small constant motor command with a feedback command completing the movement. The inverse controller was updated with training, however, such that the feedforward controller was well tuned for generating varied pulse-height commands. In short, our results show that distinctions in control strategy are likely not due to fundamental differences in the left and right hemispheres, but may be due to the degree of development of feedforward control through differential arm use.

Disclosures: J. Song: None. **K. Lee:** None. **S.Y. Schaefer:** None. **N. Schweighofer:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.19/MM5

Topic: E.04. Voluntary Movements

Support: Viterbi Graduate School Fellowship

Title: The effect of signal-dependent noise on error- and reward-based learning of an isometric force visuomotor transformation task

Authors: *V. BARRADAS PATINO[1], N. SCHWEIGHOFER[2];
[1]Biomed. Engin., [2]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: An important source of variability in human motor behavior is execution noise, which partially arises from muscle and motor command properties in a signal-dependent way. Moreover, signal-dependent noise can account for most of the variability in isometric force production tasks. On the other hand, motor variability has been found to relate to error- and reward-based learning in different ways, being greater in the latter. In this study we further explore the effects of motor variability on both types of learning by using a visuomotor scaling task in which participants control a cursor by isometrically applying force with their wrists on a load-cell. This task involves virtual reaching movements of the cursor from a central position to one of eight targets uniformly located around the central position. For each learning condition we used two groups: low force and high force, which differ in the amount of force required to reach a target during a baseline phase. This experimental paradigm allows us to control the level of noise in the task by modulating the required force magnitude, while observing the effects of the noise on learning. We hypothesized a slower adaptation to the perturbation for the high force group in the error-based learning condition since the error-signal would be noisier. However, in the reward-based condition we hypothesized faster learning rates in the high force group because the increased motor noise could be used as a substitute or a supplement to exploration noise. In the error-based learning condition the experiment consists of repetitions of the task during a baseline phase followed by a perturbation phase in which the scaling of the mapping between force and cursor position is abruptly modified. During the reaching movement of the cursor all feedback is suppressed and only the final position of the cursor and the target are shown at the end of the trial. In the reward-based condition the perturbation is gradually introduced in a closed-loop reinforcement scheme in which the perturbation is equal to the average of the 10 previous reaches. The reward is binary and it is only given when the current force is beyond the average of the previous reaches in the direction of the scaling. The results of this study could be used to provide insights into methods to enhance motor learning in individuals with deficits in

either error-based or reward-based learning mechanisms, such as stroke survivors with lesions in the cerebellum or basal ganglia, respectively, in order to design new rehabilitation therapies.

Disclosures: **V. Barradas Patino:** None. **N. Schweighofer:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.20/MM6

Topic: E.04. Voluntary Movements

Support: NSF BCS 1031899

Title: Neural Substrates of reinforcement learning in a continuous visuo-motor task

Authors: ***N. SCHWEIGHOFER**[1], S. KIM[2], T. HORIKAWA[3], S. SCHAAL[4], Y. KAMITANI[5], D. CALLAN[3];
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Abstract: Although the computational, behavioral and neural correlates of reinforcement learning in discrete tasks are well understood, much less is known about the behavioral and neural correlates of reinforcement learning in continuous tasks. Here, we perform an fMRI experiment to better understand how humans learn to select motor commands to find a target when provided positive and negative feedback. We hypothesized that subjects sequentially integrate information from reward feedback at each trial to direct the next movement, as consistent with a Bayesian model maximizing the probability of finding the target. The model allowed us to estimate the latent variables underlying the behavior such as reward-prediction error, exploration and uncertainty. For fMRI analysis, we generated a set of regressors for related information with behaviors: (1) history of rewards, (2) exploration, as estimated by the difference between actual movement direction and the model-computed direction (3) reward prediction error and (4) uncertainty, as computed by the entropy of the posterior probability distribution. The computed regressors were also applied for multi-voxel pattern regression analysis to decode the relevant information. We found significant activation maps in cortical areas such as angular gyrus (reward), parietal (uncertainty), and Right frontopolar regions (exploration).

Disclosures: **N. Schweighofer:** None. **S. Kim:** None. **T. Horikawa:** None. **S. Schaal:** None. **Y. Kamitani:** None. **D. Callan:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: German Research Foundation (DFG) Grant RA 2183/1-3

Title: Eye-hand coordination during visuomotor learning: effects of terminal visual feedback

Authors: ***M. K. RAND**, S. RENTSCH;
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Abstract: We previously examined adaptive changes of eye-hand coordination during learning of a visuomotor rotation by using continuous on-line visual feedback. Gazes during reaching movements were initially directed to a feedback cursor in early practice, but were gradually shifted toward the visual target with more practice. This result reflected a functional change of gaze control for visuomotor learning from exploring the cursor-hand relation to guiding the hand to the task goal. The present study investigated adaptive changes of eye-hand coordination when another type of visual feedback, i.e., terminal feedback was used for learning a visuomotor rotation. Young adults made reaching movements to targets on a digitizer, while looking at targets on a monitor. Visual feedback (a cursor) of hand movements was rotated and displayed on the monitor after each movement. Three rotation angles (30°, 75° and 150°) were examined in three groups to vary the task difficulty. The results showed that the 30° group gradually reduced direction errors of reaching with practice and well adapted to the applied rotation. The 75° group made large direction errors of reaching in early practice, and the 150° group applied a 180° reversal shift of hand movements; both groups overcompensated the respective rotations in late practice. Regarding eye-hand coordination, all groups gradually adapted gaze directions measured at the onset of reaching from the target area to the areas related to the final positions of reaching during the course of practice. This adaptive change was different from the one observed in the previous study with continuous feedback. Post-tests revealed that the adaptive changes of both hand and eye movements in all groups mainly reflected explicit adjustments of movement directions, whereas only the 30° group showed small implicit adaptations in both effectors. Taken together, the results indicate that gaze directions prior to reaching are adapted from the visual target to the final position of reaching based on explicit knowledge of the visuomotor rotation. Through this adaptive change, the oculomotor system supports the limb-motor system

to make precise preplanned adjustments of reaching directions for learning of visuomotor rotation under terminal feedback.

Disclosures: M.K. Rand: None. **S. Rentsch:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.22/MM8

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS092079

Title: Sensorimotor adaptation to small visual errors: error size-dependent effects on rate but not magnitude

Authors: *H. E. KIM[1], J. R. MOREHEAD[3], M. J. BOGGESS[1], W. SHWE[1], T. C. DIXON[2], D. PARVIN[1], R. B. IVRY[1];
[1]Dept. of Psychology, [2]Dept. of Bioengineering, Univ. of California, Berkeley, Berkeley, CA;
[3]Sch. of Engin. & Applied Sci., Harvard Univ., Cambridge, MA

Abstract: Sensorimotor adaptation is driven by sensory prediction errors (SPEs), the differences between predicted and actual sensory outcomes of motor commands. Recent work has shown that adaptation from SPEs operates even when its effects on performance act in opposition to task goals, and when feedback is decoupled from task performance. Evidence for the latter comes from experiments utilizing a visual error clamp, where the position of a "feedback cursor" is coupled to the radial distance of the hand, but angular trajectory is invariant with respect to the target location, thus spatially independent of hand position. This method attempts to isolate adaptation from other mechanisms. Surprisingly, learning functions during these experiments were invariant over a large range of error sizes, from 7.5° to 95°, a pattern at odds with current learning models. In the present study, we further examine constraints on adaptation using the error clamp. Previously, we observed that performance was unaffected by clamps >95°; we now employed small angular clamps, asking if learning remained invariant. Fifty participants performed center-out slicing movements along a digitizing tablet to one of 8 target locations. During the error clamp block, the angular trajectory of the cursor was fixed at either 1.75°, 3.5°, 6°, 10°, or 15° (n=10/group). This was explicitly described to the participants prior to the manipulation, and they were told to reach directly to the target and ignore cursor feedback. Learning was quantified by changes in hand angle during the clamp and by aftereffect size. All groups adapted, with hand angles shifted in the direction opposite the clamp. Aftereffects were

again equal across groups (p=0.92), approximately 15°. This effect is striking since the error signal was much smaller in most groups, and in the extreme, only off from the target by 1.75°, with cursor and target partially overlapping. In contrast to the similar aftereffects, early adaptation rates differed between groups and scaled with error size (p=0.006). Our results suggest that, while adaptation magnitude is independent of error size, initial learning rate is dependent on error size over a limited range, with the rate saturating with errors of ~10° or greater. These constraints are difficult to capture in a state space model; for example, using a fixed forgetting parameter value, *A*, of 0.99 underestimates learning rates and overestimates aftereffects for all clamp sizes. Previous models have discussed how the learning rate parameter, *B*, may vary as a function of error size; we consider extensions to this work in which error signals may be treated in a probabilistic manner, rather than in terms of magnitude.

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Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.23/MM9

Topic: E.04. Voluntary Movements

Title: Sequence specific motor learning in an immersive virtual environment

Authors: *J. BAER, J. C. STEWART;
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Abstract: Examination of sequential motor skills has largely involved single finger button presses, or minute movements of a mouse or joystick. These paradigms are capable of assessing basic motor learning principles in a laboratory setting, however, they may not translate to real-world function that requires more complex, 3-dimensional (3D) movements. Immersive virtual environments (VE) allow for systematic presentation of visual stimuli in first-person space, whole arm movements in 3 dimensions, and methodical manipulation and control of the task. The purpose of the current study was to examine implicit motor learning using whole arm reach movements in a 3D VE. Fifteen non-disabled, young participants practiced a sequential target task with the dominant, right arm over two days. Targets appeared one at a time and were presented as a red sphere. An electromagnetic marker secured to the right index finger served as the cursor and was represented as a white sphere. Participants were instructed to move the cursor to the target as quickly and accurately as possible. Targets were presented in eight-target sequences and alternated between a random sequence and a repeated sequence. A total of 144

sequences were practiced during acquisition on Day 1, and 72 sequences were completed at retention on Day 2. Each sequence was matched for difficulty using Fitts' index of difficulty and total distance covered. Total movement time of each sequence was measured and averaged for random sequences and repeated sequences. Total time to complete a sequence decreased over practice ($p<0.001$). While total time decreased for both random and repeated sequences, the decrease in time was greater for the repeated sequence ($p=0.023$), suggesting sequence learning was present. Implicit sequence learning was observed with a whole arm reaching paradigm, similar to previous tasks using simpler movement requirements. Improved ability to interact with the VE was seen, as evidenced by improvements in the random condition; however, improvement was greater in the repeated condition when an embedded sequence was present. An immersive VE may provide the opportunity to study implicit motor learning in a more real-world context that could have implications for clinical populations with arm dysfunction.

Disclosures: J. Baer: None. **J.C. Stewart:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: NSERG

Title: Effect of perturbation uncertainty on the retention of a new visuomotor relationship.

Authors: *C. CANAVERAL, F. BERRIGAN, P.-M. BERNIER;
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Abstract: The brain uses forward modeling to predict the sensory consequences of descending motor commands. In support, studies have shown that when a constant visuomotor perturbation is introduced (e.g. 15º cursor rotation), motor commands gradually change and aftereffects occur after removal of the perturbation, suggesting an update of the forward model. Here we investigate whether introducing variance in the distribution of exposed rotations (e.g. 10º, 15º, 20º) influences the acquisition and retention of new visuomotor relationship. Participants (n=30, 7 males) took part in a visuomotor adaptation paradigm, which consisted in an Adaptation phase (240 trials, cursor rotated), followed by a No vision post-test (80 trial, no cursor). During the Adaptation phase, the variance in exposed rotations was manipulated. In the Constant group (C), the rotation was constant at 15°. In the Low Variance group (LV), the cursor rotation pseudo-randomly varied between 10º (20% of trials), 15º (60% of trials), and 20º (20% of trials). In the

High Variance group (HV), the cursor rotation varied between 10º (40% of trials), 15º (20% of trials), or 20º (40% of trials). Importantly, the mean of exposed rotations was identical across groups (i.e. 15º). Reach direction was calculated as the angular difference between the hand and the target at the target radius (i.e. 10 cm). Feedback regarding task success (i.e. on or off target) was provided in binary form throughout the adaptation phase, but not in the No vision post-test. Acquisition and retention were defined as the mean accuracy in the last 80 trials of the Adaptation phase and the 80 trials of No vision post-test, respectively. Verbal reports revealed that none of the participants consciously perceived the variability in cursor rotation. Interestingly, all groups acquired the new visuomotor relationship to the same extent during the Adaptation phase, as hand trajectories did not differ across groups ($p > 0.05$). However, participants in the C group showed better retention (8.3 ± 1.4º) as compared to the LV (7.7 ± 1.8º) and the HV groups (6.8 ± 1.7º), differing significantly from the latter ($p < 0.05$). A control experiment further revealed that the observed differences in retention were not attributable to differences in task success during adaptation. These results suggest that the update of the forward model during adaptation depends upon the mean of the distribution of exposed rotations but not its variance. In contrast, retention is influenced by the variance in exposed rotations regardless of task success.

Disclosures: C. Canaveral: None. **F. Berrigan:** None. **P. Bernier:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Program#/Poster#: 332.25/MM11

Topic: E.04. Voluntary Movements

Support: NIH Grant EY021252

Title: Spatiotemporal properties of motor adaptation generalization

Authors: *W. ZHOU, J. FITZGERALD, K. COLUCCI-CHANG, K. MURTHY, W. M. JOINER;
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Abstract: Motor learning modifies both the spatial and temporal aspects of performance. These features are typically studied in isolation, leaving the spatiotemporal interaction largely unknown. Here, we systematically examined how the temporal decay of visuomotor adaptation influences the spatial generalization. In the first experiment 10 subjects made 9 cm movements to a peripheral target and a 30^{o} rotation was applied to the endpoint visual feedback of their

unseen reaching motion (rotation direction was counterbalanced). Similar to Hadjiosif and Smith (2013), we examined the decay of adaptation at the trained target over 8 different temporal delays (0, 3, 6, 10, 20, 30, 60, or 120 seconds). After each delay subjects completed 5-7 retraining movements to maintain adaptation throughout the session. We found that a single exponential represented the temporal change in behavior ($R^2 = 0.98$, decay constant of 23.7 ± 8.4 sec). In the second experiment 32 additional subjects made 9 cm movements with the same 30^o perturbation. Following training, subjects made 6 movements to a possible 19 different target locations (spaced 15^o apart, symmetric around the trained location). During these generalization movements no visual feedback was provided. After movements to 6 randomly selected generalization targets, subjects again completed 5-7 retraining movements to maintain adaptation. We used a modified Gaussian to quantify the spatial generalization of adaptation across targets locations for different temporal delays, specifically 3 generalization functions for the temporal ranges delineated from the experimental data (4.43 $\pm$ 0.5, 13.0 $\pm$ 0.4, and 25.6 $\pm$ 2.1 sec). Our results demonstrate that generalization within ± 60^o around the trained direction significantly decreased with the increase in delay and distance from the trained direction (two-way ANOVA, $P < 0.001$ in both cases). However, generalization > 60^o away from the trained location was near constant across the different delay ranges ($P > 0.05$). Interestingly, the decay at the trained target did not follow the exponential decay pattern based purely on time (experiment 1). This suggests that the spatiotemporal decay of visuomotor adaptation is a function of both the passage of time and the changing context within that time.

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Poster

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Topic: E.04. Voluntary Movements

Support: KAKENHI A26242062

KAKENHI 16J03309

Title: Short-term maintenance of motor memory induced by memory retrieval

Authors: *A. SASAKI, D. NOZAKI;
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Abstract: It is known that motor memory decays with passage of time (Hadjiosif et al., TCMC, 2014) or with performing the trained movement (Ingram et al., Curr. Biol., 2013). However, it has not been fully elucidated how these two types of motor memory decay are interrelated, which we tried to clarify in this study. In Experiment 1, participants performed reaching movements in the presence of velocity-dependent force field (FF). We used error-clamp (EC) method to quantify the level of motor adaptation (i.e., motor memory). After performing 100 FF training trials, they repeated a test set 100 times. Each test set consisted of 6 FF trials followed by 2 EC trials. The inter-trial interval (ITI) between the 2 EC trials was changed randomly from 3 to 24s so that we could investigate time-dependent and trial-dependent memory decay (ITI for FF trials was 4s). We observed a small reduction of motor memory from 1st to 2nd EC trial even if the ITI was 3 sec. However, this level was maintained for approximately 8 s, which was followed by gradual time-dependent reduction. In declarative memory, it has been known that memory rehearsal plays a crucial role in maintenance of memories (i.e. maintenance rehearsal, Craik & Lockhart, J. Verb. Learn. Verb. Behav., 1973). Similarly, we speculated that retrieving the motor memory by EC trial contributes to maintain the memory for short-term period by rehearsing the movement, which was further examined in Experiment 2. The protocol of Exp.2 was almost identical to that of Exp.1, but each test set consisted of 3 successive EC trials. The ITI between 1st and 3rd EC trials was kept constant at 16 sec. The 2^{nd} EC trial was inserted at 4, 8, and 12s from the 1^{st} EC trial. When the 2^{nd} EC trial was performed at 4s (or 12s), the time-dependent memory decay should occur from the 2^{nd} to 3^{rd} EC trial (or from the 1^{st} to 2^{nd} EC trial), because the ITI of 12s was longer than 8s for which the memory can be maintained by EC trial. In contrast, the effect of such time-dependent memory decay can be minimized when the 2^{nd} EC trial was performed 8s. Our preliminary results support this prediction, suggesting that retrieval of motor memory had an immediate effect to reduce the motor memory level, but could contribute to maintain the level for short-term period.

Disclosures: **A. Sasaki:** None. **D. Nozaki:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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Topic: E.04. Voluntary Movements

Support: KAKENHI (#26242062)

KAKENHI (#26-2174)

Title: Rotation of preferred direction of motor primitive explains the dependence of visuomotor adaptation rate on shape of visuomotor map

Authors: *T. HAYASHI[1], K. TAKIYAMA[2], D. NOZAKI[1];
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Abstract: Control of visually-guided reaching movement largely relies on feedforward controller based on visuomotor map that transforms visual target information into the appropriate motor command. We have previously demonstrated that the motor system uses the visoumotor map not only for the feedfoward control but also for movement correction across trials (visuomotor adaptation) (Hayashi et al., TCMC 2015). More specifically, we gradually increased visuomotor rotations for the movement toward two different targets (±30 degrees rotation was imposed for ±30 degrees target located from straight ahead, respectively). This procedure implicitly distorted the visuomotor map so that the actual movement direction deviated more inward (i.e., closer to 0 deg) than the target directions (inward group). In contrast, when the direction of visual rotation was reversed, the actual movement directions deviated more outward (outward group). We found that the size of aftereffect induced by a visual rotation (i.e., adaptation rate) for the movement to 0 deg target changed with the type of of visuomotor map distortion (reduced or increased for inward or outward group, respectively). Here, we investigated a computational principle to reproduce the experimental results. We found that conventional motor primitive framework, which assumes that visuomotor adaptation is accomplished by changing the weight parameters, predicted that the learning rate remained unchanged even after the visuomotor map was distorted. Thus, additional factors other than weight parameters need to be included to reproduce our previous results.Based on previous neurophysiological studies (e.g., Shen and Alexander, JNP 1997), we speculated that the additional factor would be rotations of preferred directions (PD) of motor primitives. When the PD rotations were included in the motor primitive framework, the modification of shape of visuomotor map was accomplished not only by the changes in the weight parameters but also by changes in the PD distribution. A numerical simulation with this revised model successfully reproduced the dependence of visuomotor adaptation rate on the shape of visuomotor map. These results suggest that the PD rotations play a substantial role for visuomotor adaptation.

Disclosures: **T. Hayashi:** None. **K. Takiyama:** None. **D. Nozaki:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

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NWO-VICI 453-11-001

Title: Opposite effects of reward probability on learning and motivation in a 3D visuomotor adaptation task

Authors: *K. VAN DER KOOIJ[1], K. E. OVERVLIET[2], L. OOSTWOUD-WIJDENES[3], J. B. J. SMEETS[1];
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Abstract: Rewards are motivating and have also been found to affect motor learning (e.g. Galea et al., Therrien et al., 2016). However, It is unclear whether motivational and learning effects of rewards are related. To gain insight in the dual effects of rewards, we tested how motivation and learning depend on the reward probability.
Participants (N = 600; age 8-65) performed a 3D pointing task in which they tried to 'catch' virtual flies (shown in an Oculus Rift) by pointing to the location where a fly appeared and keeping their hand still until the next fly appeared in a pseudo-random and reachable direction. The movement error was calculated as the distance between the target and the hand position when participants kept their hand still plus a ten-centimeter leftward displacement. This way, the movement error would be zero if the participant reached ten centimeters to the right of the target. Reward feedback consisted of an animation of the fly dying, 5 scored points and a rewarding sound.
The effects of reward probability and error-based feedback on learning and motivation were assessed in a 3 (reward probability) by 2 (error feedback) between-subject design. Three different reward probabilities were created (~30%, ~50%, ~70% of the trials) based on performance relative to previous trials, whereas in a control group the rewards were random (with a probability of ~50%). Participants in the 'reward only' groups never received feedback about their hand's position, whereas participants in the 'error and reward' groups saw on the (shifted) movement endpoint. Participants started with 20 baseline trials without any feedback, then performed 80 learning trials with feedback and finished with 15 retention trials without any feedback. Learning was assessed by comparing the error in the second half of the learning phase to the error in the baseline phase. Motivation was assessed with an inventory that participants completed after the 'catching' task.
We found that motivation was positively influenced by the reward probability. Learning from the error-feedback, in contrast, was negatively influenced by the reward probability: participants learned the least in the high-reward condition. When presented alone, the reward feedback did not induce learning. We did find a larger trial-by-trial change after a movement that had not been rewarded, which may be indicative of reward-based explorative behavior. We conclude that reward probabilities have opposite effects on motivation and learning.

Disclosures: **K. Van Der Kooij:** None. **K.E. Overvliet:** None. **L. Oostwoud-Wijdenes:** None. **J.B.J. Smeets:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 332.29/NN3

Topic: E.04. Voluntary Movements

Support: Wellcome Trust

The Rutherford Foundation Trust

Cambridge Trust

Title: Planning different follow-throughs, rather than their execution, activates separate motor memories

Authors: ***H. R. SHEAHAN**[1], D. W. FRANKLIN[1,2], D. M. WOLPERT[1];
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Abstract: When different follow-through movements are associated with motor skills that normally interfere, they can be learned simultaneously, suggesting that distinct future actions activate separate motor memories (Howard et al, 2015). However, it is unknown which features of the follow through, such as planning or action, are crucial for such learning. We examined four groups of human subjects reaching in a randomly selected clockwise or counter-clockwise curl field from a start target to a goal target. On some trials we applied a force channel to assess learning. For the first group, a follow-through target (to the right or left of the goal target) was displayed from the start of a trial. After the movement through the field the subjects continued on to the follow-through target (with no force field). The direction of the field applied on the first movement depended on the follow-through target. As expected strong learning was seen. For the no-follow through control group the subjects stopped at the goal target and did not follow through. As expected no learning was seen. For the third group, the follow-through target only appeared as they reached the goal target and the participants then followed through. No learning was seen in the third group, suggesting execution of follow-through alone was unable to retroactively separate motor memories. A fourth group planned a follow through movement similar to the first group but the follow-through target disappeared as they reached the goal target and they were required to abort the movement. However, on channel trials the follow through

was performed. This group therefore planned different follow-throughs but did not enact them. Despite never enacting a follow-through movement on a trial with a force-field perturbation (performing the same action as in the no-follow through control group), the fourth group were able to learn. By simply planning for follow-through movements, learning was not significantly different from the control group that planned and performed full-follow through movements. This suggests that follow-through leads to the activation of separate motor memories through motor planning, not execution.

Howard, I. S., Wolpert, D. M., & Franklin, D. W. (2015). The Value of the Follow-Through Derives from Motor Learning Depending on Future Actions. *Current Biology*, *25*(3), 397-401.

Disclosures: **H.R. Sheahan:** None. **D.W. Franklin:** None. **D.M. Wolpert:** None.

Poster

332. Reaching: Human Motor-Learning and Adaptation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

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Rhode Island Foundation 20144132

NSF CAREER 1555006

Title: The effects of cognitive aging on attentional context in visuomotor learning

Authors: *E. K. FESTA, T. WANG, W. C. HEINDEL, J.-H. SONG;
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Abstract: From birth to old age, motor learning allows us to form new sensory-motor relations and adapt to environmental or internal changes. Importantly, such sensorimotor adaption is acquired in complex environments where multiple stimuli compete for limited attentional resources. Using a dual-task paradigm in which a visuomotor adaptation task was performed with a concurrent attention-demanding perceptual discrimination task, we surprisingly found that the visuomotor adaptation acquired while attending to this perceptual distraction was retained only when a similar attention distractor was present during the recall phase. This paradoxical effect suggests that attentional state is encoded as an internal context during visuomotor learning and should be reinstated for successful recall. Our subsequent demonstration that attentional state is primarily encoded during the early phases of learning further suggests that the encoding of internal context may be driven primarily by strategic control processes rather than by more

gradual recalibration processes mediating sensorimotor adaptation. To directly examine this possibility, the present study examined the effects of cognitive aging on visuomotor adaptation within this dual-task paradigm, since previous studies have found that strategic control processes are impaired while recalibration processes remain intact in healthy elderly. We found that while consistent attentional distraction between learning and recall enhances visuomotor memory recall in the young group (replicating our original finding), the same distraction deteriorates recall performance in the elderly group. Thus, the cross-integration between sensory distraction and motor memory only occurs in the young group. These findings confirm previous findings of impaired strategic control and intact recalibration adaptation processes in healthy elderly, and provide strong support for the critical role of strategic processes in encoding attentional state as an internal context during visuomotor adaptation learning. The differential effects of internal task context on adaptation learning in young and elderly also have important implications regarding the relative effectiveness of different rehabilitation programs to real-life situations within these two populations.

Disclosures: **E.K. Festa:** None. **T. Wang:** None. **W.C. Heindel:** None. **J. Song:** None.

Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.01/NN5

Topic: E.05. Brain-Machine Interface

Support: ERC-2012AdG 320708-iCONNECT

Title: Unravelling temporal dynamics of sensorimotor cortex activity during speech for bci decoding

Authors: *E. SALARI, Z. V. FREUDENBURG, M. J. VANSTEENSEL, N. F. RAMSEY; Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: With growing interest in the neural representation of speech production for decoding with Brain-Computer Interface implants, it becomes increasingly important to understand the timing of neural responses relative to utterances. We observed that electrocorticographic (ECoG) brain activity is typically sustained for the duration of a word in the high frequency band (HFB; 65-135Hz) power. Since word duration is influenced by both the number and the duration of speech sounds, this might suggest that the temporal trace of a word contains information about the timing of individual phonemes and their durations. However, to investigate this it is important to understand the temporal dynamics of individual phonemes. Therefore, in this study

we explore the temporal dynamics of one single phoneme in the sensorimotor cortex and the effect of speech duration.
Three epileptic patients who were implanted with ECoG electrodes for diagnostic purposes participated in our study. All had coverage over sensorimotor cortex. Subjects were asked to pronounce the Dutch /i/ vowel for 1, 2 or 3 seconds. Each trial started with an indication of the duration, followed by the vowel. Each condition was repeated 15 times.
Data were preprocessed (discarding noisy electrodes, removing line-noise, common average re-referencing) and the HFB power was extracted and temporally smoothed. For electrodes that showed a significant response to the task (ca. 16% of sensorimotor electrodes), the HFB response of every trial was aligned to speech onset and the average response was calculated per vowel duration (after correction for speech offset inaccuracies).
Two models were fitted, one for sustained activity (a block function with a length equal to the vowel duration), and one for transient activity (Gaussian function).
Half of the analyzed electrodes showed a clear, vowel-duration dependent sustained HFB increase, of which 80% showed a peak at voice onset and 20% an additional peak at voice offset. From the electrodes that showed a transient response, 60% showed a peak at voice onset and 40% an additional peak at voice offset.
We show that the activity in some parts of the sensorimotor cortex is related to the duration of vowel pronunciation. Other areas only show a transient response. Sustained responses are likely to reflect either sensory input or sustained motor activity (e.g. larynx activity). Transient responses are probably linked to movement of specific articulators (e.g. lips & tongue). These findings encourage further research on decoding (intended) speech for communication BCI's, and suggest that placement of recording electrodes requires careful planning to capture relevant temporal detail.

Disclosures: E. Salari: None. **Z.V. Freudenburg:** None. **M.J. Vansteensel:** None. **N.F. Ramsey:** None.

Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.02/NN6

Topic: E.05. Brain-Machine Interface

Support: French National Research Agency (ANR-Carnot Institute)

Fondation philantropique Edmond J. Safra

Fondation Motrice

Fondation Nanosciences

Fondation de l'Avenir

Title: Preclinical chronical implantation of the wimagine ecog recording implant: a sheep study.

Authors: *C. CRETALLAZ, M. FOERSTER, F. SAUTER-STARACE, T. COSTECALDE, D. RATEL, C. GAUDE, A. LAMBERT, G. CHARVET, C. MESTAIS, N. TORRES;
CEA LETI/CLINATEC, GRENOBLE, France

Abstract: WIMAGINE is a wireless electrocorticogram (ECoG) recording implant, designed for long term human implantation, made of an array of 64 biocompatible electrodes, hermetic titanium housing and electronic boards as previously described [1]. Our objective is to test WIMAGINE in a large animal model adapted to implant size. Two female sheep have been implanted with WIMAGINE in order to validate long term *in vivo* implantation. Ethical approval was obtained from local ethical committee, in accordance with European Communities Council Directive of 1986 (86/609/EEC) for care of laboratory animals. The main advantage of the sheep model is the cranial anatomy compatibility with the WIMAGINE diameter. Premedication, anesthesia, analgesia and neurosurgical procedures were realized identically for the two sheep. The implant was placed epidurally in a 50mm craniotomy and fixed with suture thread previous to skin closure. Control ECoG recordings throughout the surgery were obtained and a post-operative scanner was performed. ECoG recordings and visual evoked potentials (VEP) were performed on each vigil animal once a week. Every two months for each animal under general anesthesia, the tibial and median nerves of each limb were stimulated by peripheral nerve stimulator and two subdermal electrodes in order to record somatosensory evoked potential (SSEP). At the end of this chronical study, histological investigations were performed post-mortem to evaluate long term effect of the implantation. Results: WIMAGINE, here presented, allowed us to perform chronic ECoG recordings for a period of 140 days after implantation. No change in signal quality has been observed. Analysis of evolution in time of neurophysiological signals recordings (ECoG, VEP and SSEP) showed a stable signal in time. Electrodes recorded information from primary sensory motor cortex, showing in SSEP phase reversal, allowing identification of the central sulcus. VEP signals could not be extracted, due to the final implant location. WIMAGINE was easily removed after 10 months implantation: no adhesion was observed between the device and the tissues in at least one sheep. The cortex underlying the implanted grid showed no macroscopic signs of damage. Conclusion: Long term SSEP and signal recordings was obtained for the first time using novel WIMAGINE implant in an ovine model, providing further support for future clinical trials. [1] Mestais, C., Charvet, G., Sauter-Starace, F., ¨ and Benabid, A. L. (2015). WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications. Neural Systems and Rehabilitation Engineering, IEEE Transactions on, 23(1), 10-21.

Disclosures: **C. Cretallaz:** None. **M. Foerster:** None. **F. Sauter-starace:** None. **T. Costecalde:** None. **D. Ratel:** None. **C. Gaude:** None. **A. Lambert:** None. **G. Charvet:** None. **C. Mestais:** None. **N. Torres:** None.

Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.03/NN7

Topic: E.05. Brain-Machine Interface

Support: French National Research Agence (ANR CARNOT)

Fondation Philanthropique Edmond J. Safra

Fondation Motrice

Fondation Nanosciences

Fondation de l'Avenir

Title: WIMAGINE: An ECoG recording implant validated for clinical trials

Authors: *G. CHARVET, C. MESTAIS, F. SAUTER-STARACE, M. FOERSTER, A. LAMBERT, N. TORRES-MARTINEZ, T. COSTECALDE, C. CRETALLAZ, D. RATEL, A.-L. BENABID;
CEA/LETI/CLINATEC - MINATEC Campus, Grenoble, France

Abstract: The WIMAGINE® implant was developed to record and wirelessly transmit ECoG (ElectroCorticoGram) signals for long term clinical applications within the framework of a Brain Computer Interface project which goal is to provide the proof of concept that it is possible to control complex effectors, such as a 4-limb exoskeleton, thanks to brain activity monitoring and decoding, to open new opportunities to motor disabled. The implant consists of a 64 biocompatible electrodes array, a hermetic titanium housing including the electronic boards and biocompatible antennae for wireless transmission of ECoG data and remote power supply [1]. During the surgical procedure, the implant will be inserted into a 50 mm craniotomy so that the electrode array is in contact with the dura mater, and the implant recovered by the skin. The design of the WIMAGINE® implant required a tradeoff between the clinical operability with minimal invasiveness, the technical performances, the regulatory compliance to implantable medical device standards, and the manufacturability. Especially, the WIMAGINE® platform satisfies the Essential requirements of the European Medical Device Directives 93/42/EEC and 90/385/EEC. In particular, WIMAGINE® is compliant to the ISO 14708-1 standards (mechanical and electrical qualification tests), the ISO 60601-1 standards (electrical security and electromagnetic compatibility tests) and the ISO 10993 standards (long-term biocompatibility). Moreover the implants are manufactured according to an ISO13485 process. The long term recording performances of the WIMAGINE® implant has been assessed on sheep, over a period of 10 months after implantation. Ethical approval was obtained for all procedures from ComEth,

in accordance with directive 86/609/EEC for care of laboratory animals. Finally, the authorization was obtained in December 2015 from the French regulatory agencies to start a clinical research protocol named “BCI and tetraplegia” including the implantation of 2 WIMAGINE implants per patient [2]. Other neurological applications requiring chronic wireless ECoG recording such as epilepsy, functional substitution or post stroke rehabilitation can be addressed. [1] Mestais, C., Charvet, G., Sauter-Starace, F., ⋯ and Benabid, A. L. (2015). WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications. Neural Systems and Rehabilitation Engineering, IEEE Transactions on, 23(1), 10-21. [2] https://clinicaltrials.gov/show/NCT02550522

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Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.04/NN8

Topic: E.05. Brain-Machine Interface

Support: National Institute of Health, NIDCD, DC014279

Pew Charitable Trusts, Pew Biomedical Scholars Program

Title: Neural decoding of attentional selection in multi-speaker environments without access to separated sources

Authors: ***Z. CHEN**[1], J. O’SULLIVAN[1], S. SHETH[2], G. MCKANN[2], A. D. MEHTA[3,4], N. MESGARANI[1];
[1]Electrical Engin., Columbia Univ. Counseling and Psychological S, New York, NY; [2]Dept. of Neurolog. Surgery, The Neurolog. Inst., New York, NY; [3]Dept. of Neurosurg., Hofstra North Shore LIJ Sch. of Med., New York, NY; [4]Feinstein Inst. for Med. Res., New York, NY

Abstract: In a natural auditory environment, most people can easily attend to a particular speaker out of many. However, this task remains challenging for those suffering from peripheral and central auditory pathway disorders. Recently, it has been shown to be possible to decode which speaker a person is attending to, by monitoring their neural activity via both invasive and non-invasive electrophysiological recordings. This has led to an upsurge in attention-aware brain-computer interfaces (BCIs) that can control smart hearable devices capable of selectively

amplifying one speaker and suppressing all others in crowded environments. Current attention decoding algorithms require explicit access to the isolated sound sources in the environment, which is not realistic. Techniques such as beamforming have been used in an attempt to isolate each speaker. However, not only do these techniques fail when there is an inadequate spatial separation between speakers, their reliance on multiple microphones has drawbacks with regards to hardware complexity and user comfort. Here, we address these challenges by using state-of-the-art single microphone automatic speech separation algorithms in which deep neural network (DNN) models are used to separate the sound sources. The output of the speech separation algorithm can then be used to decode the attentional state of the listener, and subsequently, to amplify the attended source. Using invasive electrocorticography (ECoG) data, we successfully decoded the attention of a user in seconds. Subjects were presented with two stories: one read by a male, and the other by a female. There was no spatial separation between the two. Subjects were instructed to attend to one speaker, and to periodically switch their attention between the two. We used a method known as stimulus-reconstruction to estimate the stimulus spectrogram that the patient was attending to. By assessing the similarity of this reconstruction and the separated sound sources provided by the DNNs, we obtained decoding-accuracies in excess of 90% when using 4 seconds of test data. Switches in attention could be determined between 5 and 15 seconds after the transition point. The decoder output can then be used to amplify the attended source relative to the background to assist the listener. We show that the separation algorithm used produces a speech signal that is objectively cleaner (8.97 dB increase in Signal-to-Distortion-Ratio (SDR), 45.3% relative increase in Perceptual Evaluation of Speech Quality (PESQ) score). Such an improvement has shown to significantly improve the subjective experience for the hearing impaired.

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Poster

333. Neuroprosthetics: eCoG

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Topic: E.05. Brain-Machine Interface

Support: French National Research Agency (ANR-Carnot Institue)

Fondation Motrice

Fondation Nanosciences

Fondation de l'Avenir

Fondation Philanthropique Edmond J. Safra

Title: Brain Computer Interface human platform to control a 4-limb exoskeleton based on the ECoG-recording implant WIMAGINE®: toward clinical trials

Authors: *C. MESTAIS[1], G. CHARVET[1], F. SAUTER[1], N. ABROUG[2], S. COKGUNGOR[1], T. COSTECALDE[1], M. FOERSTER[1], E. LABYT[1], B. MORINIERE[2], D. RATEL[1], M.-C. SCHAEFFER[1], N. TORRES-MARTINEZ[1], A. VERNEY[2], I. VERGARA[1], A. YELISYEYEV[1], T. AKSENOVA[1], A.-L. BENABID[1];
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Abstract: Chronic clinical Brain Computer Interface (BCI) is the challenge of our project. Our goal is to bring the proof of concept that it will be feasible for a tetraplegic subject to control a 4-limb exoskeleton EMY [1] after training, thanks to its cortical brain electrical activity decoding. The ElectroCorticograms (ECoG) signals will be recorded on 64 electrodes, and wirelessly transmitted by the WIMAGINE® implant [2]. Innovative ECoG signal decoding algorithms [3, 4], will allow self-paced control of the exoskeleton by decoding the subject's brain activity. 2-limbs 3D trajectory control and binary switch for gait cycle are previsedas commands.
Before applying the BCI platform to patients, studies on control subjects were carried out non-invasively using MagnetoEncephaloGraphy (MEG). Their brain activity was recorded by a MEG acquisition system while they were executing real or imagined limbs movements. After the decoding of single upper limb movement trajectory, multi-limb decoding was explored. In particular, decoding of real 1D trajectories for wrists and switch for leg (2 wrists, 1 wrist+1 leg, 2 wrists+1 leg) were investigated. Time-frequency features were extracted from the 36 MEG sensors facing the subject's sensorimotor area using Continuous Wavelet Transforms. Simultaneous decoding was performed.
Certified qualification tests show that the BCI platform satisfies the Essential requirements of the European Medical Device Directives 93/42/CEE and 90/385/EEC.
Finally the clinical research protocol "BCI and tetraplegia" has now started: https://clinicaltrials.gov/ct2/show/NCT02550522?term=clinatec&rank=2

[1] Eliseyev, A., Mestais, C., Charvet, G., Sauter, F.,... and Benabid, AL. (2014, August). CLINATEC® BCI platform based on the ECoG-recording implant WIMAGINE® and the innovative signal-processing: Preclinical results. In Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE (pp. 1222-1225).
[2] Mestais, C., Charvet, G., Sauter-Starace, F., … and Benabid, A. L. (2015). WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications. Neural Systems and Rehabilitation Engineering, IEEE Transactions on, 23(1), 10-21.
[3] Eliseyev, A., & Aksenova, T. (2013). Recursive N-way partial least squares for brain-computer interface. PloS one, 8(7), e69962.
[4] Eliseyev, A., & Aksenova, T. (2014). Stable and artifact-resistant decoding of 3D hand trajectories from ECoG signals using the generalized additive model. Journal of neural engineering, 11(6), 066005.

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Poster

333. Neuroprosthetics: eCoG

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Program#/Poster#: 333.06/NN10

Topic: E.05. Brain-Machine Interface

Support: DARPA/ARO Contract # W911NF-14-2-0043

Title: Adaptive identification of high-dimensional brain network dynamics to track non-stationarity and plasticity

Authors: ***Y. YANG**[1], E. F. CHANG[2], M. M. SHANECHI[1];
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Abstract: Identification of brain network dynamics is of essential importance to uncover biomarkers for neurological disorders such as depression. Further, it plays a central role in developing brain-machine-interfaces (BMIs) for adaptive closed-loop stimulation therapies of various neurological disorders. Our prior work has developed a framework to identify time-invariant linear state-space models (LSSMs) to describe spontaneous neural population dynamics, and input-output neural dynamics in response to electrical stimulation. However, brain network activity can have non-stationary and time-varying dynamics, especially when the patient's brain is monitored for a long time, e.g., using electrocorticography (ECoG). Thus the prediction power of an offline estimated time-invariant LSSM can decrease due to the non-stationarities. Consequently, we need to develop adaptive identification methods to track non-stationary dynamics in real time, with the final goal of both facilitating better estimation of biomarkers, and devising online adaptive control algorithms to effectively treat a wide range of neurological disorders. Additionally, stimulation therapy might induce plasticity and changes in network dynamics and hence an adaptive algorithm can track and account for plasticity effects. Here, within our prior LSSM modeling framework, we develop an adaptive estimation algorithm to update LSSM parameters online. The adaptive identification algorithm (i) can consistently estimate a time-varying LSSM with the same state-space basis, thus ensuring consistent estimates of biomarkers over long time periods and (ii) has low computational burden, and hence can be efficiently implemented online for adaptive closed-loop control design. We first show using numerical analyses that the proposed adaptive identification algorithm can successfully

track various types of linear time-varying dynamics. We then validate our algorithm on ECoG signal collected from high-density electrodes implanted in epilepsy patients with a co-occurring diagnosis of depression. ECoG signals were continuously recorded over weeks. We show that a time-varying LSSM identified from the adaptive algorithm achieved better prediction of ECoG spectral powers compared with an average time-invariant LSSM estimated from traditional offline LSSM identification algorithms. In conclusion, the proposed adaptive system identification method can track possible non-stationary brain network dynamics. The results have important implications for more accurate estimation of biomarkers, and adaptive closed-loop stimulation therapy for a wide range of neurological disorders.

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Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.07/NN11

Topic: E.05. Brain-Machine Interface

Support: Craig H Neilsen Foundation postdoctoral fellowship

Doris Duke Charitable Foundation Clinical Scientist Development Award

Title: Cortical activity during object grasp represents movement and force differently

Authors: *R. D. FLINT, III[1], M. C. TATE[1,2], M. W. SLUTZKY[1,3,4,5];
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Abstract: Restoration of grasping is a major priority for paralyzed individuals to achieve increased independence. Brain-machine interfaces (BMIs) have the potential to grant this restoration. Previous studies have demonstrated that finger movement and grasp aperture can be decoded in real time from cortical activity in order to control a BMI. Also, motor cortical signals are capable of exceptionally stable movement representations over long time periods, which is promising for BMI development. However, some aspects of the cortical representation of grasp are still not well understood.

Manipulating objects with one's hands requires the ability to switch between movement and isometric force. To provide natural interactions between any hand prosthesis and objects in the environment, it will likely be necessary to decode both movement and intended level of force. Studies of motor control in both humans and nonhuman primates suggest that separate cortical

processes may be responsible for controlling movement and force. Any complete description of the hand motor system requires an understanding of how this transition is achieved so smoothly and efficiently in healthy individuals.
We investigated the cortical representation of movement and force in three human subjects, during awake craniotomy procedures that also included functional mapping, prior to tumor resection. We used high-density electrocorticography (ECoG) arrays positioned over primary and pre-motor cortices to record the subjects' neural activity. The subjects completed a finger pinching task that required both movement and isometric force application.
We decoded continuous isometric force levels, as well as the kinematics of the index finger, with each recording site on the ECoG array. The sites' prediction accuracies, taken as a whole, provided a layout or map that revealed the cortical representation of the behavior within the covered region. In addition, we investigated the spectral changes that occurred in each site on the array, as the subject's behavior changed from movement to force. For each subject, we found that grasp kinematic and kinetic variables were represented differently in both spatial and spectral domains. These results may offer insight into designing BMI-controlled devices that can successfully lift heavy objects without slip, or grip delicate objects without crushing them. The ability to manipulate objects would provide a vital improvement in the lives of people living with paralysis.

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Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

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Program#/Poster#: 333.08/NN12

Topic: E.05. Brain-Machine Interface

Support: UCSD ECE Medical Devices & Systems Initiative

Title: Using deep learning techniques to decode electrocorticographic signals.

Authors: *T. PAILLA[1], K. J. MILLER[2], V. GILJA[1];
[1]Electrical and Computer Engin., Univ. of California San Diego, La Jolla, CA; [2]Dept. of Neurosurg., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Brain-machine interfaces map the relationship between cortical activity and abstract states, such as behavioral intentions. Current systems use hand-picked features like spectral powers [Shenoy, P et al., IEEE TBME, 2008] and map them to the behavioral parameters using linear decoders like Kalman filters or classification models such as LDA or SVM. While the

combination of hand-designed features and linear models have successfully driven existing BMIs, their inability to model nonlinearities suggest that there is an opportunity to substantially improve system performance. Additionally, the drawback in using hand picked features to train a classifier or a decoding model is that these features might not be optimal for the given application. We address these shortcomings of existing approaches with a deep learning framework. The hierarchical structure of deep learning architectures have the capability to learn spatio-temporal patterns, complex nonlinearities and high-level abstractions in the data. By capturing informative features while simultaneously handling noise artifacts in a data set, they eliminate the need for a two-step procedure of hand-picking features and selecting an appropriate decoding algorithm for the application.
Convolutional Neural Networks (CNNs) model spatial patterns in the data with weight semantics that captures local connectivity. In this work, we show how CNNs can be designed using domain knowledge to decode electrocorticographic (ECoG) signals. We present our results for the finger flexion dataset [Miller et. al., PLoS Comput. Biol. 2012] where subjects with implanted electrodes grids performed a task that involved flexing a finger in response to a visual cue. For the best subject, a simple three layer CNN could classify movement and rest periods with an accuracy of 92.5±1.5% (mean +/- s.e.m, 10-fold cross validation) using a single electrode. In addition to comparing the classification performance of our model to the current state-of-the art decoder, we analyze how the network parameters learnt by our model relate to traditional spectral features. We also review how these models can be generalized across subjects.

Disclosures: **T. Pailla:** None. **K.J. Miller:** None. **V. Gilja:** None.

Poster

333. Neuroprosthetics: eCoG

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.09/NN13

Topic: E.05. Brain-Machine Interface

Title: Online coordinated user-computer based decoding of electrocorticographic signals for brain-machine interfaces

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Abstract: Communication prostheses, like the P300 speller, often utilize static, computer-paced user interaction and decoding schemes to maximize communication accuracy. This rigidity in timing may limit system usability, flexibility, and ultimately user adoption. If timing constraints

could be loosened while retaining communication decoding performance, user interaction could be improved towards free-pace control. However, moving towards free-paced control increases decoding complexity and may significantly reduce performance. Thus, we explore a continuum between computer-pacing and free-pacing, and the associated classification performance. For example by using a variable stop time for user interaction based on prediction confidence, we can potentially overcome limitations imposed by fixed timing systems. To simulate the impact of variable stopping time on brain-machine interface (BMI) performance, a finger flexion experiment utilizing electrocorticographic (ECoG) electrodes was selected (Miller et. al., PLoS Comput. Biol. 2012). Six subjects (four female and two male) being treated for intractable epilepsy were implanted with a subdural ECoG grid with coverage including the motor cortex. The behavioral task involved asking the patients to move a particular finger as indicated by a visual cue, which typically consisted of 2-5 flexions in sequence. A hierarchical classifier composed of two linear discriminant analysis classifier stages, a movement detector followed by a finger classifier, is trained on discrete flexions. The high gamma power (70-150 Hz) of the neural data corresponding to sequences of flexions was provided to the model with the assumption that each flexion in a group was independent. The number of flexions provided to the model was varied to explore the interaction between stopping time and performance. For the best subject, the classifier achieved a cross-validated finger classification accuracy of 90% when all three flexions were provided to the model. This is an improvement from an accuracy of 79%, achieved when only the first flexion was provided. Across all patients, an average increase in accuracy of 6% was observed. Using these preliminary results, we develop dynamical modeling techniques to explore the continuum between computer-pacing and free-pacing BMIs.

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Poster

333. Neuroprosthetics: eCoG

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Title: Decoding naturalistic kinematic states using electrocorticography in humans

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Abstract: The high temporal and spatial resolution provided by implanted neural recording techniques, like intracranial electrocorticography (ECoG), capture neural activity that can be decoded into control signals for brain-machine interfaces (BMIs) [Wang, W. et al, PLoS, 2013; Bundy, D. et al, JNE, 2016]. A common approach for training BMIs is to learn decoding features from highly restricted and repetitive experimental tasks to accommodate practical data collection constraints and reduce behavioral variability. Generalizing decoders for naturalistic settings (e.g. [Parvizi, J. et al, Nat. Comm., 2013]) remains a relatively unexplored area for BMI applications. In this work, we present strategies for analyzing the neural correlates of naturalistic behavior by recording continuous multi-modal data from an epilepsy surgery patient undergoing ECoG in a hospital setting. Volitional movements synchronized to clinical recordings were extracted from these data. Computer vision based tracking techniques were applied to the video data to estimate the kinematics of patient hand movements across two active hours in the afternoon. As a preliminary analysis, the kinematics were split into two distinct states - rest and fast right hand movements. Machine learning techniques were applied to assess if these discrete labels can be predicted from the corresponding neural data. Specifically, we trained a linear discriminant analysis (LDA) classifier on binned high-γ power (70-110 Hz) from 22 electrodes across the frontal, temporal, and sensory-motor regions of left hemisphere to distinguish between movement and rest states for contralateral arm. Cross-validation was performed using 10 stratified folds, drawing from thousands of kinematic labels. Preliminary results demonstrated balanced binary LDA classifier performance at 0.62 ± 0.05 (mean $\pm$ std), which was significantly above chance. Movement and rest states were correctly classified with scores of 0.57 and 0.67, respectively. Our findings motivate more in-depth approaches for naturalistic neural decoding, including more detailed descriptions of behavior kinematics and nuanced modeling of neural activity.

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Poster

333. Neuroprosthetics: eCoG

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Program#/Poster#: 333.11/OO1

Topic: E.05. Brain-Machine Interface

Support: ImPACT Program of Council for Science, Technology and Innovation (Cabinet Office, Government of Japan)

JSPS KAKENHI Grant Number 15H03049

Title: Adaptation of neural population activity in rat cortex connected to ECoG-based BMI system

Authors: *M. YOKOTA[1], Y. KUNIMURA[1], T. SUZUKI[2];
[1]Osaka Univ., Osaka, Japan; [2]CiNet, Natl. Inst. of Information and Communications Technol., Suita-shi, Japan

Abstract: The brain-machine interface (BMI) has promising medical applications such as control of prostheses by thought. In a clinical application, electrocorticogram (ECoG) has attracted attention as a potential input signal for BMI because of low invasiveness and the large amount of information contained in ECoG waveforms (Yanagisawa et al., 2011). However, once the nervous system is connected to a BMI system, it will gradually adapt to the external device. Studies have reported regarding the manner in which multiunit activities of rats change in a BMI environment (Koralek et al. 2012). In this study, we investigated the manner in which neural signals, such as single-unit activity, multiunit activity, local field potentials, and ECoG waveforms, adapt to a prolonged BMI environment and examined the neuroplastic changes in rats during adaptation. A self-made flexible 32-channel electrode array composed of Parylene-C was implanted on the surface of the left motor cortex. We focused on the power of a specific band (80−120 Hz) from one channel. When the rat was able to maintain band power greater than a set threshold for a certain period, it obtained a small amount of water. We tracked the neuroplastic changes of ECoG while the rats adapted to the BMI system and monitored head movements using a 3-axis accelerometer to control motion artifacts. Rats were able to freely obtain water rewards in approximately 3-5 days. The changes in ECoG spread from the original target channel at the center over a wide range of the cortex.

Disclosures: **M. Yokota:** None. **Y. Kunimura:** None. **T. Suzuki:** None.

Poster

333. Neuroprosthetics: eCoG

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 333.12/OO2

Topic: E.05. Brain-Machine Interface

Title: Behavior classification using multi-site lfp and ecog signals

Authors: *A. O. HEBB[1], H. GOLSHAN[2], M. MAHOOR[2], J. NEDRUD[3], S. HANRAHAN[3]; [1]Colorado Brain and Spine Inst., Englewood, CO; [2]Univ. of Denver, Denver, CO; [3]Colorado Neurolog. Inst., Englewood, CO

Abstract: Deep Brain Stimulation (DBS) is an effective therapy that alleviates the motor signs of Parkinson's disease (PD). Existing DBS is open loop, providing a time invariant stimulation pulse train that may generate cognitive, speech, and balance side effects. A closed loop DBS system that utilizes appropriate physiological control variables may improve therapeutic results, reduce stimulation side effects, and extend battery life of pulse generators. Furthermore, by customizing DBS to a patient's behavioral goal, side effects of stimulation may arise only when they are non-detrimental to the patient's current goals. Therefore, classification of human behavior using physiological signals is an important step in the design of the next generation of closed-loop DBS systems. Ten subjects who were undergoing DBS implantation were recruited for the study. DBS leads were used to record bilateral STN-LFP activity and an electrocorticography (ECoG) strip was used to record field potentials over left prefrontal cortex. Subjects were cued to perform voluntary behaviors including left and right hand movement, left and right arm movement, mouth movement, and speech. Two types of algorithms were used to classify the subjects behavior, support vector machine (SVM) using linear, polynomial, and RBF kernels as well as lp-norm multiple kernel learning (MKL). Behavioral classification was performed using only LFP channels, only ECoG channels, and both LFP and ECoG channels. Features were extracted from the time-frequency representation of the signals. Phase locking values (PLV) between ECoG and LFP channels were calculated to determine connectivity between sites and aid in feature selection. Classification performance improved when mulitsite signals were used with either SVM or MKL algorithms. Our experiments further show that the lp-norm MKL outperforms single kernel SVM-based classifiers in classifying behavioral tasks.

Disclosures: **A.O. Hebb:** None. **H. Golshan:** None. **M. Mahoor:** None. **J. Nedrud:** None. **S. Hanrahan:** None.

Poster

333. Neuroprosthetics: eCoG

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Topic: E.05. Brain-Machine Interface

Support: T32 NINDS Fellowship

NSF Career Award

NIH R01 NS096008

Title: Electrocorticographic features of therapeutic deep brain stimulation in Tourette Syndrome.

Authors: *J. B. SHUTE[1], E. OPRI[1], R. MOLINA[2], J. ROSSI[3], K. FOOTE[4], M. OKUN[5], A. GUNDUZ[2];
[1]BioMedical Engin., [2]Electrical Engin., [3]Neurosci., [4]Neurosurg., [5]Neurol., UF, Gainesville, FL

Abstract: Deep brain stimulation (DBS) is an invasive neuromodulatory therapy intended for the treatment of movement and psychiatric disorders. Tourette syndrome (TS) is a neuropsychiatric movement disorder characterized by motor and vocal tics. DBS is under investigation as a therapy for TS and early studies have been promising, but several hurdles lie in the path of developing successful treatment plans. One of the main difficulties associated with DBS is identification of ideal stimulation parameters (voltage, frequency, pulse width, and electrode contact). To address this issue, we sought to identify electrophysiological signatures of therapeutic deep brain stimulation in an effort to automate deep brain stimulation programming. Two patients with Tourette syndrome were implanted with bilateral DBS electrodes in the centromedian thalamus (Cm-Pf) and bilateral electrocorticographic strips over the primary motor cortex (M1). Patients were followed for 6 months and data was collected at each month. At each visit, a trained programmer identified high frequency “therapeutic” deep brain stimulation settings. A set of power matched low frequency “nontherapeutic” settings were also identified. Stimulation was delivered to thalamic leads and electrophysiological data was collected from M1. Phase amplitude coupling (PAC) (Tort, 2011) from M1 electrodes was calculated for each trial. Increases in PAC between alpha phase and gamma amplitude were observed within M1 contacts during and following therapeutic stimulation in both patients. The same PAC pattern was also observed when no stimulation was applied, but it was significantly lower than during ($p < .02$) and following ($p < .03$) therapeutic stimulation. The administration of charge balanced nontherapeutic stimulation did not significantly change M1 PAC when compared to no stimulation. No significant changes in power spectral density (i.e., amplitude modulation) between the conditions was observed. Herein, we hypothesized and demonstrated that DBS induces changes in phase amplitude coupling in downstream cortical targets and that these

findings could be a marker of effectiveness. We observed a gradient in M1 PAC as a function of stimulation frequency and we also observed that charge-balanced non-therapeutic settings did not elicit this signature pattern. The close association between cortical signatures and clinically defined therapeutic settings may allow for development of a mapping procedure that could accelerate the identification of optimal stimulation settings. Additional tests to evaluate this signature for larger sets of therapeutic and nontherapeutic stimulation settings are underway.

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Poster

333. Neuroprosthetics: eCoG

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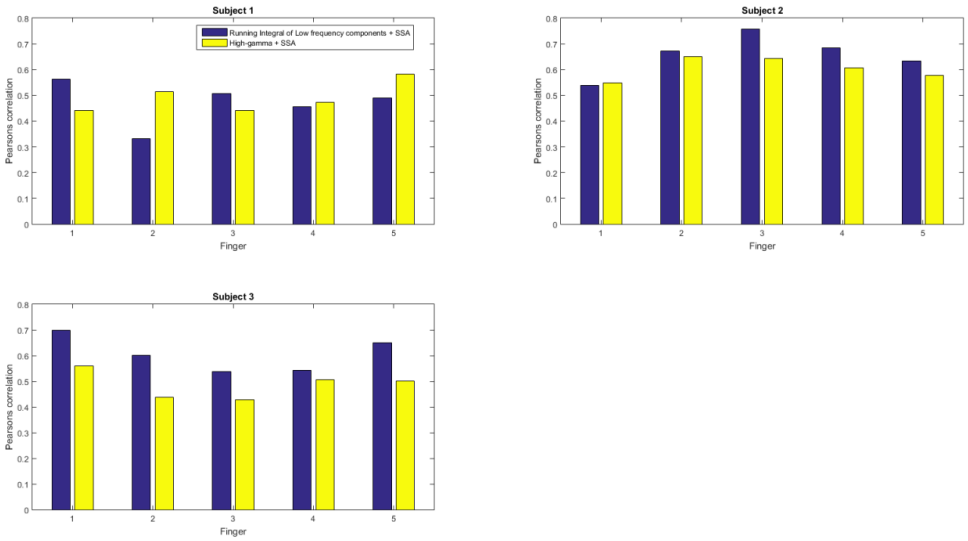
Program#/Poster#: 333.14/OO4

Topic: E.05. Brain-Machine Interface

Title: Using low frequency components to predict speed and position of fingers during execution of a motor task.

Authors: *J. F. DELGADO SAA;
Univ. Del Norte, Barranquilla, Colombia

Abstract: It is well known that cortical activity in the frequency band of 70 Hz to 200 Hz, known as high-gamma, correlates with the dynamics executed activity. In particular, in motor tasks, the envelope of high-gamma has been used for determining the position of limbs in two and three dimensions and prediction of repetitive finger flexion, among other many other applications. The literature shows that in motor tasks, low frequency components do not provide as much information as high-gamma for prediction of classification of the movements. In this work, using Electrocorticographic recordings of 3 patients while repetitive finger flexion are performed, we show that low frequency components (<8Hz) correlate with the speed of the flexion. Having an estimation of the speed of the movement, we show that the time running integral of these low frequency components predict the position of the finger. In order to compare the prediction power of the low frequency components and high-gamma, we trained for each case, a model based on simultaneous sparse approximation (SSA). The SSA method was selected because imposing sparsity on the model prevents over-fitting. The results show that when the actual movement of the fingers, measured with a data-glove, is compared to the prediction provided by both models, the model that uses the time running integral of the low frequency components obtains an averaged correlation value higher that the model that uses high-gamma.



Disclosures: **J.F. Delgado Saa:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 334.01/OO5

Topic: E.05. Brain-Machine Interface

Title: Effects of speed tuning on trajectory decoding

Authors: ***Y. INOUE**[1,2], X. ZHOU[3], A. B. SCHWARTZ[2];
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Abstract: Movement speed acts as a gain factor on the cosine tuning of motor cortical firing rate to movement direction. Failure to account for this effect can lead to decoding problems when using firing rates from a population of cells to predict movement trajectory. This may be especially problematic when the sample of recorded cells has preferred directions that are not distributed uniformly. To better characterize this problem, we used a set of coefficients to fit the following equation 1: $FR=b_0+b_xV_x+b_yV_y+b_sS$; where Fr is firing rate, V_x and V_y are the x and y components of arm velocity and S is the speed of the arm. Empirical data from a 2D center-out task were selected from a prototype neuron and used to fit the coefficients of the equation. A set

of firing rates were then calculated for a population of simulated neurons by choosing b_x and b_y to produce different preferred directions. The average speed profile from the empirical data was used in the simulations.
Decoding was performed using the population vector algorithm. Each unit's contribution to the population vector was calculated using the simulated firing rates from Eq. 1 in the conventional cosine tuning model: $FR=b_{r0}+b_{rx}D_x+b_{ry}D_y$, where D_x and D_y are the x and y components of target direction. b_{r0}, b_{rx}, and b_{ry} were found through regression. Each unit's contribution was a vector in the direction b_{rx}, b_{ry} with a magnitude, $M= (FR-b_{r0})/mag$, where mag is the magnitude of the vector $[b_{rx}, b_{ry}]$.
Two simulated populations were created. The first was constructed by choosing b_x and b_y to be uniformly distributed. The second population was non-uniform, b_x and b_y were chosen only from one half of the unit circle. Population vectors were constructed from each population to each target using the empirical speed profiles and Eq. 1 for firing rates and the conventional cosine model for each contribution. We measured the angle error, time length, trajectory length and population vector magnitude for the 2 populations. The trajectories for all targets were fairly straight in uniform condition. Across targets, duration, trajectory length, and angle error were not significantly different. The population vector length was highly correlated to speed. In contrast, for the non-uniform condition, the initial portions of the trajectories of all targets were skewed by the over-represented preferred directions in the sample. Compared to the uniform condition, population vector magnitudes were less related to speed.
A failure to account for speed tuning can lead to significant decoding distortion of both speed and direction when the sampled population has a non-uniform directional distribution.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Title: Adaptation of motor planning activity in monkey motor, premotor and parietal cortices during bci control of 3d reaches

Authors: *E. FERREA[1], P. MOREL[1], M. BERGER[1], A. GAIL[1,2,3];
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Abstract: Brain Computer Interfaces (BCIs) have been used as experimental paradigms probing sensorimotor adaptation in absence of haptic feedback by controlling the relationship among end-effector and neuronal activation. Since proficient BCI control relies on the adaptation of neural networks, previous studies have pointed-out the importance of designing decoders that take into account how the brain adapts during on-line control. However, such learning process might not be limited exclusively to the on-line control phase or to neurons that directly drive the decoder. To probe the structural specificity of neural sensorimotor adaptation, we here test whether BCI learning acts locally or modifies a distributed frontoparietal network that is not directly controlling the decoder. For functional specificity, we test whether adaptation occurs not only during movement control, but also during planning.
Neuronal activity was recorded from a macaque monkey implanted with 2x32 floating multi-electrode arrays (FMAs), in each of three cortical areas M1, PMd, and PRR. A Kalman-Filter decoder was trained by regressing neuronal firing rates from a subset of M1 single and multi-unit with hand velocity (50 ms steps) while the monkey manually performed a memory-guided center-out reach task in a 3D virtual-reality environment with 8 targets arranged on the vertices of a 12 cm cube. During the following BCI-control task the animal had to control the cursor directly via the decoder. We compared the directional modulation depth (MD) in early (first 50 trials, initial learning phase) vs late trials (last 80 trials, steady-state performance) of the neurons not used by the decoder during BCI control. MD was defined as the difference between the average firing rates for the preferred vs non-preferred target, normalized by the MD under manual control separately in the planning and movement periods. For each period, only units that were tuned under manual or BCI control were included in the analysis. We found that BCI learning decreased the modulation depth of non-controlling neurons not only in M1, where the controlling neurons were located, but also in the remote PRR area, where none of the neurons contributed to the decoder. Moreover, neural adaptation occurred not only during BCI movement control but also affected movement planning activities. Our results indicate that BCI learning might include an explicit learning strategy or modified motor-control policy reflected in modified motor planning and that taking into account planning activity could be beneficial for the design of cognitive prosthetics decoders, particularly in patients with major deficits in motor control.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Title: Coordinate frames for encoding reach movements by single neurons in the human posterior parietal cortex.

Authors: *M. JAFARI[1], T. AFLALO[1], N. POURATIAN[2], E. ROSARIO[3], D. OUELLETTE[3], K. PEJSA[1], R. ANDERSEN[1];
[1]Caltech, Pasadena, CA; [2]Univ. of California Los Angeles, Los Angeles, CA; [3]Casa Colina Centers for Rehabil., Pomona, CA

Abstract: We have shown that motor intentions can be decoded from human posterior parietal cortex (PPC) for brain-machine interfaces. However, we have yet to determine the coordinate frames in which these intentions are encoded. Are intentions coded with respect to the subject's gaze, current imagined limb position, intended target location, or a combination of these. Or, is it encoded in world or body coordinates in which case gaze and imagined limb position have no effect. Knowing the answers to these questions has the potential to improve implementations of closed-loop neural prosthetic applications by ensuring that neural decoding algorithms are tailored to the way intention related information is actually encoded in the neural population. Here we explored the reference frames of motor intentions in neural populations recorded in PPC of a tetraplegic subject participating in a human clinical study. We then examined the impact of the cortical regions' reference frames on population decoding of the subject's intentions. We used a delayed-reach paradigm in which we systematically varied hand, eye and target position in order to determine the reference frame of reach intentions during planning and execution epochs. A trial begins by instructing the gaze-fixation point and initial hand position at one of four possible locations. As the subject is paralyzed, we asked the subject to imagine her hand at the instructed position. Following a delay period of 2.75-3.25 seconds, the target was presented at one of four possible locations. After a second delay, a go cue instructed the subject to imagine a reach to the cued target. We used gradient and SVD analysis (Pesaran et al. 2006) on the planning and go epochs to determine the relationship between neural firing and behavioral

variables. This design allowed us to characterize whether the target of a reach is coded relative to the direction of gaze (gaze-centered), the position of the hand (hand-centered), the position of the hand relative to the eye (relative), or is body/world centered. We recorded from 421 neurons across 5 sessions. During the execution of the imagined movement, the largest proportion (50%) of tuned neurons were found to code the intended target relative to the imagined starting position of the hand and thus behaved as though specifying the intended direction of movement although we found evidence for more mixed representations as well, especially during the motor planning phase.
Pesaran et al. 2006, Neuron 51, 125-34.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Program#/Poster#: 334.04/OO8

Topic: E.05. Brain-Machine Interface

Support: MIUR

FIRB 2013,RBFR132BKP

Title: Decoding objects and grips from the medial posterior parietal cortex of the macaque

Authors: M. FILIPPINI[1], R. BREVEGLIERI[1], E. CHINELLATO[2], A. BOSCO[1], *P. FATTORI[1];
[1]Univ. of Bologna, Bologna, Italy; [2]Middlesex Univ. London, London, United Kingdom

Abstract: Nowadays, neuro-decoders are widely used by researchers to analyze and model neural data with the aim to understand how our brain dynamically processes the neural information or to control neuro-prosthetic devices like robotic hands. Here we investigated whether objects and grips can be reliably decoded offline using relatively small numbers of neurons from area V6A. This area is a grasping area of the medial posterior parietal cortex (Fattori et al., 2010 J Neurosci, 30(1):342-9). Two Macaca fascicularis were trained to perform an instructed-delay reach-to-grasp task in dark and in light. Different objects were grasped in a constant spatial location: a ball, a handle, a ring, a plate, and a cylinder-in-groove. Population neural activity (N=19 neurons), approximated as mean firing rates, were extracted from time intervals corresponding to the vision of the objects, the delay before the movement, and the grasp

execution, and used to train a simple Bayes classifier to decode objects and grip types. Decoder recognition rates were well over the chance level for all the epochs tested (62 to 96%). Futhermore, we detected a different decoding accuracy related to task condition, whether the grasping action was accomplished in dark or in light. Noteworthy is the ability of our classifier to discriminate grasp types in the delay before reach-to-grasp initiation, well in advance with respect to movement onset, with a timing potentially suitable to send signals to the computer interfaces well before the movement needs to be articulated. Futhermore, to understand how visual and motor codes change during the time course of the task, from object vision to grasping execution, we performed a generalization analysis where codes learned during object vision and movement intervals were applied to the delay before movement execution. Results demonstrate how a 'visual' code is converted in a 'motor' code in the first part of the delay, and with different time course according to the presence or to the absence of visual information in the task. Present results show that it is possible to reliably decode objects and grips off line form a relatively small number of V6A neurons. This suggests that, in addition to dorsolateral brain areas traditionally decoded for grasping, also the neural signals from the dorsomedial visual pathway can be a good substrate to feed the neural prostheses for object grasping. This adds a novel brain area in the panorama of the cortical sites targeted for brain-computer interfaces able to perform correct prehensile actions.

Disclosures: **M. Filippini:** None. **R. Breveglieri:** None. **E. Chinellato:** None. **A. Bosco:** None. **P. Fattori:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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UCSF Wheeler Center for the Neurobiology of Addiction

CDMRP W81XWH-14-1-0510

PVA 2978

Title: Decoding for brain-machine interfaces with a new, unsupervised-learning algorithm

Authors: ***J. G. MAKIN**, J. E. O'DOHERTY, P. N. SABES;
Ctr. for Integrative Neurosci., Univ. of California, San Francisco, San Francisco, CA

Abstract: Neuro-prosthetic brain-machine interfaces (BMIs) use neurological recording devices and a set of "decoding" algorithms to transform brain activity directly into real-time control of a machine. Decoding algorithms for continuous tasks (e.g., movement of a robotic arm or a cursor) generally treat neural data (e.g., vectors of spike counts in small temporal windows) as noisy observations of an ongoing dynamical process, corresponding to the dynamical state of the object to be controlled. That is, they are _dynamical filters_, typically some variant on Kalman's celebrated algorithm (KF). The KF, however, although fairly robust in practice, is optimal only when the relationships between variables are linear and the noise is Gaussian, conditions sure to be violated in most BMIs.
To overcome these limitations, we apply a new filter, the "recurrent exponential family harmonium" [rEFH; Makin et alia 2015], that explicitly takes into account the Poisson statistics of spike counts, and allows for arbitrary nonlinearities in the maps between dynamical states, and between those states and the neural response. The parameters of the rEFH are acquired through unsupervised learning, in this case on spike counts recorded with Utah arrays from the primary motor (M1) and primary somatosensory (S1) cortices of a rhesus macaque. A static linear map between the latent states of the rEFH and the kinematic state is subsequently acquired with supervised learning (regression).
We compare offline reconstruction of reaches in the plane for the rEFH; the standard KF; and a recently introduced variant ("neural dynamical filter," NDF) whose parameters are also acquired with unsupervised learning. We find that the rEFH improves velocity decoding by an average of 20% over its nearest rival.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Program#/Poster#: 334.06/OO10

Topic: E.05. Brain-Machine Interface

Support: NSF GRFP

Title: Closed-loop pairing of motor cortex activity and phasic VTA activation reinforces specific spatiotemporal activity patterns

Authors: *V. R. ATHALYE[1,2], F. J. SANTOS[1], J. M. CARMENA[2], R. M. COSTA[1];
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Abstract: Over novel skill learning, animals initially produce large trial-to-trial variability in both movement and motor control neural activity which decreases with training, resulting in the consolidation of particular movement and neural activity patterns. The neural circuit mechanisms underlying neural spatiotemporal pattern consolidation are not well understood.
Phasic dopaminergic activation can substitute for natural reinforcements in promoting behavioral selection, and dopamine is critical for the generation of novel actions and for modulation of neural variability within corticostriatal circuits. Thus, we asked: is pairing arbitrary task-relevant neural activity patterns in primary motor cortex (M1) with phasic activation of dopaminergic cells in the Ventral Tegmental Area (VTA) sufficient to reinforce the task-relevant patterns?
We combined a mouse operant Brain-Machine Interface (BMI) paradigm (Koralek*,Jin*,et al 2012) with closed-loop optogenetic excitation of VTA. During training, the BMI algorithm translated the activity of two arbitrarily-defined ensembles of 2-4 units from M1 into an auditory tone. By decreasing firing rate modulation of the first ensemble while concomitantly increasing the modulation in the second ensemble, mice would drive the audio feedback toward low frequencies. (The reverse modulation would drive high audio frequencies.) When mice produced the rare task-relevant activity pattern to produce the target audio tone, they received a train of blue laser pulses, resulting in the stimulation of dopaminergic cells in the VTA contralateral to the recording site.
We trained TH-Cre mice expressing channelrhodopsin-2 in VTA (ChR2 group, N=10) and YFP in VTA (YFP group, N=6) for 4 days consisting of 20 min daily sessions. Over training, we found that the reinforced audio tone was produced significantly more often in ChR2 animals and does not change in YFP animals. Further, we found that the probability distribution over audio tones shifts towards the rewarded tone in the ChR2 animals but does not change in YFP. Finally, we found that increases in co-variability of the task-relevant ensembles predict improvements in BMI performance for the ChR2 animals but not YFP.
These results show that the closed-loop pairing of motor cortex activity with phasic dopaminergic VTA activation leads to the reinforcement of specific spatiotemporal activity patterns, suggesting a neural circuit mechanism for pattern consolidation during natural skill learning.

Disclosures: **V.R. Athalye:** None. **F.J. Santos:** None. **J.M. Carmena:** None. **R.M. Costa:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Program#/Poster#: 334.07/OO11

Topic: E.05. Brain-Machine Interface

Title: Does the brain learns to control robotic limbs using sparse representations?

Authors: C. KONNARIS[1], F. MEHRABAN POUR BEHBAHANI[1], *A. A. FAISAL[2];
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Abstract: A central question in motor control is to understand how the brain controls the multiple degrees of freedom of our body. We use learning to control an robotic extra limb to test for our brain's motor representations. Previous approaches to answer this question fall broadly into two categories: (1) optimisation of a cost function and (2) dimensionality reduction. We offer here an analysis of natural human hand movement data combining the two ideas: 1. optimisation of a cost function on neural activity to encode movements and 2. dimensionality reduction in the kinematic space so that motor control takes place in a low-dimensional subspace of possible joint configurations, through the notion of sparse coding of movement. The spatial structure of movements - and evidence for low-dimensional control manifolds - has typically been investigated using global latent variable models (PCA, Santello 1997, Ingram at al 2008, PPCA, Belic & Faisal, 2015). We presented a new algorithm for a sparse dictionary approach that extract time-scale and amplitude invariant patters from kinematic time-series of arbitrary dimensionality (Thomik, Fenske & Faisal, 2015). Three aspects set our approach apart from previous work: (1) data efficiency, (2) consideration of spatiotemporal instead of only spatial structure and (3) automatic estimate of the latent dimensionality. Our method can be understood as both an extension and an improvement on the techniques promoted in sensory neurosciences. The hypothesis of sparse encoding of motor output has been raised as a possibility but not tested against other representations. We hypothesise that sparse encoding of movement may account for observed grasp-type specificity in neurons in F5/PMv areas and may help understand the neural mechanisms of learning by observation. In order to test our hypothesis, we have designed a robotic teleoperation experiment that involves human subjects performing bimanual tasks where one hand is teleoperating a dexterous robotic hand using a CyberGlove (which tracks each joint of the human hand). Using this robotic platform we can directly compare the learning performance of subjects under three conditions for mapping the subjects' finger movements to drive the artificial fingers: 1) direct mapping 2) PCA-based control 3) Sparse motor representation control. We hypothesise that subjects teleoperating the robotic hand through a control representation that matches their own cortical hand representation will experience the same learning rates as when directly operating the robotic hand. Conversely, if the control representations are "alien" to their cortical representations, than learning times will be longer.

Disclosures: C. Konnaris: None. **F. Mehraban Pour Behbahani:** None. **A.A. Faisal:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Program#/Poster#: 334.08/OO12

Topic: E.05. Brain-Machine Interface

Support: ARC DE140101375

Title: Computational capacity as a function of network size

Authors: *C. KERR[1], S. DURA-BERNAL[3], R. J. MENZIES[2], C. MCLAUCHLAN[2], S. J. VAN ALBADA[4], D. J. KEDZIORA[2], S. NEYMOTIN[3], W. W. LYTTON[3];
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Abstract: There is strong circumstantial evidence that organisms with larger nervous systems are capable of performing more complex computational tasks. Yet few studies have investigated this effect directly, instead typically treating network size as a fixed property of a simulation while exploring the effects of other parameters. Recent studies have found that network performance does increase modestly with network size, but larger networks also required longer training times to achieve a given performance. Here, we address the relationship between network size and computational capacity by using a spiking model of motor cortex to direct a virtual arm towards a target. The reaching task was performed by a two-joint virtual arm controlled by four muscles. These muscles were controlled by a neural model that consisted of Izhikevich neurons in three populations: a proprioceptive population, receiving input from the current arm position; a motor population, used to drive the arm muscles; and a sensory population, serving as the link between the proprioceptive and motor populations. The model was trained to reach the target using exploratory movements coupled with reinforcement learning and spike-timing dependent plasticity (STDP). The model was implemented using NEURON. A major challenge in scaling network size is that not all properties of the network can be held constant. While first-order properties (such as average firing rate) can be maintained, there are limitations in preserving second- and higher-order statistical properties. Thus, we explored different ways of scaling the connectivity of the network, including (a) preserving connection probability, scaling connection weight to be inversely proportional to model size, and increasing the variance of the external drive; and (b) reducing connection probability to preserve average node degree and leaving other parameters unchanged. In addition, we explored scaling each of the neuronal population groups versus only scaling the sensory (processing) population group. Large differences were observed in network dynamics and statistics based on different scaling choices. However, the relationship between network size and task performance was significant only for certain choices of model parameters. Task performance is highly sensitive to the

network's metaparameters, such as STDP learning rates. These must be optimized specifically for different network sizes; otherwise, differences in suitability of these parameters overwhelm the advantages of larger networks. Thus, while network size does affect computational capacity, the relationship is dependent on the manner in which the scaling is implemented.

Disclosures: **C. Kerr:** None. **S. Dura-Bernal:** None. **R.J. Menzies:** None. **C. McLauchlan:** None. **S.J. van Albada:** None. **D.J. Kedziora:** None. **S. Neymotin:** None. **W.W. Lytton:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Topic: E.05. Brain-Machine Interface

Support: NSF CAREER Award CCF-1453868

Cal-BRAIN Award 62636622

Title: A new modeling framework for multiscale neural activity underlying behavior

Authors: *H. ABBASPOURAZAD, M. SHANECHI;
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Abstract: Given the significant advances in neurotechnology in recent years, we can now measure the brain using simultaneous multiscale neural recordings. For the same behavior, we can record multiple scales of neural observations such as spiking activity, local field potentials (LFP), and electrocorticography (ECoG). Prior work has extensively studied the modeling and decoding of brain states given a single scale of these observations. For example, motor brain-machine interfaces (BMI) have decoded movement intention using either spikes or LFP, and in some instances ECoG. The ability to record simultaneous multiscale activity introduces the new challenge of modeling and decoding neural activity at multiple scales to maximize the amount of extracted information. This could lead to more accurate BMIs, for example for control of neuroprosthetics. To enable multiscale decoding, we first need to develop a modeling framework that describes how the brain state is simultaneously represented across scales. This is a difficult problem as the statistical profiles and the time-scales of multiscale observations could be significantly different. Here we develop a new framework for modeling of multiscale neural activity consisting of spikes, LFP, and ECoG. Our framework constructs a multiscale state-space model and fits this model to neural observations. The state-space model consists of a state model that describes the dynamics of the hidden brain state and a multiscale observation model

consisting of a mix of linear Gaussian and nonlinear point process likelihood functions. To fit this model, we need to develop a new system identification approach that learns the parameters of the nonlinear multiscale observation model when the underlying brain states are hidden. We develop a novel multiscale system identification algorithm using expectation-maximization (EM). Our novel EM algorithm finds the maximum likelihood estimate of the model parameters. We first derive all the mathematical equations for this algorithm and show that they enable accurate estimation of the true parameters. We then perform extensive analyses to demonstrate the goodness of fit and robustness of our modeling framework for describing the encoding of behavior in neural activity. The proposed multiscale modeling framework has significant implications for the exploration of the fundamental dependencies across multiscale representations of brain states, and for the development of brain-machine interfaces.

Disclosures: H. Abbaspourazad: None. **M. Shanechi:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Topic: E.05. Brain-Machine Interface

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Title: Adaptive multiscale brain machine interface decoders

Authors: *H.-L. HSIEH, M. SHANECHI;
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Abstract: Brain-machine interfaces (BMI) use a single scale of neural activity, such as spiking activity, local field potentials (LFP), or electrocorticography (ECoG) to decode the brain state, such as intended movement kinematics. However, recording multiple scales of neural activity simultaneously has become possible given the advances in neurotechnology. Such simultaneous recording provides the opportunity to decode the hidden brain state using multiple scales of neural activity to improve decoding accuracy. But multiscale decoding is challenging for three reasons. First, the inherent statistical characteristics of spikes and LFP/ECoG are different, with spikes being discrete and LFP/ECoG being continuous signal modalities. Second, the time-scales of these signals are different; while spikes have a millisecond time-scale, the time-scale of LFP/ECoG are much slower and on the order of tens of milliseconds. Third, given the

demonstrated benefit of learning the decoder models in closed-loop BMI operation, the parameters of the multiscale statistical models need to be learned concurrently and adaptively in real time. Here we develop a multiscale adaptive BMI decoder that addresses all these challenges. We use a linear state-space model to describe the movement dynamics as in prior work. However, to describe the various neural signal modalities, we build a novel multiscale observation model composed of both linear Gaussian and nonlinear point process likelihood functions. To learn the parameters of the multiscale state-space model, we have recently devised a new expectation-maximization algorithm in open loop. To further enable parameter learning in closed-loop, here we develop a new multiscale adaptive decoder that both estimates the movement kinematics and adapts the multiscale model parameters in real time. Our multiscale adaptive decoder extracts information from ECoG/LFP and spikes simultaneously, while operating at the fast time-scale of the spikes. The decoder specializes to an adaptive Kalman filter (KF) when no spikes are recorded, and to an adaptive point process filter (PPF) when no ECoG/LFP are recorded. It thus provides a unified framework for BMI decoding. Using simulations, we show that the decoder can track the true multiscale model parameters in real time. Also, comparing to PPF decoding of spikes alone or KF decoding of LFP/ECoG alone, the multiscale decoder significantly improves the error and accuracy performance while running at the fast millisecond time-scale of the spikes. This new adaptive multiscale decoding framework has the potential to improve BMI control by combining information from multiple scales of neural activity.

Disclosures: H. Hsieh: None. **M. Shanechi:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Title: Increasing brain-machine interface performance by using discrete state selection with hidden Markov models

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Abstract: Communication brain-machine interfaces (BMIs) aim to restore efficient communication to those with neurological injury or disease by decoding neural activity into control signals. These control signals can be analog (e.g., the velocity of a computer mouse) and discrete (e.g., clicking an icon). A major component of a communication BMI is conveying the selection of a target, such as a key on a virtual keyboard. To date, the highest-performance communication BMIs select a target by hovering over it with an analog cursor for a set amount of time (e.g., Nuyujukian et al., IEEE TBME 2015). Although discrete state selection algorithms based on linear discriminant analysis have demonstrated the ability to decode a discrete "click" state to select the target (e.g., Kim et al., IEEE TNSRE 2011), these algorithms do not perform as well as simply hovering over the target for a set amount of time (Gilja*, Pandarinath*, et al., Nature Medicine 2015).

A better approach to achieving reliable, accurate and fast target selection may be with hidden Markov models (HMMs; Nuyujukian et al., SFN 2012). We performed closed-loop BMI experiments with two monkeys, where we decoded threshold crossings from intracortical multielectrode arrays implanted in primary motor cortex and dorsal premotor cortex. We incorporated an HMM to perform discrete state selection in parallel with a continuous decoder (ReFIT-KF; Gilja*, Nuyujukian*, et al., Nature Neuroscience 2012). We found that both monkeys were able to achieve significantly higher communication performance when targets were selected with the HMM rather than hovering over the target (13.9% increase in achieved bitrate in Monkey J, 4.2% in Monkey R, $p < 0.01$). Further, we found that the transition model of the HMM was crucial to achieving this performance. Specifically, we observed that the HMM performed significantly better than a quadratic discriminator using only the emissions process of the HMM (14.8% increase in achieved bitrate in Monkey J, 16.5% in Monkey R, $p < 0.01$). Finally, we found that discrete state selection with an HMM resulted in the highest achieved peak-bitrates we have measured for these monkeys across years of experimental sessions (6.5 bps for Monkey J, 5.7 bps for Monkey R). This parallel ReFIT-KF + HMM decoding approach has been translated in human clinical trials, achieving the highest-reported typing rates (Pandarinath et al., SFN 2014) and allowing intuitive use of an Android tablet (Nuyujukian et al., SFN 2015). Together, these results demonstrate that high-performance discrete state decoding with HMMs can be beneficially incorporated into a communication BMI to achieve new state-of-the-art levels of performance.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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SCOPE

MIC 152107008

Title: Volitional modulation of neuronal activities among multiple neuron groups via neuronal operant conditioning

Authors: *K. SONG, S. TAKAHASHI, Y. SAKURAI;
Grad. school of brain science, Doshisha Univ., Kyoto-Hu, Japan

Abstract: Recording neuronal activities intracortically and utilizing those as source signals is one of the effective strategies for better operation of brain-machine interfaces(BMIs), allowing animals to control external prosthetic devices without bodily movements. Recent studies on BMIs have postulated that volitional modulation of neuronal activities is essential for precise and efficient control of prosthesis via BMIs, also indicating plastic changes in the activities of the recruited neurons. Therefore, understanding neuronal dynamics induced by BMIs, especially correlated and interactional activities among widely distributed neurons, is very important to develop higher performing BMIs. However, it has not been clearly demonstrated if the operantly conditioned firings of a neuron group affect those of other neuron groups. In this regard, we focused on changes in activities of multiple neuron groups during neuronal operant learning.
We recorded activities of multiple neuron groups via multiple bundles of microwires implanted in the motor cortex of each behaving rat. Experiment sessions started after spikes from at least 2 neuron groups were identified. In pre-learning stage, the rats could get reward at randomized timing only during LED lights were turned on. In this manner, the rats were trained to recognize the waiting period as well as go period in which rats could get reward. In learning stage, the rats engaged in neuronal operant learning. During go period of learning stage, the rats were rewarded whenever firing of the neuron group 1 detected in real-time with a BMI system satisfied preset criteria. After learning of the neuronal operant task with the neuron group1 was completed, the rats conducted the identical neuronal operant task using firing of the neuron group 2.
Data of each of the neurons and the neuron groups were analyzed offline. Firing rates and delays

to reward delivery were employed to examine the hypothesis that neuronal operant learning of neuron group 1 affects the conditioned modulation of the neuron group 2. A preliminary results examining the hypothesis will be reported in detail.
It has been said that the most critical problem of neuronal operant conditioning using a small number of neurons is its limited life as sources of signals for volitional motor outputs to control a neuroprosthesis. For this reason, studying interactional effect of neuronal operant conditioning on multiple neuron groups, compensating the limited life of source signals from them, is worthwhile to manage the problem in BMIs. The result of this study is expected to contribute to advances in neurorehabilitation and the development of BMIs in daily life.

Disclosures: K. Song: None. **S. Takahashi:** None. **Y. Sakurai:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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BWF

Title: Self-recalibrating brain-computer interfaces based on population subspace alignment

Authors: *A. D. DEGENHART[1,2,5], W. E. BISHOP[5,6], E. R. OBY[1,2,5], E. C. TYLER-KABARA[2,3,4,9], A. P. BATISTA[1,2,5], S. M. CHASE[5,7], B. M. YU[5,7,8];
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Abstract: A key problem limiting the clinical translation of intracortical brain-computer interface (BCI) technology is that of stability. Over time, neural signals recorded by penetrating microelectrode arrays can change due to a number of factors, including glial scarring, electrode micro-motion, and mechanical failure. To combat these changes, BCI systems typically rely on explicit daily recalibration of their decoding algorithms to recover satisfactory control. Recalibration procedures require the user's participation and may be burdensome in a clinical setting.

To overcome this shortcoming, we present an algorithm for decoding a continuous control signal which performs automatic recalibration by leveraging the low dimensional structure found in neural population activity. We make the assumption that the day to day relationship between a low-dimensional representation of neural activity and intended BCI movements is constant, even if the set of neurons recorded and the characteristics of the signals vary from day to day. By finding the alignment between low-dimensional spaces of the population activity estimated at different points in time, decoding parameters can be automatically updated based only on observation of new neural activity and without knowledge of intended movement kinematics. This allows recalibration to occur in the background and requires no time or effort on the part of the user.

We assessed performance of the self-recalibrating algorithm in a series of closed-loop BCI experiments with a Rhesus macaque implanted with a Blackrock array in primary motor cortex (M1). Experiments began with the calibration of a well-controlled "baseline" decoder. As the neural activity within a single experimental session is often stable, we simulated recording instability by perturbing the neural activity using: (1) baseline shifts, where a random constant offset was added to the firing rate of each neuron, (2) silencing, where the firing rates of a subset of neurons was set to zero, (3) swaps, where the activity of a subset of neurons was replaced with that of held-out neurons, or (4) combinations of baseline shifts, silencing, and swaps which might mimic clinically severe recording instabilities. In 30 of 32 experiments, we find that the self-recalibrated decoder was able to significantly improve performance in the presence of the perturbation, returning control to pre-perturbation levels or better in 11 of these sessions. Furthermore, we found that use of the algorithm in the absence of artificially-generated neural instabilities did not adversely alter performance. This work has the potential to increase the viability of BCI systems for clinical use.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Topic: E.05. Brain-Machine Interface

Support: NSF GRFP

Title: Distinct subspaces emerge in neuroprosthetic control during different tasks

Authors: ***P. KHANNA**[1], V. R. ATHALYE[2,1], S. GOWDA[1], R. M. COSTA[2], J. M. CARMENA[1];
[1]UC Berkeley, Berkeley, CA; [2]Champalimaud Ctr. For the Unknown, Lisbon, Portugal

Abstract: Recent evidence shows the presence of stable neural correlations during neuroprosthetic control. It remains unclear whether neural correlations are static properties of the motor neural circuitry related to learning a specific effector (decoder), or whether they are flexible and can be optimized depending on task goals. Some studies suggest that using the same subspace defined by neural correlations for a new task ought to be easier than generating a new subspace. To directly test how neural subspaces change across task contexts, 1 rhesus macaque uses a brain-machine interface to neurally control a cursor in two different tasks. The subject performs a centerout task and a centerout obstacle avoidance task. Neural correlations are prominent during centerout task performance. To separate correlations from uncorrelated activity we decompose neuron spike counts into a summation of a low-dimensional shared component designed to capture the subspace of neural correlations, and a high-dimensional component. By doing this decomposition online and using only the low-dimensional shared component to drive the prosthetic cursor we can discern how much the shared subspace drives task performance. Compared to using the full neural input, when using only the shared component, cursor speed and time to target increase while path error remains constant. We then test if this centerout low-dimensional subspace remains if the subject performs a new obstacle-avoidance task. We find that with the same decoder, a new, distinct obstacle task subspace emerges that has more dimensions and does not significantly overlap with the centerout task subspace. Using the component of neural activity lying in the new obstacle task subspace as a decoder input slightly improves performance in the obstacle task. Further, using only neural activity lying in the centerout subspace during obstacle performance makes obstacle task performance worse and does not shift the used obstacle subspace towards the centerout subspace.In conclusion, despite identical decoders and effectors, different task goals result in the emergence of different shared subspaces. This finding is surprising given that subjects were fully capable of generating a solution to the obstacle task using the centerout task subspace. With the neuroprosthetic paradigm we confirm that these subspaces capture most relevant variance for performance. We

also demonstrate that using the subspace from one task in another task does not induce a shift towards the used subspace demonstrating the robustness of task-specific correlations.

Disclosures: **P. Khanna:** None. **V.R. Athalye:** None. **S. Gowda:** None. **R.M. Costa:** None. **J.M. Carmena:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

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Title: Effects of vagus nerve stimulation on cortical activity and excitability in the nonhuman primate

Authors: ***S. ZANOS**[1], S. MOORJANI[1], S. SABESAN[2], E. E. FETZ[1];
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Abstract: Vagus nerve stimulation (VNS) is used clinically to treat some cases of intractable epilepsy. In addition, it has been suggested that VNS may facilitate adaptive cortical reorganization after ischemic stroke. However, the cortical mechanisms behind these actions remain elusive, especially in humans and in the nonhuman primate (NHP). We studied the stimulation-elicited effects of VNS on cortical activity and cortical excitability in a NHP. We implanted a NHP with a left VN cuff electrode and multiple epidural and intracortical electrodes bilaterally in sensorimotor cortex, parietal cortex and prefrontal cortex. While the NHP generated left or right wrist torques for reward, we delivered VNS at different current intensities and stimulation schedules, including tonic-VNS at different frequencies and burst-VNS at different pulsing frequencies and pulse counts. In order to quantify VN stimulus-elicited effects, we compiled stimulus-triggered averages of cortical activity. Widespread evoked cortical responses (time- and phase-locked) were seen bilaterally, in most implanted cortical sites. These responses consisted of positive and negative waves at various latencies, up to 500 ms post-stimulus. Time-

locked responses were registered primarily on the right hemisphere and involved short-latency increases in the power of field potential frequencies below 50 Hz. The magnitude of these responses was dependent on the VNS parameters, with larger current intensities, pulsing frequencies and pulse counts associated with larger responses. In order to quantify the effect of VNS on cortical excitability, we delivered electrical cortical stimulation at different times after the delivery of VNS and registered cortico-cortical evoked potentials (CCEPs), whose amplitude is proportional to cortical excitability at the time of stimulation. We found widespread suppression of cortical excitability, bilaterally. Cortical sites with larger VNS-elicited responses were associated with more VNS-elicited suppression. Suppression of excitability occurred as early as 100 ms post-VNS and lasted for several seconds. In conclusion, left VNS elicited widespread time- and phase-locked responses to multiple cortical areas. It also elicited suppression of the amplitude of CCEPs, a measure of cortical excitability. These effects lasted from hundreds of milliseconds to several seconds.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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PVA 2978

Title: Quantifying the information rate of sensory feedback for neuroprosthesis

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Abstract: Brain-machine interfaces (BMIs) show great promise for restoring motor abilities in persons with movement disorders, spinal cord injury and limb loss. However, BMIs have not yet been able to provide a degree of dexterity that approaches normal movements. There are likely multiple reasons for this, but it is notable that proprioception is absent for BMIs, leaving the user to rely on vision alone. As a result, there has been recent interest in providing prosthetic sensations directly to the brain through cortical stimulation. While this work is still in its infancy, one challenge that has immediately arisen is that of quantifying the degree of performance improvement that the prosthetic sensation provides. In particular, adequate methods are lacking for quantifying the information rate of sensory feedback modalities for the execution of movements.

To address this gap, we employed a critical stability task (CST) to quantify closed-loop sensorimotor performance (Quick, Card, Whaite, Mischel, Loughlin, & Batista, 2014). We experimentally manipulated the visual feedback, both to diminish its reliability and to allow for the delivery of quantifiable rates of information. We then tested the performance of human and non-human primate subjects with these feedback regimes and estimated the task-relevant sensory feedback rate as a function task performance.

The CST requires subjects to control, moment by moment, the state of an unstable dynamical system using one or more feedback modalities. The index of performance is then the maximum level of instability at which the user is able to control the system. To quantify the information rate of the visual feedback used to perform this task, we discretized its delivery in time and space. This allowed us to model the relationship between the CST performance and the visual information rate on a subject-by-subject basis. The goal is to use this model to estimate the information flow of any feedback modality--natural or artificial. Similarly, by applying the same method on the input space of the system we were able to compute the information rate of the controller (i.e. motor command) as well.

We collected pilot data with human and macaque subjects and found that CST performance depends on the information rate of the signal, validating the approach. The functional dependence is well modeled by a sigmoid, with a peak inflection at about 15 bit/s. Based on these results, and those of Quick et al, we propose that the CST can thus be used to evaluate the sensory and motor performance of any closed-loop control system, providing a generic, validated estimate of information rate for comparing the performance of different feedback and control schemes.

Disclosures: J.D. Rechenmann: None. **J.E. O'Doherty:** None. **P.N. Sabes:** None.

Poster

334. Neuroprosthetics: Network and Motor Processing

Location: Halls B-H

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Topic: E.05. Brain-Machine Interface

Support: the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (No. 2013R1A2A2A04014987)

Title: Decoding the bimanual movements in non-human primate using hybrid-regression method

Authors: ***H. CHOI**[1], J. LEE[1], S. LEE[1], I. KIM[1], K. AHN[2], K. LEE[2], D. JANG[1];
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Abstract: BCI (brain-computer interface) is a promising technology that can provide communication tools to paralyzed patient. Especially, about the arm movement tools using motor BCI, various studies have been performed because it is intuitive. The unimanual research has been well studied compared to bimanual study. The bimanual brain state has known as different from the unimanual one. The conventional method used in unimanual seems to be insufficient to decode bimanual movement. Thus, the needs for new method has been raised. In this paper we suggest the hybrid-regression method combining brain state classification and hand trajectory predicting algorithm for unimanual and bimanual movement, to apply in real world BCI. Two micro electrode patches (32 channels) were inserted over duramater on rhesus monkey's brain covering premotor cortex, primary motor cortex, and primary somatosensory cortex. six motion sensors (IMU: Inertial Measurement Unit) were attached to both wrist, upper arms, and back of the shoulder. The monkey performed 3 types of arm movement tasks which were divided in 3 by the light color and the type. We contemporary recording the arm movement signals and brain signals for 2hours, 4 times a week. The signal was analyzed off-line using MATLAB. The brain signal and movement signals were preprocessed to scalogram which represents time frequency power, and Cartesian (xyz) position respectively. To computing the correlation coefficient between the arm movement and brain signal, we used hybrid-regression algorithm. For training set, the 1s of scalogram is categorized and stacked by movement state every 50ms. When the new brain signal recorded, it was transformed to scalogram and classified by type of the movement using the training sets. Then, the classified scalogram was decoded by each movement training set. As results, the hybrid-regression method showed improved arm movement decoding performance and significant and stable decoding rate over several months at bimanual tasks. This technique could be applied to arm movement BCI in real world and the various neuro-prosthetics fields.

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Poster

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2014DFG32580

Title: Dorsal premotor area of the macaque monkey encodes internal grasp movement planning

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Abstract: Recently, some studies demonstrated that neuron activity of the dorsal premotor cortex (PMd) showed selectivity for different hand gestures during planning period, which suggested that PMd is involved not only in arm reaching but also in hand grasping. However, it is difficult to identify whether the neuron selectivity results from visual cues related to object shapes or hand preshapes because different objects were asked to be grasped by using specific gestures in their experiments. In this study, we designed a free-choice task where a monkey was trained to autonomously choose one of two gestures (Power or Hook) to grasp the same object after a holding period for planning. Neural signals of PMd were collected by microelectrode array when the monkey was performing the task. With single unit activity analysis, we found that 18% (45/250) of units in PMd showed significant difference for the two gestures during movement planning period. In some of these units, the selectivity of their neuron activity disappeared immediately after the movement onset. We further examined the selectivity of neural activity on the neuron population level with a support vector machine (SVM) classifier. The gesture classification accuracy obtained from the neural activity of these tuning neuron subset could reach up to 93.5% during the planning period. Our results infer that the selectivity of partial PMd neurons during the planning period of grasp movements was closely related to the internal action selection or movement preparation rather than the external visual cues such as size or orientation of target objects.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Topic: E.05. Brain-Machine Interface

Title: Predicting decision outcomes from single realizations of lateral prefrontal cortex neuronal activity

Authors: *C. BOULAY[1,2], F. PIEPER[3], M. LEAVITT[4], J. MARTINEZ-TRUJILLO[5,6], A. SACHS[1,2];
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Abstract: Neurons in the lateral prefrontal cortex (LPFC) encode sensory and cognitive signals, as well as commands for goal directed actions. Thus, this brain region might be a good signal source for a goal-selection brain-computer interface (BCI) that decodes the intended goal of a motor action previous to its execution. Toward the development of a goal-selection BCI, we set out to determine if we could decode saccade goals from single-trial LPFC neuronal activity.
We recorded neuronal spiking activity from microelectrode arrays implanted in area 8A of the LPFC of two adult macaques while they made visually guided saccades. The rewarded target was indicated by a colour cue and we changed periodically the rule that associates colour and rewarded direction. We extracted neuronal firing from single-trial LPFC activity in the pre-saccade period and predicted saccade targets using support vector machines with 10-fold cross-validation. We performed unsupervised dimensionality reduction using Gaussian process factor analysis (GPFA) to reduce the neural dimension and principal components analysis (PCA) to reduce the temporal dimension (30 time points to 3 windowing functions).
Eight-class and binary decision outcomes were decoded reliably from pre-saccadic single-trial LPFC activity. Decoder performance was unchanged or improved after dimensionality reduction despite 40-fold size reduction and accounting for only 35% of the full dataset variance. This result suggests that the latent structure of the sampled neuronal population revealed by GPFA and the temporal activity patterns revealed by PCA were involved in the task.
Both behavioural performance and decoder performance were poor immediately after colour-target rule changes and they both improved as the monkey acquired the new rule. This result suggests that task-relevant information processing and the representation of the intended saccade goals in the LPFC are both subject to plasticity during learning.

These results provide further evidence that LPFC neurons encode intention and suggest that LPFC activity can be used as a signal source for a goal-selection cognitive BCI.

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Poster

334. Neuroprosthetics: Network and Motor Processing

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Title: Brain-spinal interface to alleviate gait deficits in rats: Direct-proportional neuromodulation

Authors: *M. BONIZZATO[1], G. PIDPRUZHNYKOVA[1], G. COURTINE[1], S. MICERA[1,2]; [1]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [2]The BioRobotics Inst., Scuola Superiore Sant'Anna, Pisa, Italy

Abstract: The sensorimotor cortex of rats monitors locomotion through a continuous exchange of information with the spinal neuronal network that generates locomotor patterns. A spinal cord injury (SCI) disrupts this communication, resulting in severe motor deficits. Brain computer interfaces provide the technology to link cortical motor states to spinal cord stimulation protocols in order to restore this communication, and thus alleviate motor deficits.To explore this possibility, we studied whether locomotor related information could be extracted from neuronal ensemble modulation recorded in the hindlimb sensorimotor cortex of rats with contusion SCI. To enable locomotion of the paralyzed rats, we delivered electrochemical stimulation over the lumbar spinal cord. We found that not only the swing and stance phases of gait but also certain kinematic features related to voluntary control of walking could be decoded from the spiking activity of cortical neurons. For example, the activity of the cortical neuronal population at foot-off explains up to 70% of the variance of the step height that will occur during the subsequent

swing phase.We then used these findings to develop neuromodulation protocols that directly linked cortical motor activity to the onset and proportional adjustment of epidural electrical stimulation during locomotion. This brain-spinal interface improved the robustness and quality of leg movements during voluntary locomotion overground. This neuroprosthesis provides the opportunity to enhance the communication between the cortex and spinal cord during gait rehabilitation after SCI in order to augment neuroplasticity and thus motor recovery.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: CABMC (Control of Animal Brain using MEMS Chip) funded by Defense Acquisition Program Administration (UD140069ID)

Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137)

Title: A novel rat movement control for rat-robot using electrical stimulation of basal ganglia

Authors: *C. KOH[1,2], H. PARK[2], J. SHIN[1,3], C. KONG[1], M. YUN[1], W. CHANG[1], H. JUNG[1], H.-C. SHIN[2], J. CHANG[1,3];
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Abstract: Background: Rat-robot is a kind of animal model that conducts specific movement following operator's command through artificial stimulation. In the past decade, many rat-robot models have been studied after its first introduction in 2002. However, most of these studies still depend on the virtual reward and cue to motivate rats to follow the instruction. These models require relatively complex procedures and have diverse effects in rats. Hence, to tackle these limitations, we propose a novel rat-robot movement control model based on basal ganglia stimulation.
Material and methods: Three male adult Sprague-Dawley rats (350 – 450 g) were used in this experiment. Stimulation electrodes were implanted in the bilateral basal ganglia for delivering

stimulations under ketamine anesthesia (50mg/kg). Stimulations (train duration: 200ms, pulse duration: 0.2ms, pulse interval: 4ms) were presented by Model 2100 (A-M systems, Inc., WA, USA)
Result: Our proposed rat-robots show contralateral turning behavior following the electric stimulation in onside of the basal ganglia. The rat turning angle varies upon the amplitude of electrical stimulation, and we chose the intensities that induced more than 40 degrees (70 – 160 **μ**A). In T maze test, our rat-robots showed overall 99.0% success rate. Our results clearly show that the new rat-robot can be an alternative for traditional reward-based model.
Conclusion: In this pater, we proposed a new non-training based rat-robot model using electrical stimulation in the basal ganglia. Our model is ready to perform tasks immediately after recovery from the surgery and does not need any training or maintaining procedures. Furthermore, the turning angle can be controlled by fine amplitude variation. Therefore, the basal ganglia stimulation method can be a more accurate control of rat movements.
Acknowledgement: This study was supported by the grant from CABMC (Control of Animal Brain using MEMS Chip) funded by Defense Acquisition Program Administration (UD140069ID) and Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137).

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Poster

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Topic: E.05. Brain-Machine Interface

Support: Shriners Hospital Grant 85900

Title: Decoding intended gait modifications from the hindlimb sensorimotor cortex

Authors: *M. MEYERS, K. A. MOXON;
Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA

Abstract: The hindlimbs present a unique challenge to the design of decoders for brain-machine interfaces. While it is well documented that decoding algorithms can be used to extract details of the intended forearm position from the motor cortex, the same cannot be said of hindlimb position. A fundamental difference is the role of the motor cortex. The cortex is directly involved in planning and executing the complex, goal-directed movements of the forearms. Hindlimb

position follows a stereotyped locomotor pattern hypothesized to be controlled by the spinal cord. The role of the motor cortex in this case is to modify this pattern. This hypothesis is consistent with the fact that attempts to directly decode details of hindlimb position from the cortex have been met with relatively little success while the phase of the step cycle can be decoded. Within this framework, one would expect a decoder to identify the intent to make and the parameters of a modification to the gait cycle, rather than specifics of hindlimb position. Given this understanding of the encoding of hindlimb movements, we developed a three stage decoder to identify changes in the gait cycle.
Female Long-Evans rats walked on a treadmill. To remove any potential information about gait cycle from the intact forelimbs, rats walked bipedally and were weight-supported. Rats were conditioned to step over obstacles connected to the treadmill belt that required them to modify their gait. Once conditioned, rats were implanted stereotaxically with bilateral 16 channel multielectrode arrays in layer V/VI of the sensorimotor cortex hindlimb representation. After recovering from surgery, rats were placed back into the task and simultaneous neural and video recordings were acquired.
Off-line, a three-stage decoder was trained and then evaluated on different segments of the recordings. First, a classifier was applied to a sliding window to determine the phase of the step cycle. At identified stance to swing transitions, the second stage was triggered – a classifier to determine whether a modification (i.e. step over an obstacle) was intended. Finally, if an intended modification was detected, a third stage decoded the amplitude of the change (quantified using the extracted toe coordinates). Each stage was evaluated and performed above chance.
The above chance performance of the decoder stages demonstrate that it is possible to decode the intent to make a modification to the gait and the amplitude of that modification on a single-trial basis. Additionally, these results further support the hypothesis that the motor cortex is involved in voluntary modifications to gait.

Disclosures: **M. Meyers:** None. **K.A. Moxon:** None.

Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: Scientific Research (B) (23300238)

Title: Training effect of position perceptibility in forward and backward leaning posture using a balance-board for the elderly

Authors: *K. FUJIWARA[1], N. KIYOTA[2], H. TOYAMA[1], A. HYODO[1], F. SATO[3]; [1]Kanazawa Gakuin Univ., Kanazawa, Japan; [2]Japan Hlth. Care Col., Sapporo, Japan; [3]Kanazawa Univ., Kanazawa, Japan

Abstract: The following findings have been suggested for dynamic balance of the elderly: 1) backward fall tended to occur during horizontal floor oscillation, 2) postural controllability pivoting at the ankle while arms flexion was declined, and 3) increase in the muscle strength of the triceps surae (TS) was not related to the improvement of dynamic balance. Therefore, we expected that for the elderly, backward position perceptibility pivoting at the ankle may be declined, and investigated the training effect of the perceptibility using a balance-board.
Subjects were 20 healthy elderly adults, aged from 60 to 79 years. In the training, subjects stood on the balance-board with their metatarsal heads or medial malleolus (about 70% or 25% distance from the heel in relation to foot length (FL)) positioned on the axis (about 2 cm) of the board. They maintained forward or backward leaning posture for 2 sec by shifting their center of foot pressure onto the axis, which could make the board horizontally. This training at each position was repeated 100 times per day for a month. Before and after the training period, position perceptibility at 2 target position (70 and 25%FL) was measured with eyes closed and the knee, hip and trunk fixed. Subjects voluntarily moved their standing position by forward or backward leaning from their quiet standing (QS) position until the buzzer sounded (i.e., target position), and then maintained and perceived the position for 2 sec. After returning to the QS position, they reproduced the target position without the buzzer and maintained the position for 2 sec. This trial was repeated 10 times per each position. Position perceptibility was evaluated by absolute error of reproduced position to each target position. Plantar- and dorsi-flexor strength, and muscle thickness of TS by ultrasound were measured.
Only before the training, the absolute error at 25%FL position was larger than 70%FL. At 70%FL position, significant training effect on the absolute error was not found. At 25%FL position, the absolute error after the training was significantly smaller than before. Plantar- and dorsi-flexor strength, and TS muscle thickness significantly increased by the training. No significant correlations were found between the absolute error at both positions and muscle strength ($r < 0.3$).
These results demonstrated that for the elderly, 1) position perceptibility was lower in backward leaning posture than in forward, 2) training using the balance-board was effective for the position perceptibility in backward leaning posture, and 3) increase in the muscle strength of TS was hardly related to the improvement of the position perceptibility.

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Poster

335. Posture and Gait: Afferent Control

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Support: US ARO 64929EG to AJS

DoD SC140089 to GMS

Shriners Hospital for Children #86000 to GMS

Title: Viral expression of excitatory DREADDs in dorsal root ganglia induces reflex hyperexcitability

Authors: *B. D. ROBERTSON[1], M. A. LEMAY[1], G. M. SMITH[2,3], A. J. SPENCE[1]; [1]Dept. of Bioengineering, Temple Univ., Philadelphia, PA; [2]Lewis Katz Sch. of Med., Temple Univ., Phladelphia, PA; [3]Shriners Hosp. Pediatric Res. Ctr., Temple Univ., Philadelphia, PA

Abstract: In recent years, researchers have demonstrated that amplification of reflex activity via electrical stimulation (e-stim) yields improved functional recovery from spinal cord injury in humans and animals. Unfortunately, e-stim requires implantation of a device with a limited lifetime/functional window, and is not inherently selective for proprioceptors and large diameter exteroreceptors thought to be responsible for enhanced recovery. Recent advances in chemogenetics have led to the creation of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). When activated by Clozapine-N Oxide (CNO), inhibitory DREADDs modulate the activity of Potassium channels, inducing hyper-polarization of neurons, and therefore inhibiting spiking. An excitatory DREADD (hM3Dq) causes burst firing and presumed depolarization through a different G-protein coupled pathway. Because expression of DREADDs can be controlled both genetically and through targeted delivery, they offer a unique solution to targeted modulation of reflex activity. We transfected rats with Adeno-associated virus serotype 2 (AAV2) expressing an excitatory DREADD (hM3Dq) under the human synapsin (hSyn) promoter via direct injection into L3-L6 dorsal root ganglion (DRG). We observed a loss of frequency dependent inhibition for the monosynaptic Hoffman (H) reflex (an electrophysiological hallmark of hyperexcitable reflex activity) 30 minutes post IP injection of CNO (2mg/kg). We also observed the return of frequency dependent inhibition in H-reflex 90 minutes post CNO exposure. This demonstrates the ability of DREADDs to modulate sensory afferent activity without necessitating an implantable device, and paves the way for chemogenetic modulation of spinal circuitry thought to be responsible for the recovery of rhythmic stepping and body weight support in SCI individuals. To our knowledge, this is the first

reported use of DREADDs for modulating peripheral neural circuitry in an intact animal preparation.

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Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant (SDP)

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Title: Correlation of plantar-surface pressure and lower limb muscle activity during gait

Authors: ***S. D. PERRY**, B. MCGREGOR;
Kinesiology & Physical Educ., Wilfrid Laurier Univ., Waterloo, ON, Canada

Abstract: Impaired plantar-surface sensation is associated with a number of changes in gait characteristics. However, to date the relationship between plantar cutaneous mechanoreceptors and the muscles of the lower leg has not been quantified during gait. Observing this relationship will provide insight into how the body uses foot pressure to adapt to changes in walking terrain and other instabilities during gait. Twenty-one (10 male, 11 female) healthy young adults were recruited from the university population (mean age= 22, SD=3.37). Each participant completed 10 normal walking and 20 randomized uneven terrain walking trials along a 10m walkway. Participants were equipped with 8 electromyography (EMG) electrodes (AMT-8, Bortec Inc., Calgary, AB) on the left and right legs, and wore standardized shoes containing size-matched pressure sensors (Medilogic Inc., Germany). Uneven terrain conditions were induced using wooden wedges placed in the walking path at each step location. Uneven terrain (UT) conditions were slanted platforms in the anterior, posterior, lateral and medial directions (relative to the stance foot) in order to assess a variety of plantar pressure situations. A significant negative correlation was found between loading rates under the contralateral great toe and the magnitude of tibialis anterior (TA) during normal walking in both short and long latency periods, as well as in posterior, lateral and medial UT conditions. A significant positive correlation was found between the loading rates under the ipsilateral heel and the magnitude of the TA activity in

normal walking, posterior and lateral UT conditions in the short and long latency response periods. Lateral and medial UT conditions showed altered muscle activity in the TA in order to respond faster to the medial-lateral perturbations and to increase the surface area in contact with the walking surface for improved cutaneous input. The anterior UT condition showed a significant positive correlation between TA magnitude and the contralateral great toe loading as the COM was slowed due to the upward slope and a stronger push off was required. Alternatively, the posterior UT condition showed increased TA activity, thought to be required to maintain a slowed dorsiflexion on a downward slope to prevent tripping and maintain a comfortable COM speed. This study provides evidence of a relationship between plantar cutaneous mechanoreceptors (foot pressure) and the magnitude of muscle activity during gait.

Disclosures: S.D. Perry: None. **B. McGregor:** None.

Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: NIH Grant HD053367

Title: Does ankle proprioception modulate muscle recruitment during locomotor-related leg movements in chick embryos?

Authors: *S. SUN, N. S. BRADLEY;
Biokinesiology and Physical Therapy, USC, Los Angeles, CA

Abstract: Chick embryos spontaneously produce repetitive limb movements (RLMs) characteristic of locomotion 3-5 days before hatching. During RLMs, flexor and extensor muscles are alternately active. However, flexor muscles appear to be more consistently recruited across multiple cycles than extensors, suggesting flexor motor pools are more readily driven than extensor pools. We speculated that the extremely flexed posture and movement *in ovo* may generate proprioceptive input to motor pools and account for differences in flexor and extensor muscle recruitment. It is known that proprioceptive afferents can code extreme atypical leg posture and movement prior to hatching. It is not known if proprioception contributes to muscle recruitment during RLMs under normally constrained posture and movement *in ovo*. Synchronized electromyographic and kinematic recordings were performed during spontaneous leg movements *in ovo* 1 day before hatching. Activity of ankle flexor (tibialis anterior, TA) and extensor muscles (lateral gastrocnemius, LG) was recorded bilaterally. After recording for ≥ 2

hrs, the left TA or LG tendon was cut (tenotomy). Recording continued for an additional ≥ 2 hrs. Employing a within-subject design, TA and LG burst parameters during RLMs were compared pre- and post-tenotomy in the left and right ankle (10 TA, 10 LG tenotomies). Neither TA nor LG tenotomy altered recruitment of ankle muscles during RLMs. A similar number of RLM sequences was generated pre- and post-tenotomy in left and right ankles. On average, TA burst counts were twice that of LG counts pre- and post-tenotomy in both ankles. On average, left and right TAs were recruited in approximately 97% of RLM sequences, both pre and post TA or LG tenotomy. In 25-42% of left and right sequences, only the TA was recruited; whereas, in 2-5%, only the LG was recruited pre- and post-tenotomy. Burst and cycle durations, and normalized burst amplitude were similar between muscles and across conditions. Our results suggest that proprioception does not contribute to ankle muscle recruitment during RLMs under normal postural conditions *in ovo*. The TA was more readily recruited than the LG pre- and post-tenotomy. Further, all recruitment parameters were similar pre- and post-tenotomy for both the left and right ankle muscles. Our findings do not rule out the possibility that input from intact ankle muscles compensated for the lost proprioception. Nonetheless, loss of input from homologous primary and secondary afferents suggests that differences in flexor and extensor recruitment during RLMs are at least partially attributed to central neural mechanisms.

Disclosures: S. Sun: None. **N.S. Bradley:** None.

Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Title: Balancing sensory inputs: sensory reweighing of vision and ankle proprioception during a bipedal posture task

Authors: *C. S. LAYNE, R. KABBALIGERE, B.-C. LEE;
Dept Hlth. & Human Performance, Univ. Houston, Houston, TX

Abstract: Multisensory integration is driven by a process of sensory reweighting during which each input is assigned a weight depending on the current functional state of a particular sensory system, the task itself and the context in which it is being performed. The primary aim of this study was to determine which of the two inputs between ankle proprioception and vision is upweighed during a postural control task when the two inputs provide conflicting information pertaining to direction of body sway. Achilles tendon vibration and visual flow were used to create sensory conflict, which produced center of pressure (COP) sway in opposite directions

when applied independently. The baseline conditions (1) consisted of eyes open quiet stance condition, eyes closed with vibration applied on the Achilles tendons (2) and eyes open with visual flow (3). The experimental condition simultaneously combined vibration and visual flow. COP excursions were recorded in 10 healthy young adults to evaluate the magnitude and direction of sway produced by vibration and/or visual flow. Additionally, lower body joint kinematics were evaluated to understand the multi-segmental strategies and their adaptation to the various sensory manipulations. The results showed that visual flow moderated the extent of backward COP and ankle angular displacement produced when vibration was applied independently. Additionally, visual flow was also found to reduce the extent of predominant hip strategy generated by ankle vibration. The findings show that visual input plays a significant role in maintaining stability and that ankle proprioception is downweighed during conflicts between vision and proprioception. This has important implication for balance training using controlled visual flow in patients with balance disorders and elderly.

Disclosures: **C.S. Layne:** None. **R. Kabbaligere:** None. **B. Lee:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.06/QQ2

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant

Title: The influence of physiological arousal on human lower limb cutaneous reflexes during treadmill walking

Authors: ***M. ZABACK**[1], B. C. HORSLEN[1], T. W. CLEWORTH[1], L. COLLINGS[1], C. LANGLET[4], T. INGLIS[1,2,3], M. G. CARPENTER[1,2,3];
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Abstract: Electrically evoked cutaneous reflexes are modulated in a phase- and task-dependent manner during human gait [1]. These reflexes have been shown to be facilitated when walking with the threat of perturbation [2]. However, it remains uncertain whether changes in cutaneous reflexes can be attributed to a general arousal response (as seen in other sensory systems [3]), or are a context-specific response to an expected perturbation. Therefore, this study investigated the influence of physiological arousal on human lower limb cutaneous reflexes during treadmill

walking. Nine healthy young adults completed trials of treadmill walking while viewing emotionally-charged pictures from the International Affective Pictures System [4] that included blocks of 30 high arousal (SAM arousal rating = 6.94±1.10) and 30 low arousal (SAM arousal rating = 5.17±0.86) pictures (each shown for 8 s). Reflexes were evoked at heel contact, mid-stance, and toe-off at random intervals throughout each trial with electrical pulse trains (5x, 200Hz, 2x radiating threshold, ~70-90 trains per trial) to the sural nerve. Electromyography (EMG) of the ipsilateral soleus (SOL), medial gastrocnemius, tibialis anterior (TA), biceps femoris (BF), and vastus lateralis (VL) was recorded. EMG was sampled at 1000hz, rectified, trigger averaged, and referenced by subtraction of the corresponding EMG of non-stimulated steps. Reflex amplitude was quantified by calculating the area of the referenced EMG for each muscle between reflex onset and offset. Physiological arousal was quantified as mean electrodermal activity (EDA) of the hand. Highly arousing pictures evoked significant increases in EDA ($p<0.01$). Medium-latency reflexes were consistently detected (onset range=70.4-132.7ms) during toe-off in the SOL (n=8) and TA (n=7) and during mid-stance in the VL (n=8) and BF (n=6). Mild reflex facilitation was observed in the SOL and BF during high compared to low arousal trials, but was not statistically significant (Z-change=0.32, p=0.17; Z-change=0.12, p=0.18, respectively). No reflex changes were observed in TA or VL. The mild changes observed while walking under arousing conditions are similar to the changes reported when standing at the edge of an elevated platform [5]. This suggests that cutaneous reflexes during walking are not strongly modulated by a general arousal response, but are more likely influenced in a context-specific manner (e.g., preparing to recover from an expected perturbation [3]).
Research funded by NSERC
[1] Zehr et al. (1998) *J Physiol* [2] Haridas et al. (2005) *Brain Res* [3] Horslen et al. (2013) *J Neurophysiol* [4] Lang et al. (2008) *IAPS manual* [5] Horslen et al. (2014) *ISPGR*

Disclosures: **M. Zaback:** None. **B.C. Horslen:** None. **T.W. Cleworth:** None. **L. Collings:** None. **C. Langlet:** None. **T. Inglis:** None. **M.G. Carpenter:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.07/DP06 (Dynamic Poster)

Topic: E.06. Posture and Gait

Title: Walking through aperture with visual information obtained at a distance

Authors: ***T. HIGUCHI**, D. MUROI;
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Abstract: The present study addressed whether visual information about the width of an aperture, obtained at a distance, would be sufficient to guide safe passage through it without collision. For this purpose, we asked twelve young participants to walk while holding a 66-cm horizontal bar and passed through an aperture without vision from 3m in front of the aperture. The results showed that, for narrow apertures (the relative widths were 0.8 and 1.0 times the bar length), for which body rotation was necessary to avoid collision, the rate of collision without vision was about 40-50%. This was mainly due to insufficient (i.e. smaller magnitude) body rotation. Particularly, for the 1.0-time aperture, four participants passed through the aperture with no body-rotation. The body-midpoint deviated to the side without vision; this caused collision particularly for wide apertures (1.2 and 1.4 times the bar length). Obtaining dynamic visual information (i.e., optic flow) while making two steps forward to the aperture to reach a 3m distance from the aperture did not contribute to improve behavior to avoid collision. These results suggest that visual information obtained at a distance was not sufficient to perfectly represent distance from the aperture and an aperture width relative to the body. With the visual information obtained at a distance, the space necessary for passage is underestimated.

Disclosures: **T. Higuchi:** None. **D. Muroi:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.08/QQ3

Topic: E.06. Posture and Gait

Title: Gait characteristics of children walking barefoot and with socks. ii. tandem walk

Authors: *C. W. CHAU, B. ALTHAUS, K. DE MARREE, E. PRIMUS, H. ZURITA; Nazareth Col. of Rochester, Rochester, NY

Abstract: Previous results showed that children walking at a fast speed with socks displayed a more unstable gait than when walking barefoot, suggesting that socks not only affected cutaneous input but also increased slippage. The purpose of this study is to compare the characteristics of gait in 4- and 5- year- old (yo) children between walking barefoot and with socks during regular and tandem walking. Twenty-two typically developing children (11 male and 11 female) walked on the GAITRite® (CIR system Inc. NJ), a portable walkway (3x14ft) embedded with electronic pressure sensors that record footprints. Each subject completed 3 trials of self-selected walking speed (regular walking) and along a 4" taped line placed along the center of the GAITRite mat (tandem walking) with socks and barefoot. The video footage of tandem walking trials was analyzed for missteps, defined as when any part of the foot falls

outside of the tape. Paired T-tests were used to compare gait parameters while walking barefoot and with socks during regular and tandem walking. The results showed that velocity, cadence, step length, and cycle time were comparable during regular walking with socks as compared to walking barefoot. During tandem walking with socks as compared to tandem walking barefoot, there was a decrease in velocity (-0.5 cm/sec) and cadence (-5.2 steps/min), an increase in base of support (+0.4cm), and a significant increase in missteps (+ 9% of total steps). The foot length and width were comparable between walking barefoot and with socks. Fewer missteps (-3%) and a greater decrease in velocity (-16 cm/s) and cadence (-23 steps/min) were found in 5-yo as compared to 4-yo children during tandem walking with socks. The effect size of velocity (0.57) and cadence (0.76) in 5-yo indicates moderate clinical significance. Consistent with existing literature in cats and previous findings in children, the similarity of spatiotemporal gait parameters between the sock and barefoot conditions during regular walking suggests that cutaneous input does not exert marked effects on undemanding locomotion. Spatiotemporal changes with concomitant increase in missteps during skilled locomotion (tandem walking) while wearing socks as compared to barefoot suggest a less stable gait pattern. It is likely that tandem walking increased mediolateral instability and placed an increased demand on balance control, relying more on cutaneous input for stability. The 5-yo children demonstrated fewer missteps along with clinically significant compensatory changes, suggesting that developmental maturation played a role in adapting the locomotor pattern to external constraints.

Disclosures: C.W. Chau: None. **B. Althaus:** None. **K. De Marree:** None. **E. Primus:** None. **H. Zurita:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.09/QQ4

Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant

Title: Sensori-motor responses evoked by continuous, aperiodic Achilles tendon vibration during standing

Authors: *R. L. MILDREN[1], R. M. PETERS[1], G. J. MCKENDRY[1], M. G. CARPENTER[1,2,3], J.-S. BLOUIN[1,2,4], T. INGLIS[1,2,3];
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Abstract: Recordings from single muscle spindle afferents have demonstrated that spindles within the triceps surae muscles are sensitive to small ankle rotations (i.e., magnitudes that imitate natural sway) in both active and passive states (Peters et al. 2015). However, the role of triceps surae muscle spindles in standing balance is still debated. Muscle spindle reflex pathways are typically characterized through motor responses evoked by brisk tendon taps or joint rotations that perturb the spindle ensemble (Yavuz et al. 2014). Our primary objective was to characterize spindle reflex pathways by superimposing a continuous, aperiodic modulation of triceps surae spindle firing rates during quiet standing. Our secondary objective was to describe the frequency characteristics of lower limb spindle sensori-motor interactions.

We delivered continuous stochastic vibration (0-100 Hz) to the Achilles tendons of six healthy young adults during quiet stance on a force plate. Ongoing muscle activity in the soleus (Sol), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tibialis anterior (TA) was recorded using surface EMG. We manipulated the tendon vibration amplitude [root-mean-square (RMS) accelerations 20, 15, 10, and 5m/s^2] and subsequently the frequency bandwidth (subdivided the 0-100 Hz bandwidth into five 20 Hz sections) to further probe the reflex scaling and frequency characteristics. Relationships between the stimulus acceleration and muscle responses were estimated in both the frequency (coherence) and time (cross-covariance) domains. Frequency- and time-domain correlations were also assessed between the stimulus acceleration and somatosensory cortex activity via EEG. For comparison, discrete taps (one cycle at 30 Hz, RMS acceleration 25m/s^2) were delivered to the Achilles tendon and the stimulus-triggered average EMG and EEG response latencies were contrasted against response latencies in the cross-covariance.

In all muscles (Sol, MG, LG, and TA), EMG was significantly coherent with the stochastic tendon vibration. The strongest coherence was observed in Sol, where the coherence bandwidth was significant (exceeding 95% confidence limits) between ~10 and 90 Hz. The latency of the first cross-covariance peak (~40ms) approximately corresponded to the latency of the peak response in the stimulus-triggered average (~40ms). Our findings provide novel information about the time and frequency characteristics of spindle sensori-motor interactions during quiet standing.

Research funded by NSERC.

References:

Peters et al. *Int. Soc. Posture and Gait Res.* Oral abstract O.2.6, 2015.

Yavuz et al. *J Neurophysiol* 111: 602-612, 2014.

Disclosures: R.L. Mildren: None. **R.M. Peters:** None. **G.J. McKendry:** None. **M.G. Carpenter:** None. **J. Blouin:** None. **T. Inglis:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.10/QQ5

Topic: E.06. Posture and Gait

Title: Visual influence on balance response during locomotion

Authors: *T. D. FETTROW, H. REIMANN, J. JEKA;
Kinesiology, Temple Univ., Philadelphia, PA

Abstract: We are interested in identifying the neural control mechanisms underlying the maintenance of balance during locomotion. Recent work illustrates a systematic balance mechanism in response to a vestibular perturbation, inducing an illusory fall, in the medial and lateral directions in a healthy population. The balance response consists of a shift of the stance foot center of pressure towards the direction of the illusory fall (ankle strategy), and a subsequent placement of the swing foot in the same direction (stepping strategy). Here we investigate the properties of the balance response with regard to a visual perturbation.
Subjects walked on an instrumented treadmill at a comfortable speed surrounded by a stationary visual scene consisting of 300 white triangles randomly distributed across a 180 degree black background. Every 10-13 steps at heel strike, the visual scene shifted to the right or left at 1m/s for 600ms, inducing an illusory fall.
Preliminary results show center of pressure modulation starting ≈250ms after the scene begins to shift. The maximum center of pressure excursion using the ankle strategy shifts in the direction of the illusory fall. Approximately 150ms after initiation of the ankle strategy, the step response shifts in the direction of the illusory fall, completing the balance response.
Although visual information is thought to be processed slower than vestibular information, these results suggest there is no difference in processing vestibular or visual information relative to a balance response. Considering the complexity of locomotion, the balance response may be inherently multisensory, requiring input from all sensory systems before a response can be initiated, leading to a relatively longer latency response than expected from any single modality alone.

Disclosures: **T.D. Fettrow:** None. **H. Reimann:** None. **J. Jeka:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

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Topic: E.06. Posture and Gait

Support: F32NS080393

HD32571

Title: Toe flexor reinnervation results in frontal plane motor deficits during slope walking in the cat

Authors: *M. A. LYLE, E. KAJTAZ, T. R. NICHOLS;
Sch. of Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: Muscle self-reinnervation of the triceps surae has been shown to cause permanent ankle yielding during downslope walking. The permanent ankle yielding has been attributed to a task dependent reliance on autogenic length dependent feedback, which is permanently lost after muscle self-reinnervation. The deficits reported after triceps surae reinnervation have been limited to the sagittal plane. Here, we tested the hypothesis that self-reinnervation of the toe flexor muscles, which have sagittal plane motor actions at the ankle and toes and frontal plane action at the ankle, will exhibit length dependent motor deficits in both the sagittal and frontal planes. Muscle self-reinnervation was achieved by nerve transection and immediate repair to the flexor hallucis longus (FHL) muscle in isolation and in separate cats combined with flexor digitorum longus (FHL+FDL). Joint kinematics and kinetics were collected for a period up to 25 weeks after surgery allowing complete motor recovery, followed by a terminal surgery during which intermuscular length and force dependent spinal reflex circuitry were examined. It was found that the stretch reflex was lost in the reinnervated muscles, but intermuscular force feedback remained intact. Preliminary results suggest that self-reinnervation of FHL only and FHL+FDL had no sagittal plane kinematic deficits during down slope walking 25 weeks after surgery. While no frontal plane ankle deficits were observed in the FHL only reinnervation, FHL+FDL self-reinnervation resulted in a very obvious foot abduction/twisting during down slope walking. Level and upslope walking analyses are ongoing. Qualitative evaluation suggests difficulty during the push-off phase during upslope walking. Spinal circuitry analyses indicate loss of autogenic length feedback but preservation of force dependent feedback. While the lost autogenic feedback from toe flexors was expected to result in motor deficits at the toes and ankle, this preliminary data suggest length feedback from the toe flexors does not significantly influence sagittal plane kinematics during downslope walking. However, these preliminary data suggest length dependent feedback from FHL+FDL has an important frontal plane stabilizing

role at the ankle in the cat, particularly during slope descent. This study was supported by F32NS080393 to MAL and HD32571 to TRN.

Disclosures: **M.A. Lyle:** None. **E. Kajtaz:** None. **T.R. Nichols:** None.

Poster

335. Posture and Gait: Afferent Control

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Program#/Poster#: 335.12/QQ7

Topic: E.06. Posture and Gait

Support: NSERC (Canada) Grant

Title: The effect of light touch on standing sway when the touch reference is unreliable

Authors: ***J. E. MISIASZEK**[1,2,3], J. W. VANDER MEULEN[3], T. SHIVA[3];
[2]Neurosci. and Mental Hlth. Inst., [3]Fac. of Rehabil. Med., [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Light touch (<1 N) reduces sway when standing with eyes closed. If the light touch reference is displaced a balance correction is evoked with the first exposure of the disturbance, despite the absence of a balance disturbance per se. This finding suggests that light touch provides sensory information that is incorporated into balance control. However, balance corrections are no longer observed with subsequent touch displacements, but are met with arm movements instead. In this study we asked, “Does lightly touching an external reference reduce sway when participants become aware the reference is unreliable?” Sway was compared before and after exposure to repeated touch displacements in four groups of participants. Participants were either Naïve and informed the touch reference was stable, or Aware that the touch reference could move. Half of the participants in each group were tested with their eyes open (EO) and the other half with eyes closed (EC). Participants stood on a foam block placed atop a force plate from which the position of the center of pressure (CoP) was calculated. Sway area, measured as the area of a 95% confidence ellipse of the CoP position, and mean sway velocity were calculated over 60 s periods. In addition, the mean amplitude of the full-wave rectified electromyographic activity from tibialis anterior and soleus were. For participants tested in the EC conditions, sway area increased after the touch reference was repeatedly moved, compared to before the presentation of the perturbations, regardless of whether the participants were Naïve or Aware. However, the larger sway area was less than that observed during EC standing without touching the reference. In contrast, for participants tested in the EO conditions repeated displacement of the touch reference had no effect on sway area. Light touch reduced sway,

relative to standing without touch, equally before and after the reference became unreliable. We suggest that when a touch reference becomes unreliable the contribution of this sensory cue to the regulation of balance is reduced, leading to the increase in sway observed in the absence of vision. However, in the absence of another, more reliable spatial reference (vision) light touch cues continue to be utilized, but to a lesser degree. The lack of difference in the EO conditions suggests that in the presence of a more reliable spatial reference (vision) the reduction in sway associated with light touch might serve a different purpose, such as facilitating the skilled task of maintaining precise touch.

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Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: NIH grant 5R01NS072343-02

DARPA contract N66001-11-C-4171

Title: Dorsal root ganglia stimulation elicits behavioral responses during translational postural perturbation

Authors: *L. E. FISHER[1], K. KING[2], W. CUSACK[3], A. NANIVADEKAR[2], R. GAUNT[1], D. WEBER[2];
[1]Physical Med. and Rehabil., [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]St. Jude Med., Sylmar, CA

Abstract: An effective somatosensory neural interface (SSNI) is necessary to restore sensory feedback in limb prostheses. The dorsal root ganglia (DRG) are attractive targets for stimulation because they are the only structures in which primary afferent (PA) neurons can be accessed in isolation from motor efferents. To develop an effective SSNI at the DRG, it is necessary to understand the parameters of stimulation that achieve desired behavioral effects. To do so we analyzed limb kinematics, ground reaction forces, and EMG responses from the hindlimb of a cat during translational postural perturbations where stimulus location, amplitude, and latency between stimulation and perturbation onsets were experimental variables. Awake, behaving cats underwent translational perturbations following electrical stimulation at the DRG. EMG electrodes were implanted in the flexor and extensor muscles of the hip, knee, and ankle and

used to record muscle activity during headward and tailward perturbations. Markers attached to the hip, knee, ankle, and metatarsophalangeal joints were used to track hindlimb motion. Force transducers in the platform measured ground reaction forces under each paw. Animals were implanted at the L6 & L7 DRG with penetrating, multichannel microelectrode arrays that were used to deliver microstimulation to activate Group I or Group II/Aβ PA neurons. A nerve cuff electrode was implanted around the sciatic trunk to measure evoked antidromic activity in response to stimulation through each channel of the MEA in the anesthetized animal. Channels that activated Group I PA neurons were identified based on the conduction velocity of the antidromic response (i.e. 75-120 m/s). Each week, a stimulation channel was selected based on the minimal current required to drive an antidromic response in a Group I PA neuron. These specific channels were then selected to deliver current-controlled biphasic stimulation 50-150 milliseconds prior to platform perturbation at suprathreshold amplitudes. Stimulating the DRG results in coordinated and repeatable effects on postural responses to platform translations. These effects varied with selection of stimulation electrode, amplitude, and latency. Inhibitory and excitatory responses were observed in the EMG, and these tended to be grouped according to the flexor or extensor actions of the hindlimb muscles. These changes in EMG activity were also reflected in the ground reaction force for the stimulated limb. The distinct and repeatable effects on EMG activity were graded with stimulation amplitude, and suggest reliable recruitment of specific reflex responses in the spinal cord.

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Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

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Topic: E.06. Posture and Gait

Support: EICCI from the Cluster of Excellence 227 (CITEC), DFG

DFG grant BU 857-14

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Title: Force sensing and muscle synergies in insects: adapting proprioception to motor action in serially homologous legs

Authors: *S. N. ZILL[1], J. SCHMITZ[2], A. BŰSCHGES[3], S. CHAUDHRY[1];
[1]Anat. and Pathology, J.C. Edwards Sch. Med., Huntington, WV; [2]Biol. Cybernetics, Univ. of Bielefeld, Bielefeld, Germany; [3]Zoological Inst., Univ. of Cologne, Cologne, Germany

Abstract: The effects of proprioceptive sense organs on motor actions are not constant but are modified by the nervous system to be task-specific. We have studied how force feedback from leg sense organs in insects affects the patterns of activation of muscle as synergists. Campaniform sensilla are receptors that monitor forces as strains in the exoskeleton. Our previous studies showed that, in generating substrate grip, receptors of both proximal and distal leg segments produce activation of the same group of synergist muscles. In the present study we test whether the similar mechanisms are present in serially homologous (front, middle and hind) legs, as they produce diverse forces in posture and locomotion. Groups of receptors were stimulated mechanically (half sine or ramp and hold waveforms) and motor activities were recorded myographically. Animals were induced to seek substrate grip by eliciting righting responses. Stimulation of campaniform sensilla reinforced muscle synergies in all legs. Activation of groups of muscles of distal leg segments was relatively constant: stimulation of the tarsal or trochanteral sensilla reinforced activities of leg muscles (flexor tibiae, retractor unguis) that act to generate substrate grip. However, the strength of activation of proximal leg muscles varied. Stimulation of tarsal sensilla in middle or hind legs produced strong depressor activation but this effect was weak or absent in front legs. In addition, while positive force feedback from trochanteral sensilla to the trochanteral depressor was present in all legs, the gain varied systematically: data to date indicate that the gain is highest in hind legs, weaker in middle legs and variable in occurrence in front legs. Preliminary experiments also suggest that positive feedback to other muscles may also occur from activation of trochanteral campaniform sensilla. Both the strength and sign of muscle activation in different legs may, therefore, reflect their use in behavior and the specific forces they generate. Current experiments are also testing the effects of changes in parameters (amplitude, waveform) of the force stimulus on sensory and motor discharges: use of dynamic stimuli (without a static hold phase) can decrease sensorimotor adaptation and reduce the effects of viscoelasticity in the exoskeleton. In sum, our studies support the idea that force feedback can reinforce muscle synergies to ensure that substrate adhesion is rapidly established after substrate contact, providing a stable point for force generation. However, the process is task-specific and adapted to the specific use of the legs in behavior.

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Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

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Program#/Poster#: 335.15/QQ10

Topic: E.06. Posture and Gait

Support: NIH NINDS R01NS086973

Title: Role of knee joint afferents in rat locomotion

Authors: *C. ALESSANDRO, F. BARROSO, M. TRESCH;
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Abstract: Mechanoreceptors within the knee joint are involved in neural circuitries that potentially regulate flexor and extensor muscles. However, the actual role of these receptors in task regulation is still not clear. Various works reach contradictory conclusions, with some of them observing profound impairments after silencing knee joint receptors in cats. In this poster we will present preliminary results of a systematic study to assess whether, and to which extent, knee joint afferents affect task execution.

We analyzed locomotion kinematics of a set of rats walking on a treadmill at a range of speeds and inclines before and after reversible deafferentation of the knee joint. Suppression of joint receptors has been obtained by injecting local anesthetic (lidocaine, 0.05 ml) into the joint capsule. We have compared locomotion kinematics before, 30 minutes after and 2 hours after injection (when the effect of the local anesthetic is minimal) for each subject. We compared the effects elicited by the local anesthetic to those produced by equal volume injections of saline into the knee joint in the same animals.

The administered quantity of local anesthetic results in an effective suppression of joint receptors, as shown in separate acute experiments, and does not affect nearby peripheral nerves. Volumes of 0.1 ml or higher caused notable spillover to nearby nerves, and consequent paralysis of distal muscles. Such injections caused obvious and significant impairments to locomotor kinematics, observable as a substantial foot drop. Similar large effects on distal muscles were never observed with the smaller volumes of lidocaine.

We recorded the 3D kinematics of the hindlimb across at least 20 cycles of locomotion. Standard kinematic measures (hip, knee, ankle, and metatarsophalangeal angles) and functional parameters (cadence, percentage of stance, stance duration) were extracted for each step and used to characterize the effects of lidocaine and saline injections. In the 3 animals examined thus far, lidocaine and saline had no obvious effect on any of these kinematic measures. We are currently examining the role of knee joint afferents in a wider range of subjects and behavioral conditions, both at the kinematic and muscle activation levels. If the results obtained so far were

confirmed, they would suggest that sensory receptors from the knee do not play a substantial role in regulating locomotor performance.

Disclosures: **C. Alessandro:** None. **F. Barroso:** None. **M. Tresch:** None.

Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: Massachusetts Technology Transfer Center Seed Fund Award

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Title: Enhancement of ankle position and force controls contributes to balance improvement

Authors: ***S.-C. YEN**[1], M. POLETTI[1], A. FARJADIAN[2];
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Abstract: Deficits in ankle proprioception are often associated with poor balance control. The ability to adjust ankle position and force output in response to balance threat is reduced when muscle spindles and Golgi tendon organs are impaired. We developed an ankle robotic trainer (ART) to train ankle proprioception and improve balance control. The ART is interfaced with the user with a virtual "maze" game. The user uses the ankle to control the ART as a joystick to virtually move an object through the maze as fast as possible while minimizing wall collisions, which is a task that demands accurate and precise ankle control. The game has two modes: position control and force control. In the position control mode, the user controls the virtual object by changing the direction and magnitude of ankle motion. Such control largely requires proprioceptive feedback from muscle spindles. In the force control mode, the user controls the virtual object by changing the direction and magnitude of isometric force output from the ankle muscles. Such control largely requires proprioceptive feedback from Golgi tendon organs. We hypothesized that repetitive practice of the position control and force control through the virtual game will improve balance control. To test this hypothesis, we recruited 9 subjects with chronic ankle instability, which is a population known to have poor balance control. They practiced the position control and force control games using the ART for 6 sessions in 3 weeks. Subjects' balance was assessed before and after the training using the Biodex Balance System. After the training, subjects showed significant improvements in ankle movement time (measured by the time used to complete the virtual game), ankle movement accuracy (measured by the number of

wall collisions when the subject played the virtual game), and balance control (measured by Biodex stability index). Regression analysis revealed that the improvement in balance can be mainly predicted by the improvement in the movement time when subjects played the virtual game with either position control or force control. Two possible mechanisms may explain the improvement. First, the training repetitively stimulates muscle spindles and Golgi tendon organs, which may enhance their ability to detect changes in ankle position and tension in a balance task. Second, through the training individuals may enhance the ability to adjust ankle position and force outputs according to the proprioceptive feedback received. Our results provide preliminary evidence to suggest that repetitive practice of ankle position control and force control could improve balance.

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Poster

335. Posture and Gait: Afferent Control

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Conselho Nacional de Desenvolvimento Cientifico e Tecnologico - CNPq

Title: Role of muscle spindle feedback in the swing movement dynamics and foot placement during walking

Authors: *W. P. MAYER[1,2], W. G. TOURTELLOTTE[3], T. AKAY[1];
[1]Dept. of Med. Neurosci., Dalhousie University, Brain Repair Ctr. - AMAP, Halifax, NS, Canada; [2]Dept. of Morphology, Federal Univ. of Espirito Santo, Vitoria, ES, Brazil; [3]Dept. of Pathology and Neurol., Northwestern Univ., Chicago, IL

Abstract: During walking, each step consists of stance and swing phases. During stance, the foot is on the surface and moves from the anterior extreme position (AEP) to the posterior extreme position (PEP). During swing, the foot is in the air and moves from PEP to AEP, where it touches down on the surface and starts the next stance phase. Previous experiments (Akay et al, 2014; PNAS 111:16877) demonstrated that *Egr3* knock out mice (*Egr3-KO*), in which muscle

spindles (MSs) are absent, can walk on a treadmill at 0.2m/s with subtle abnormalities. Specifically, it was observed that the activity of the ankle flexor muscle (tibialis anterior, TA) is active throughout the swing phase in contrast to wild types in which TA activity is terminated mid-swing. The prolonged TA activity is expressed in exaggerated lifting of the foot during swing phase. Additionally, *Egr3-KO* mice are severely compromised when challenged to walk on a horizontal ladder that would require precise foot placement from rung to rung. Here we hypothesize: "**the MS feedback is necessary for the precisely timed TA offset required to place the foot in a predefined AEP location at the end of swing phase during walking.**" We first measured the average distance of hind leg AEP (HLAEP) distance relative to fore leg PEP (FLPEP) as 4 mm with a standard deviation (SD) of 2 mm in wild type (WT) mice during treadmill walking at 0.2 m/s. This distance did not change, even when the swing was perturbed mechanically (5 mm, SD: 3 mm, $p=0.09$ after t-test) or through electrical stimulation of the saphenous nerve (3 mm, SD: 1 mm, $p=0.21$), both causing stumbling corrective response (SCR). The average HLAEP-FLPEP distance in *Egr3-KO* mice was not only significantly larger (14 mm, $p<0.001$) but it was also more variable (SD: 5 mm, $p<0.001$ after f-test) than in WT mice during unperturbed walking. When swing phase was perturbed by mechanical stimulation in *Egr3-KO* mice, the HLAEP-FLPEP distance did not change significantly from unperturbed swing movements in *Egr3-KO*s, however trajectory of foot movement became more variable. We are currently analyzing the EMG data from SCR experiments with wild type and *Egr3-KO* mice, to address the question of how the muscle activation pattern is related to the targeting phenomenon. Nevertheless, our data suggest that normal swing movement trajectory during walking and precise foot placement at AEP requires MS feedback.

Disclosures: **W.P. Mayer:** None. **W.G. Tourtellotte:** None. **T. Akay:** None.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.18/QQ13

Topic: E.06. Posture and Gait

Support: NIH Grant P01 HD-32571

NIH Grant R01 EB-012855

Center for Human Movement Studies at Georgia Institute of Technology

Title: Modulation of input from paw cutaneous afferents and quadriceps-sartorius stretch afferents differentially affects lateral static and dynamic stability during cat split-belt locomotion

Authors: *H. PARK[1], R. MEHTA[2], S. P. DEWEERTH[1,3], B. I. PRILUTSKY[2];
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Abstract: Motion dependent sensory input from paw cutaneous afferents and muscle proprioceptors allows animals to maintain stable locomotion in the changing environment (Bouyer, Rossignol 2003; Akay et al. 2014). The goal of this study was to investigate how modulation of paw cutaneous input and input from stretch sensitive afferents from quadriceps and sartorius muscles affect static and dynamic stability of walking in the cat. Four cats performed 80-s bouts of locomotion on a split-belt treadmill (Bertec, USA) with different speed ratios between left and right belts – 0.4:0.4 m/s, 0.4:0.6 m/s, 0.4:0.8 m/s. These locomotor conditions were repeated while tactile cutaneous input from paw pads of right fore- and hindlimbs was changed by anesthetic injections or/and electrical stimulations of distal tibial or/and sural nerves (</= 1.2T) during right hindpaw contact. In a separate set of experiments, the above experimental conditions were repeated after stretch reflex was removed from the right quadriceps and sartorius by muscle self-reinnervation (Cope et al. 1994). Center of mass (CoM) position of the cat was determined from 3D recordings of 28 markers on the cat body and known inertial body segment parameters (Hoy, Zernicke 1985). Center of pressure (CoP) was derived from paw positions on the treadmill and measured ground reaction forces. Static stability was determined as the shortest distance between the vertical projection of CoM and the boundary of the support area in the lateral direction, whereas the margin of dynamic stability was defined as the distance between CoP and the extrapolated center of mass (xCoP, Hof et al. 2007). With the equal belt speeds, paw anesthesia increased margins of static and dynamic lateral stability due to more lateral placements of the affected paws and shifting CoM in the same direction. Combining paw anesthesia with electrical stimulations of distal tibial or sural nerves tended to reduce these effects. During split-belt locomotion with unequal belt speeds, margins of static lateral stability decreased due to shifting CoM towards the slow moving belt, while margins of dynamic stability did not change. Paw anesthesia and quadriceps-sartorius self-reinnervation increased margins of dynamic lateral stability at all speed ratios owing to more lateral paw placements of the affected limbs. Thus in more challenging locomotor conditions (unequal belt speeds or reduced sensory input), cats adopt motor strategies that allow for increased dynamic lateral stability.

Disclosures: H. Park: None. **R. Mehta:** None. **S.P. DeWeerth:** None. **B.I. Prilutsky:** None.

Poster

335. Posture and Gait: Afferent Control

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 335.19/QQ14

Topic: E.06. Posture and Gait

Title: Effects of increased arm swing cued by a wearable device on gait parameters

Authors: *E. D. THOMPSON[1], H. REIMANN[1], T. FETTROW[1], P. AGADA[1], S. WEISS[1], M. LEE[1], J. JEKA[1,2];
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Abstract: Gait deficits such as small steps and decreased speed are effects of many neurological pathologies, including stroke and Parkinson's disease. These impairments place patients at increased risk of falls, functional decline, and loss of independence, and are often aggressively treated in preventive or post-injury rehabilitation. However, carryover of improvements from physical therapy to community mobility can be inconsistent and cognitively demanding, and may be beyond the capability of some patients. Those who have poor awareness of their limb position have been shown to benefit from external cues to improve walking. However, most commercially-available technology for monitoring movement (such as fitness trackers or mobile apps) cannot deliver specific feedback about limb position or joint angle, limiting their usefulness to these populations. Here we present preliminary testing of a device to cue a larger arm swing during walking, and assess the effects of this device on gait parameters in a healthy young population.

Healthy young adults participated in this preliminary study, walking on a self-paced treadmill with reflective markers on their feet, wrists, and low back. Each subject also wore a wireless device on each wrist to measure and cue arm swing using commercially available accelerometers, gyroscopes, and vibrating motors. Arm swing amplitude and gait parameters (velocity, cadence, step length and width, trunk lean) were assessed during baseline walking. A target arm swing angle was then calculated, corresponding to 120% of the mean swing for each arm during the baseline trials. Following calculation of the target angles, ten two-minute walking trials (five cued, five uncued) were performed in randomized order. During cued trials, a vibration indicated the target angle had been reached, and subjects were instructed to swing their arm until they received this vibratory cue. For uncued trials, no vibration or specific subject instructions were given.

All subjects demonstrated significant increases in arm swing, gait speed, and step length on cuing trials versus baseline. Increases in gait speed and step length were maintained in the subsequent uncued trials, while arm swing returned to baseline level. We observed variable differences in step width between subjects or conditions. We observed no significant differences in lateral or anterior-posterior trunk lean between conditions.

These results indicate that vibratory arm swing cues delivered by a portable sensor system can significantly impact gait parameters. Further research is needed to determine possible uses of this device to improve gait in people with neurological deficits.

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Poster

335. Posture and Gait: Afferent Control

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Marie Curie International Outgoing Fellowship PIOF-GA-2013-624861

Title: Rorb interneurons act as essential spinal filters for the refinement of motor movements

Authors: *S. C. KOCH, M. GARCIA DEL BARRIO, A. DALET, G. GATTO, M. GOULDING;
The Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: Studies to date point to essential roles for sensory feedback in shaping the locomotor output. Proprioceptive inputs arising from muscles and tendons, as well as cutaneous input from the skin have been shown to modify stepping behaviour allowing for tuned responses to environmental cues. These effects have been attributed to spinal integration, but how this sensory information is relayed and filtered within the spinal cord, and the mechanisms that elicit the sensory-evoked refinement of the motor output have not been fully described. We have identified a subpopulation of spinal dorsal inhibitory interneurons that can be genetically targeted by the expression of the nuclear orphan receptor *RORβ*. Using a combinatorial approach involving genetic manipulation, behavioural analysis, anatomical tracing and electrophysiology, we find that RORβ interneurons selectively inhibit cutaneous and proprioceptive afferent input into the spinal cord. Deletion of *RORβ* from inhibitory interneurons in the dorsal horn results in an altered motor gait, which is driven by aberrant cutaneous and proprioceptive input. Pharmacological silencing of afferent input from the periphery is sufficient to attenuate the behavioural phenotype highlighting the important function of inhibitory RORβ interneurons in the integration of cutaneous and proprioceptive sensory input for normal locomotion. As such, these neurons provide a critical piece in the puzzle of the sensory-motor interface.

Disclosures: S.C. Koch: None. **M. Garcia Del Barrio:** None. **A. Dalet:** None. **G. Gatto:** None. **M. Goulding:** None.

Poster

335. Posture and Gait: Afferent Control

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Program#/Poster#: 335.21/QQ16

Topic: E.06. Posture and Gait

Title: The sensory origin of the perception of heaviness at the shoulder

Authors: *D. PHILLIPS, A. KARDUNA;
Human Physiol., Univ. of Oregon, Eugene, OR

Abstract: The sense of heaviness may arise from two sources: 1) sensory receptors located in the periphery and/or 2) the centrally generated motor command. Evidence from contralateral force matching studies with fatigue and contralateral muscles at different lengths indicate that the sensation arises from the central command. However the fatigue model is unable to differentiate between central and peripheral effects of fatigue and changes in muscle length may affect the response of proprioceptors. We propose the use of a suprascapular nerve block model during a contralateral elevation force matching task at the shoulder. The suprascapular nerve block creates a disassociation between the shoulders in incoming afferent information but does not affect the efferent command. Also the motor capacity in the blocked shoulder will be reduced due to the loss of the supraspinatus muscle compared to the unblocked shoulder. We hypothesize that the matching limb will overshoot the reference target force in the blocked limb. Five subjects performed the contralateral force matching task at the shoulder without the nerve block. Subjects were seated so that their arms were elevated to 90° and their wrists were strapped to load cells. Vision of the environment was occluded with a virtual reality headset which also provided visual feedback to the force target for the reference arm. After maintaining the target force level for 1s, subjects matched the perceived force with the opposite limb. Errors in force (N) were converted to torque errors (Nm). Errors were calculated as constant error (CE) indicating the amount of under or over shoot (bias) of the target and root mean square (RMS) error indicates accuracy. Four trials for three targets (120%, 140%, 160% of baseline torque to hold the arm at 90° elevation) were recorded in a randomized order. CE improved with higher load targets, 120% (M = 3.6, SEM = 0.7), 140% (M = 2.7, SEM = 0.6), 160% (M = 1.5, SEM = 0.5). RMS error became more accurate at higher loads. This is consistent with previous research on ipsilateral force matching protocols at the shoulder.

Disclosures: D. Phillips: A. Employment/Salary (full or part-time): University Of Oregon. **A. Karduna:** A. Employment/Salary (full or part-time): University of Oregon.

Poster

335. Posture and Gait: Afferent Control

Location: Halls B-H

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Program#/Poster#: 335.22/QQ17

Topic: E.06. Posture and Gait

Support: Hima and Jive Fellowship in Computer Science for International Students

Title: A spinal reflex based neuromuscular model of human locomotion investigated against unexpected disturbances

Authors: *S. SONG, H. GEYER;
Robotics Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The control mechanism of the human spinal cord in generating muscle activations for locomotion has been studied over decades. One of the main hypotheses is that central pattern generators (CPGs) produce the background activations, and spinal reflexes produce compensatory activations against disturbances. However, the contribution of CPGs in human locomotion has not been verified. To this end, we investigate this hypothesis using a previously proposed CPG-free, proprioceptive-reflex-based neuromuscular simulation model. It has been demonstrated in physics simulation that the model generates human-like kinematics, dynamics and muscle activations. Furthermore, the same control with different control parameters generates various locomotion behaviors, such as running, negotiating slopes and stairs, and changing walking directions. Here, we examine the reflex based model with a range of unexpected disturbances to test the necessity of CPGs in explaining the responses of humans during walking. Five disturbance experiments from the literature, which encompass most artificial (epidural stimulation, tendon tap, and local joint stretches) and natural disturbances (trip and slip) used in human gait experiments, are replicated in simulation with the reflex based neuromuscular model, and its responses (immediate changes in muscle activations) are compared with those reported from the human experiments. The response trends over the gait cycle and disturbance intensities of the model matches well with those of humans against all disturbances; excluding the noticeably defective responses, which also can be improved within the proprioceptive reflex based control structure, about 80% of the properly scaled model responses fall within 1 standard deviation of human responses. However, a fundamental change in the control structure seems necessary to explain the response amplitudes of humans; the response amplitudes of the model are similar to those of humans only for artificial disturbances and are much smaller for natural types of disturbances. Adding cutaneous reflex pathways to amplify the activities of the proprioceptive reflexes of the model may explain the large human responses against natural types of disturbances. Interestingly, the current study suggests to include more

reflexes to the CPG-free reflex based model, leaving obscure the functionality and necessity of CPGs in human locomotion.

Disclosures: S. Song: None. **H. Geyer:** None.

Poster

335. Posture and Gait: Afferent Control

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Program#/Poster#: 335.23/QQ18

Topic: E.06. Posture and Gait

Support: NS-057228

Title: A novel work loop approach for decoding sensory information in afferent nerves during cyclic muscle contractions.

Authors: G. S. SAWICKI[1], *P. NARDELLI[2], T. C. COPE[2];
[1]Biomed. Engin., NC State Univ. and UNC Chapel Hill, Raleigh, NC; [2]Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: Classical studies using passive stretching paradigms strongly indicate that muscle spindle receptors (IA and Group II) encode muscle parameters including length/velocity while Golgi tendon organ receptors (IB) encode muscle force. However, data from afferent nerves of muscle-tendon units acquired during *in situ* passive stretching paradigms may not provide an accurate representation of the *in vivo* conditions during locomotion in a freely moving animal. First, muscle is not always passive during locomotion. Second, stretch in series elastic tissues (*i.e.,* tendon and aponeurosis) decouples length changes of the whole muscle-tendon from length changes experienced by the component muscle fascicles and spindle receptors [1]. Finally, during locomotion, length change patterns are cyclical and arise from the interaction of muscle-tendon forces and limb/body dynamics [2]. Each of these facts motivated us to develop an updated framework for re-evaluating the roles muscle receptors in encoding sensory information during locomotion.
We recently developed two novel muscle-tendon (MT) work loop protocols that incorporated the use of sonomicrometry to directly measure muscle length changes within the MT (*i.e.,* decoupling muscle from muscle-tendon) [1, 2] and a 'smart-ergometer' programmed to enforce the dynamics of a virtual limb/body load (*i.e.,* inertial and gravitational forces) [2]. These innovations capture salient features of 'real-world' locomotion and provide an opportunity to circumvent the confounding issues involved in passive stretching protocols for decoding sensory information in afferent nerves.

Here, we demonstrate the feasibility of *in situ* experiments that employ a MT work loop approach to study rat plantarflexor muscle-tendons (*e.g.,* soleus and gastrocnemius) under locomotion-like conditions while directly recording MT force and length from an ergometer, muscle fascicle length from implanted sonomicrometry crystals and instantaneous firing rates from either IA, IB or group II afferent nerves using a glass pipette electrode implanted in the dorsal root of the spinal cord. We demonstrate that directly controlling the phase when muscle is active during a contraction cycle it is possible to generate a myriad of conditions that exhibit unique decoupling between force and length change dynamics—providing the opportunity systematically decode information in muscle receptors in a context that approaches conditions during 'real-world' locomotion.

[1] Sawicki GS, Robertson BD, Azizi E, Roberts TJ., *J Exp Biol.* (2015). doi: 10.1242/jeb.121673.
[2] Robertson BD, Sawicki GS. *Proc Natl Acad Sci U S A.* (2015). doi: 10.1073/pnas.1500702112.

Disclosures: **G.S. Sawicki:** None. **P. Nardelli:** None. **T.C. Cope:** None.

Poster

335. Posture and Gait: Afferent Control

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Topic: E.06. Posture and Gait

Support: NSERC Discovery Grant

Title: Human microneurography reveals muscle spindles encode small/slow ankle movements associated with standing

Authors: *J. INGLIS[1], R. M. PETERS[2], B. H. DALTON[3], J.-S. BLOUIN[2];
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Abstract: Owing to the recent observation of only small, paradoxical muscle movements of the plantar flexors during quiet stance, the contribution of calf muscle spindles during standing balance has recently been questioned (Loram & Lakie, 2002; Loram et al., 2009). To investigate whether calf muscle spindles are sensitive enough to provide useful feedback during the small, slow ankle movements associated with quiet stance, we collected microneurographic recordings from human lower-limb muscle spindles. Muscle spindle afferents were recorded from the tibial

(n=12) and common fibular (n=3) nerves in 9 healthy, young adults. Currently our dataset consists of 5 muscle spindles from lateral gastrocnemius (LG), 2 from medial gastrocnemius (MG), 4 from soleus, 2 from intrinsic foot muscles, and 3 from tibialis anterior (TA). When a muscle spindle was identified, the foot was loaded into an actuated ankle platform instrumented with a load cell. Participants were exposed to traditional ramp-and-hold stretches having specific displacement, velocity and acceleration parameters, both with and without a low-level background contraction. To examine how each of these kinetic variables is signalled in the afferent spike train, a matrix of ankle movements comprising angular displacements of 2 to 6° (± 0.02°), angular velocities of 10, 25, and 50°/s (± 2°/s), and angular accelerations of 50 to 1000°/s² (± 25°/s²) were explored. Preliminary results indicate clear coding of ankle angular displacement and velocity in the static discharge rate and early dynamic response, respectively, while coding of acceleration is less evident. Participants were further exposed to continuous ankle plantarflexion-dorsiflexion movements with a power spectrum replicating that of quiet stance (peak-to-peak amplitude = 0.7°; bandwidth = 0 to 0.5 Hz; mean power frequency = 0.28 Hz). 3/4 Soleus spindles, 2/2 MG spindles, and 2/5 LG spindles coded for such small amplitude ankle movements, and coherence estimates suggest that the order of triceps surae muscle spindle sensitivity to ankle plantar flexion-dorsiflexion is soleus (most sensitive), MG (median), and LG (least sensitive). Intriguingly, this order of sensitivity resembles the order of muscle activation during standing balance, with the soleus tonically active, MG having ballistic “catch and throw” activation, and LG remaining relatively silent.

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References:

Loram & Lakie. *J Phys* 545.3: 1041-1053, 2002.
Loram et al. *Med Sci Sports Exerc* 41: 198-204, 2009.

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Poster

336. Neural Control of Respiration II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 336.01/QQ20

Topic: E.08. Respiratory Regulation

Title: Changes in protein expression and ventrolateral medulla network properties accompanying *In utero* mu-opioid agonist and/or nociceptin receptor antagonist exposure

Authors: *N. M. MELLEN[1], B. GOURÉVITCH[3], J. M. CAI, 40202[2], N. TOPORIKOVA[4]; [1]Kosair Children's Hosp Res. Inst., [2]Univ. of Louisville, Louisville, KY; [3]NeuroPsi, UMR CNRS 9197, Paris, France; [4]Washington and Lee Univ., Lexington, VA

Abstract: Opioid exposure during pregnancy causes neonatal abstinence syndrome (NAS), whose symptoms include autonomic dysregulation, likely due to cellular and network counteradaptations to prolonged mu-opioid receptor (MOR) activation. In addition, in utero opioid exposure results in hypomyelination, which may account for developmental delays in opioid-exposed children. Acute NAS symptoms are currently treated with morphine, which likely exacerbates hypomyelination. Because opioids inhibit oligodendrocyte differentiation via action at nociceptin receptors (NOP) rather than MORs, coadministration of an opioid agonist and an NOP antagonist might enable management of opioid dependence while blunting hypomyelination. To assess the feasibility of this approach, the effects of in utero exposure to methadone (MTD; 3 mg/kg/day) and/or the NOP antagonist JTC-801 (1 mg/kg/day), administered to the dam from E7 onwards by addition to drinking water were studied. Changes in myelin-related protein expression levels were assessed via Western blot analysis of brain tissue harvested at P7, P12, and P21, which revealed MTD-induced inhibition on expression of myelin proteins, and JTC-801-induced recovery of myelin proteins, when compared to control tissue. Changes in neuronal and network excitability were assessed via optical recordings of autonomic regulatory networks in ventrolateral medulla in the sagittally sectioned rat hindbrain preparation (P0-P2) at 3 (physiological), 6, and 9 mM $[K^+]_o$ In control, and JTC-801 exposed pups, manipulations of $[K^+]_o$ did not significantly alter respiratory period, but in pups exposed to both MTD and JTC-801, respiratory period was significantly shorter at all $[K^+]_o$. In addition, in JTC-801 exposed pups, inspiratory duration was significantly lengthened. These findings are evaluated within the context of a generic model of respiratory rhythmogenesis, in which phasic and tonic synaptic inputs interact to modify respiratory period and inspiratory duration.

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Poster

336. Neural Control of Respiration II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 336.02/RR1

Topic: E.08. Respiratory Regulation

Title: Inflammation alters cell expression in the respiratory regions of neonatal brainstem of rats

Authors: *C. G. WILSON[1], R. JOHNSON[2], S. MURRAY[2];
[1]Ctr. for Perinatal Biol., [2]Loma Linda Univ., Loma Linda, CA

Abstract: Sepsis, or systemic infection, and the effects of inflammation represent a multi-billion dollar healthcare burden costing thousands of dollars per patient. Pre-term infants, especially, are at high risk for systemic infection due to an underdeveloped innate immune system. They are also at risk for respiratory tract infection due to in utero exposure to bacteria, during normal vaginal birth or after birth. Airway infection results in immune upregulation, including the production of early pro-inflammatory cytokines interleukin-1 beta (IL-1beta), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFalpha). Our laboratory has previously shown that lipopolysaccharide (LPS) injected into the trachea of neonatal rats causes changes in IL-1beta, IL-6 and TNFalpha expression in the autonomic brainstem regions responsible for the control of breathing including. These regions include the *nucleus tractus solitarii* (NTS), rostroventral medulla, and the hypoglossal motonucleus (XII) (Balan et al. 2011, Gresham et al. 2012, Jafri et al. 2013, Johnson et al. 2016). We hypothesize that, in addition to cytokine upregulation, inflammatory stimuli cause cell-specific changes in number and morphology of neurons, astrocytes, and microglia. We have shown that the number of neurons in cardiorespiratory regions do not change in response to LPS injection. Our recent stereological studies show that there is no significant difference in astrocyte expression in animals that have been treated with LPS (0.05 mg/kg). Unbiased stereology counts between sham and saline ($p=0.3$), saline and LPS ($p=0.4$), and LPS and sham ($p=0.9$) showed no significant difference in cell numbers between treatments. We are currently assessing the number of microglia and determining if the early pro-inflammatory cytokines, IL-1beta, IL-6 and TNFalpha, are upregulated in specific cell types.

Disclosures: C.G. Wilson: None. **R. Johnson:** None. **S. Murray:** None.

Poster

336. Neural Control of Respiration II

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Program#/Poster#: 336.03/RR2

Topic: E.08. Respiratory Regulation

Support: CIHR grant TGS-109219 to BRS

CIHR Studentship to VS

Title: Effects of melatonin, 5-hydroxytryptamine & acetazolamide on hypoxia induced depression of field excitatory postsynaptic potentials in rat hippocampal CA1 neurons

Authors: N.-G. KANG, J. HWANG, Q. NGUYEN, V. SUEN, *B. SASTRY;
Univ. British Columbia Fac Med., Vancouver, BC, Canada

Abstract: Central neutrons can be subjected to hypoxia by hemorrhagic strokes, ischemic heart attacks, sleep apnea, high altitudes, etc., leading to functional compromise. Since prolonged hypoxic insult can cause irreversible damage, agents that facilitate a recovery from the early effects of hypoxia can be useful therapeutically. Melatonin and its precursor 5-hydroxytryptamine (5-HT) are implicated in fostering and regulating sleep. Acetazolamide, a carbonic anhydrase inhibitor, is used to treat high-altitude sickness and central sleep apnea. In this study, using the male Wistar rat (3-4 week old) hippocampal slice preparation, we examined the effects of hypoxia on the stratum radiatum stimulation-induced field excitatory postsynaptic potential (fEPSP) in the CA1 pyramidal region. Whether melatonin, 5-HT and acetazolamide alter the actions of hypoxia on the fEPSP was also tested. In controls, hippocampal slices from 3-4 week old Wistar rats were exposed to N_2/CO_2 until the fEPSP was depressed to 50% of the pre-exposure amplitude and then back to carbogen until the fEPSP returns to pre-hypoxia control size for at least 5 min. The procedure was then repeated for two more times. In one series of test experiments, the above protocol was followed while slices were exposed to 1 – 30 μM melatonin. Melatonin, in concentrations of 10 and 30 μM, significantly ($P<0.05$) accelerated the recovery from the hypoxia-induced depression of the fEPSP. In other test series, slices were exposed to 5-HT (2.5 - 10 μM) or acetazolamide (0.5 - 5 mM). Neither drug accelerated the recovery of the fEPSP from hypoxia-induced depression. These results indicate that melatonin may be useful in restoring excitatory synaptic transmission from hypoxic alterations. Whether the agent is useful in treating sleep apnea, high altitude sickness, etc., needs further investigation.

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Poster

336. Neural Control of Respiration II

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Program#/Poster#: 336.04/RR3

Topic: E.08. Respiratory Regulation

Support: National Institute of Health HL104101

Connecticut Department of Public Health Grant 150263

Title: Bicarbonate-dependent inhibition of chemosensitive neurons of the retrotrapezoid nucleus.

Authors: *C. M. GONÇALVES, F.-S. KUO, E. DANIEL, D. K. MULKEY;
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Abstract: Chemoreception is the mechanism by which the brain regulates breathing in response to changes in tissue CO_2/H^+. A brainstem region called the retrotrapezoid nucleus (RTN) is an important site of chemoreception. The mechanisms of RTN chemoreception involve direct H^+-mediated activation of chemosensitive neurons and indirect modulation of chemosensitive neurons by CO_2/H^+-evoked ATP release from astrocytes. However, the extent to which ATP contributes to RTN chemoreception is questionable; some studies report that ATP release from astrocytes is required for RTN neurons to sense changes in CO_2/H^+, whereas other studies showed that disruption of neuronal H^+ sensors eliminated the ventilatory response to CO_2. Based on evidence that CO_2 is required for ATP release by RTN astrocytes, we chose to investigate the purinergic contribution to RTN chemoreception by comparing firing responses of RTN neurons to graded acidifications in the presence and absence of CO_2 and HCO_3^-. Brainstem slices (300 μm) from rat pups (7-12 days postnatal) were incubated in a bicarbonate (26mM) buffered solution equilibrated with 5% CO_2 (pH=7.3) and cell-attached recordings were used to identify RTN chemoreceptors by their characteristic response to CO_2, i.e., low activity under control conditions (0.7 ± 0.1 Hz) and high activity in 10% CO_2 (1.7 ± 0.3 Hz) or 15% CO_2 (2.2 ± 0.4 Hz). Unexpectedly, we found that switching to a bicarbonate-free HEPES buffered solution decreased activity by 0.9 ± 0.1 Hz. This inhibition was reversible, repeatable and retained in the presence of CNQX, gabazine, and strychinine. We also found that in HEPES, acidifications to pH=7.0 and pH=6.9 increased activity by 0.65 ± 0.2 and 0.8 ± 0.2 Hz, respectively, suggesting that RTN chemoreceptors respond more vigorously to hypercapnic acidosis compared to an equivalent acidification in HEPES buffer. The mechanisms underlying this response likely involve changes in intracellular pH since baseline activity in prolonged HEPES exposure (~40 min) returned to near control levels. However, KCNQ channels also regulate activity of chemosensitive RTN neurons and since these channels have been shown to be inhibited by HCO_3^-, we wanted to determine if the inhibitory response to HEPES transition involves activation of KCNQ channels. We found that bath application of a selective KCNQ channel blocker blunted the HEPES-mediated inhibition by 0.83 ± 0.3 Hz. These findings are consistent with the possibility that CO_2-evoked ATP release from astrocytes contributes in part to RTN chemoreception and suggest that HCO_3^- controls baseline activity of RTN neurons by regulating KCNQ channel activity and possibly influencing intracellular pH.

Disclosures: C.M. Gonçalves: None. **F. Kuo:** None. **E. Daniel:** None. **D.K. Mulkey:** None.

Poster

336. Neural Control of Respiration II

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Title: Hypercapnic acidosis induces glutamate, D-serine, and ATP release from caudal brainstem astrocytes in culture

Authors: *M. OLIVARES[1], V. DONOSO[1], R. CONTRERAS[1], G. ZUÑIGA[1], J. P. HUIDOBRO-TORO[1], I. LLONA[1], R. VON BERNHARDI[2], J. EUGENÍN[1];
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Abstract: Homeostatic regulation of breathing is achieved through feedback information provided by peripheral and central respiratory chemoreceptors. Peripheral chemoreceptors sense $O_2/CO_2/H^+$ in the arterial blood and central chemoreceptors CO_2/H^+ in the interstitial fluid of the CNS and cerebrospinal fluid. In the brainstem, respiratory chemoreceptors are found, among other sites, in the retrotrapezoid nucleus (RTN), nucleus tractus solitarius (NTS), ventral lateral medulla, medullary raphe nucleus (RN), and preBötzinger complex. Astrocytes release gliotransmitters on response to several stimulus. In fact, ATP is released by pH/CO_2 sensitive astrocytes in response to acidosis of the RTN, but in caudal medullary chemosensory nuclei like the NTS and the RN, the purinergic antagonists application does not affect the response to hypercapnia. In the present work, we evaluated whether cultured astrocytes from the caudal brainstem (without including the RTN) or cerebral cortex are able of releasing glutamate (Glu) and D-serine (D-Ser), together with ATP and its derivatives like ADP, AMP and adenosine (ADO), in response to hypercapnic acidosis (exposure to air enriched with 10% CO_2). Two days old CF1 mouse neonates were anesthetized (3% isoflurane), decapitated, and their brains were extracted, disaggregated, and cultured in DMEM-F12 medium equilibrated with air containing 5% CO_2 at 37°C for 2 weeks. During experiments, DMEM-F12 medium was replaced by artificial cerebrospinal fluid. Astrocytes cultures were exposed to 5% CO_2 for 45 min (basal condition), followed by 10% CO_2 for 45 min (hypercapnic acidosis) and finally, returned to 5% CO_2 at 37°C. Samples were collected at 5, 15 and 30 min at basal and hypercapnic conditions.

The concentrations of Glu, D-Ser, ATP and derivatives were measured using high-performance liquid chromatography technique. ATP released from brainstem astrocytes increased 3- fold during first 5 min of hypercapnic acidosis compared to the basal condition whereas Glu and D-Ser increased 3-fold at 30 min of hypercapnia. ADP and AMP concentrations increased during hypercapnic acidosis. By contrast ADO concentration increased once the maximum release of ATP was achieved. In contrast to medullary astrocytes, cortical astrocytes did not release Glu, D-Ser, ATP or its derivatives in response to hypercapnia. Our results indicate that caudal brainstem astrocytes, but not cortical astrocytes are able of release Glu, D-ser and ATP as the main gliotransmitter in response to hypercapnia.

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Poster

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Title: Suppression of astrocytic activation by arundic acid prevents severe hypoxia-induced seizure and death in mice

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Abstract: Mild hypoxia increases ventilation. However, severe hypoxia induces seizure, ventilatory depression and eventually death. This phenomenon in animals may be assumed as a

model of Sudden Unexpected Death in Epilepsy (SUDEP) that is the sudden, unexpected, non-traumatic, and non-drowning death of a patient with epilepsy. Although SUDEP is a serious threat in patients with epilepsy, the precise mechanism of SUDEP remains unclear. We consider that ventilatory disturbance accompanied by seizure underlies the pathophysiology of SUDEP. In the present study we intended to clarify the mechanism of ventilatory depression and death accompanied by seizure. Recent progress in glial physiology has suggested that astrocytes play an active role in the generation of seizure activity. We investigated the hypothesis that astrocytes are involved in occurrence of severe hypoxia-induced seizure, by examining the responses of EEG and ventilation to severe hypoxia before and after administration of arundic acid, an inhibitory modulator of astrocytic function. We analyzed the data obtained from a series of hypoxia experiments of which main data were reported as another study. During this series of experiments 18 mice died, and their data were excluded from that study. This time we analyzed these once excluded data. They were conscious, spontaneously breathing, adult male mice. We recorded EEG and measured ventilation by whole body plethysmograghy. The oxygen concentration in the chamber was monitored with an oxygen analyzer. After recording EEG and ventilation in room air, the gas in the recording chamber was switched to severe hypoxic gas mixture (6% oxygen, nitrogen balanced) until seizure and ventilatory depression occurred, followed by a switch back to room air. The responses of EEG and ventilation to hypoxia were tested firstly after i.p. injection of DMSO alone as a vehicle, and secondly after administration of arundic acid (100 mg/kg) in DMSO with 60 min interval. Severe hypoxia induced seizure and ventilatory depression in all mice examined. Arundic acid affected neither EEG nor ventilation in room air. Severe hypoxia initially increased ventilation, followed by occurrence of seizure, reduction of ventilation and eventual death. Eleven mice died during or after loading hypoxia before administration of arundic acid, and 7 mice died after administration. Arundic acid delayed the occurrence of seizure and death. We suggest that astrocytes are involved in occurrence of severe hypoxia-induced seizure and death. Arundic acid may be effectively used to prevent SUDEP in high risk patients.

Disclosures: **I. Fukushi:** None. **K. Takeda:** None. **J. Horiuchi:** None. **Y. Okada:** None.

Poster

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CNPq (304873/2014-4)

Title: Effect of nicotinic antagonism in the commissural nucleus of the solitary tract on the respiratory responses to hypercapnia

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Abstract: We have previously demonstrated that nicotinic receptor antagonism in the commissural nucleus of the solitary tract (cNTS) decreased the tachypneic response to peripheral chemoreflex activation. In the present study we investigated whether nicotinic receptor antagonism in the cNTS modify the sympathetic and respiratory responses to central chemoreflex activation. Decorticated arterially-perfused *in situ* preparations of male Holtzman rats (60-90 g, n=8) were obtained to record from thoracic sympathetic (tSN), phrenic (PN), hypoglossal (HN) and abdominal (AbN) nerves. Hypercapnia (10% CO_2) was applied in the perfusion solution of the preparation during 15 min. Mecamylamine (MEC; nicotinic receptor antagonist) microinjections (5 mM, 40-60 nL) or saline (40-60 nL) were performed in the cNTS 5 min after the beginning of the hypercapnia and the changes in the nerves activities were analyzed 8-10 min after the injections. The exposure to hypercapnia increased the pre-inspiratory time in the HN, generated late-expiratory (late-E) bursts in the AbN (not observed in all respiratory cycles), decreased the PN frequency and produced a sympathoexcitatory response. We observed that the MEC in the cNTS attenuated the HN pre-inspiratory response (424±24 vs before MEC 585±64 ms, $p<0.05$) and increased the frequency of late-E bursts in AbN (18±2 vs before MEC 13±1 bpm, $p<0.05$) elicited by hypercapnia. On the other hand, MEC microinjections did not modify the hypercapnia-induced changes in PN frequency and tSN activity. Saline in the cNTS did not change the hypercapnia-induced responses. The present data indicate that cholinergic neurotransmission in the cNTS, via activation of nicotinic receptors, differentially modulates the hypoglossal and abdominal responses to central chemoreflex activation.

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Poster

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Title: Decrease in central chemosensitivity after perinatal fluoxetine exposure is associated with changes in serotonin receptors contributions

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Abstract: Serotonin (5HT) plays a role in neurotrophism, neuronal apoptosis and cell differentiation and the administration of serotonin reuptake inhibitors during pregnancy alters offspring serotonin levels (Alwan and Friedman, 2009)
We postulate that an increase of serotonin levels during the perinatal period may alter the brainstem respiratory network in mouse neonates. We found that mice exposed to perinatal fluoxetine have a decreased ventilatory response to CO_2, due to a reduction in chemosensory nuclei activation and impairment of central respiratory chemoreception from postnatal day 8 (P8) to adulthood (Bravo et al., 2016). In this work we investigated whether perinatal fluoxetine exposure can modify the respiratory effects induced by activation of 5HT receptors within the raphe nucleus (RN, pre-synapse) and at the ventral respiratory column (VRC, post-synapse). Osmotic minipumps were implanted to CF1 dams on day 5-7 of pregnancy, to deliver fluoxetine (7mg Kg^{-1} day^{-1}) for 28 days. Fictive respiration was recorded in brainstem slices from P8 neonates at VRC, while glutamate and 5HT agonists where applied in pre- and post-synaptic areas Glutamate injected into RN increased the respiratory frequency (fR) at the same magnitude in control and fluoxetine-exposed slices. In control slices, 5HT 10 and 30 μM, injected into the raphe nucleus, inhibited the fR more than 5HT 100 μM. By contrast, in fluoxetine-exposed slices 5-HT 10 and 30 μM increased fR. Such fR increases were higher than that observed with 5-HT 100 μM, Additionally, 5HT1AR selective activation of RN decreased fR in a concentration dependent way in control slices but no in those perinatally exposed to fluoxetine. 5HT2AR selective activation of VRC (post-synaptic region) produced similar respiratory effects in control and fluoxetine exposed slices. Our results indicate that perinatal fluoxetine exposure decreased the contribution of 5HT1A in presynaptic area (raphe nucleus) while that of 5HT2A receptors at the postsynaptic region seems to be unchanged. These functional impacts of perinatal fluoxetine,should motivate a vigilant attitude in clinics leading to the revision of the safety/risk ratio in the perinatal use of fluoxetine. Alwan S, Friedman JM (2009) Safety of selective serotonin reuptake inhibitors in pregnancy. CNS Drugs 23:493-509. Bravo K, Eugenin JL, Llona I (2016) Perinatal Fluoxetine Exposure Impairs the CO Chemoreflex: Implications for Sudden Infant Death Syndrome. Am J Respir Cell Mol Biol.

Disclosures: **K.A. Bravo:** None. **J. Eugenín:** None. **I. Llona:** None.

Poster

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Topic: E.08. Respiratory Regulation

Support: FAPESP

CAPES

CNPq

Title: Respiratory chemoreception´s neuroplasticity in a rat model of Parkinson's Disease

Authors: L. M. OLIVEIRA[1], M. TUPPY[1], T. S. MOREIRA[2], *A. T. TAKAKURA[1]; [1]Dept of Pharmacology, Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Dept of Physiol., Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Parkinson´s disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra compacta (SN). The main motor symptoms are tremor at rest, bradykinesia, classic rigidity and postural instability. However, we can observe non-motor symptoms as neuropsychiatric, sleep and breathing disorders. Previous study has already demonstrated that in 6-hydroxydopamine (6-OHDA)-model of PD there is a reduction in the number of Phox2b neurons in the retrotrapezoid nucleus (RTN) and a decrease in the respiratory response to hypercapnia 40 days after PD-induced in awake animals. This functional deficiency is restored 60 days after 6-OHDA injection and here we tested the hypothesis that A6 noradrenergic cells could be a candidate to restore this deficiency in this model. Minute Ventilation (V_E) in response to hypercapnia (7% CO_2) was assessed one day before and 60 days after bilateral 6-OHDA (24 μg/μl) or vehicle injections in the striatum and in the A6 in Male Wistar rats. Bilateral injections of 6-OHDA decreased catecholaminergic neurons by 86% and 83% in the SN and A6, respectively. In animals with lesion in the SN and A6 (N = 6/group) there is a reduction in the ventilatory response to hypercapnia 60 days after the toxin injection (785 ± 18 vs. vehicle: 1417 ± 177 ml/kg/min). In another group of rats after 40 or 60 days of injections of 6-OHDA into the striatum (N = 4/ group), the rats were exposed to hypercapnia or normocapnia for 3 hours and there was a time-dependent reduction in the number of hypercapnia-induced-Fos-ir cells in the RTN region (40 days: 38 ± 3 and 60 days: 8.5 ± 0.9 vs. vehicle 78 ± 3 cells). However, in PD rats, hypercapnia was able to induce Fos-ir cells time-dependent in the A6 region (40 days: 46 ± 4 and 60 days: 94 ± 22 vs. vehicle 1 ± 1 cells). Our data suggest that A6 noradrenergic neurons can be a candidate to assume the chemoreceptor function in a rat model of PD.

Disclosures: **L.M. Oliveira:** None. **M. Tuppy:** None. **T.S. Moreira:** None. **A.T. Takakura:** None.

Poster

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Title: The raphe chemosensory amplifier: a novel amplifier network model for respiratory control

Authors: K. KEPLINGER[1], S. A. CAMPBELL[1], B. E. TAYLOR[2], *M. B. HARRIS[3,2]; [1]Applied Mathematics, Univ. of Waterloo, Waterloo, ON, Canada; [2]Inst. of Arctic Biol., Univ. of Alaska Fairbanks, Fairbanks, AK; [3]Dept Biol. and Wildlife, Univ. Alaska Fairbanks, Fairbanks, AK

Abstract: An important function of the respiratory control system is to match breathing to metabolic demand by detecting CO_2 levels in tissues and reflexively modulating breathing. Of particular interest have been associations between serotonergic dysfunctions and respiratory system pathologies. We have shown that serotonergic disruption alters ventilatory sensitivity to CO_2 and that GABAergic plasticity can rescue a system from serotonergic dysfunction. To explain this behavior, a novel respiratory model is presented and constructed using the Neural Engineering Framework (NEF) and its software implementation, *Nengo*. Our model is constructed from two populations of first-order chemosensory cells, reciprocally connected CO_2-activated 5HT neurons and CO_2-inhibited GABA neurons, and a second-order interneuron receiving inputs from the first-order cells. We propose these cells together form a local network we are describing as a raphe chemosensory amplifier (RCA). The validity of this model is reinforced by duplicating the known network and it's output under various conditions; serotonergic dysfunction, GABAergic dysfunction, and serotonergic dysfunction following CO_2

challenge. All neurons are modeled as leaky-integrate-and-fire (LIF) neurons with time constants appropriate to their firing rates. Theoretical synaptic connections and intrinsic firing rates are compared to *in situ* firing observations. We model connections between the RCA, central rhythm generators and motor neuron pools to emulate patterns of phrenic nerve discharge under normocapnia and hypercapnia. This *in silica* model robustly reproduces experimental observations, verifying the qualitative RCA model previously based on experimental observations. Furthermore, this model demonstrates the versatility of the NEF for combining hard-coded biological elements with theoretical elements, abstracted to state space.

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Poster

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Support: Thomas Hartman Center for Parkinson's Disease Research at Stony Brook University

Title: Characterization of acute intermittent hypoxia (AIH)-induced respiratory activity in spontaneously breathing 6-OHDA SN-lesioned Parkinson's Disease rat model

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Abstract: Respiratory dysfunction in Parkinson's Disease (PD) patients manifests as a variety of altered breathing patterns that are suggested to result from impairment of central respiratory control. To date, only a limited number of studies have used a rat PD model to begin to explore respiratory aspects of PD. To begin to address this issue and better characterize the respiratory phenotype of PD, we have begun a series of experiments in the 6-hydroxydopamine (6-OHDA) neurotoxin-induced unilateral SN lesion rat model. For the current experiments, we examined the inspiratory motor (diaphragm EMG) responses to and following a single bout of acute intermittent hypoxia (AIH) consisting of three 5-min episodes of hypoxia (12% O_2 in a balance of N_2) separated by 5-min intervals of normoxic recovery (21% O_2) in spontaneously breathing urethane-anesthetized adult female rats. AIH exposure is well known to elicit a robust increase in inspiratory motor output that persists beyond the cessation of the stimulus, resulting in respiratory spinal motoneuron plasticity that is referred to as long-term facilitation (LTF). Here we examined AIH-induced respiratory LTF in this rat model of PD. As expected, in control

(vehicle injected) rats, we observed a long lasting increase in diaphragm EMG amplitude (of 12-40% above baseline levels) that persisted up to 90-min following cessation of the intermittent hypoxic exposures. In contrast, in 6-OHDA SN-lesioned rats (at 1-4 weeks post-lesion), there was an attenuation of the AIH-induced diaphragm EMG amplitude response (typically ≤10% above baseline levels). No frequency LTF was seen in either control or SN-lesioned rats. These preliminary observations suggest that (compared to control rats), respiratory LTF induced by 12% O_2 is blunted in 6-OHDA-lesioned rats. While additional experiments are needed to identify the mechanisms underlying this difference, we suggest that the 6-OHDA-lesioned rat/rodent is a viable model for studying respiratory abnormalities in PD.

Disclosures: I.C. Solomon: None. **W.F. Collins:** None.

Poster

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Support: FAPESP

CNPQ

CAPES-PROEX

NIH

Title: Activation of cholinergic receptors the pedunculopontine tegmental nucleus suppresses respiratory activity in urethane-anesthetized and awake rats.

Authors: *C. R. SOBRINHO[1], J. D. LIMA[2], A. C. T. TAKAKURA[3], D. K. MULKEY[5], T. S. MOREIRA[4];
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Abstract: The pedunculopontine tegmental nucleus (PPTn) contributes to the regulation of behavioral states and various motor systems including respiratory activity. Activation of cholinergic receptors in the PPTn has been shown to induce a REM-sleep-like state and depress respiratory activity, thus providing a useful means of studying state dependent control of

breathing. Here, we used this approach by injection carbachol (cholinergic agonist) into the PPTn while recording respiratory activity in urethane-anesthetized and awake male Wistar rats. In anesthetized rats (N = 5/group) respiratory activity was measured by recording genioglossus and diaphragm muscle activity. We found that unilateral PPTn injection of carbachol (10 mM - 100 nl) reduced genioglossus amplitude and frequency by 78.3 ± 12.7 and 49.2 ± 20.0%, respectively. This treatment also decreased diaphragm frequency by 31.1 ± 20.5%. In unrestrained awake rats, we measured respiratory activity by whole body plethysmography and found that unilateral carbachol injections into the PPT also decreased minute ventilation (315 ± 105, vs. control: 604 ± 114 ml/kg/min) by decreasing frequency (60 ± 8, vs. control: 102 ± 8 breaths/min) but not tidal volume. These results are consistent with previous evidence and understanding the neurotransmitter basis for these changes may be relevant to the pathogenesis of central sleep apnea.

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Poster

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Title: ATP-mediated specialized control of vascular tone in the retrotrapezoid nucleus

Authors: *V. E. HAWKINS[1], A. TRINH[1], A. C. TAKAKURA[2], I. C. WENKER[1], T. DUBREUIL[1], M. T. NELSON[3], T. S. MORRIERA[4], D. K. MULKEY[1];
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Abstract: Cerebral blood flow is highly sensitive to changes in CO_2/H^+, with increased CO_2/H^+ causing vasodilation and increased blood flow. Tissue CO_2/H^+ is also the main stimulus for breathing by activating chemosensitive neurons in brainstem respiratory centers such as the retrotrapezoid nucleus (RTN). Interestingly, RTN astrocytes support chemoreception by providing a CO_2/H^+-dependent purinergic drive that enhances activity of chemosensitive

neurons. Considering that CO_2/H^+-induced vasodilation would accelerate removal of CO_2/H^+ and potentially counteract the drive to breathe, we hypothesize that CO_2/H^+-evoked ATP release from astrocytes will prevent CO_2/H^+-vasodilation in the RTN, and thus avoid CO_2/H^+ washout, further enhancing chemoreceptor function. Therefore, the aim of this study is to determine whether purinergic signaling in the RTN provides specialized control of vascular tone in a manner that contributes to the drive to breathe. We used adult rat brainstem slices to measure RTN arteriole diameter responses to CO_2/H^+ or the mGluR agonist t-ACPD under control conditions and when ATP-purinergic receptors are blocked with suramin. Consistent with our hypothesis, we found that exposure to CO_2/H^+ or t-ACPD decreased arteriole diameter under control conditions but not during purinergic receptor blockade. Conversely, CO_2/H^+ and t-ACPD acted as potent vasodilators of cortical arterioles. Consistent with our in vitro data, we found in anesthetized adult rats that exposure to high CO_2 constricted pial vessels in the region of the RTN by 13 ± 2 % under control conditions but not after application of PPADS. These results suggest purinergic regulation of vascular tone contributes to RTN chemoreception. To test this further, we applied a vasoconstrictor (phenylephrine) and vasodilator (nitroprusside) to the ventral surface while measuring the ventilatory response to CO_2. We found that phenylephrine and nitroprusside increased and decreased the ventilatory responses to CO_2, respectively. These findings demonstrate that purinergic signaling in the RTN maintains vascular tone during high CO_2/H^+ and disruption of this mechanism decreases the ventilatory response to CO_2. Together, these results expand our understanding of how RTN astrocytes control breathing by showing that CO_2/H^+-evoked ATP release also contributes to chemoreception by preventing CO_2/H^+-induced vasodilation.

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Poster

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Cnpq

CAPES

Title: Adenosine in the retrotrapezoid nucleus inhibits breathing in rats

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Abstract: Introduction: It is important to consider that the actions of ATP are not determined solely by its actions in the P2 receptors. ATP signaling is best considered as a three-part system which effects are determined from a dynamic interaction between the signaling actions of ATP and ADP at P2 receptors, the spatial distribution of ectonucleotidases that differentially metabolize ATP into ADP, AMP and adenosine (ADO), and the signaling actions of ADO at P1 receptors. Moreover, little is known about ADO effects during hypercapnia and its involvement in central respiratory chemoreflex. A brainstem region called the retrotrapezoid nucleus (RTN) contains a population of CO_2/H^+-sensitive neurons that appears to function as an important chemoreceptor. **Aim:** Despite ATP is quickly breakdown in ADO, the aim of our study is to understand how ADO acts in the RTN during chemoreflex activation. **Methods:** Respiratory activity of urethane-anesthetized or awake rats was measured in response to RTN injections of ADO during exposure to hypercapnia (7% CO_2) (CEUA-ICB/USP: n° 68/ fls. 33/ liv. 3). **Results:** In anesthetized rats, bilateral injections of ADO (10-50 mM - 100 nl) in the RTN reduced the increase in diaphragm frequency (Dia_{EMG} 10 mM: 11±3; 25 mM: 8.5±3; 50 mM: 8.6±2, vs. vehicle: 40±2%) and amplitude (Dia_{EMG} 10 mM: 110±6; 25 mM: 107±4; 50 mM: 106±3, vs. vehicle: 193±13%) and genioglossus amplitude (GG_{EMG} 10 mM: 174±11; 25 mM: 142±10; 50 mM: 143±7, vs. vehicle: 250±9%) and abdominal frequency (Abd_{EMG} 25 mM: 33±3; 50 mM: 34±2, vs. vehicle: 50±1 bpm) elicited by hypercapnia (10% CO_2). In conscious rats, bilateral injection of ADO (10 mM - 100 nl) in the RTN also attenuated (1168 ± 34, vs. vehicle: 1437 ± 16 ml/kg/min) the increase in ventilation during hypercapnia. **Conclusion:** These results suggest that, at the RTN level, ADO can exert an inhibitory effect on breathing. **Financial support:** FAPESP, CNPq, CAPES/PROEX.

Disclosures: B. Falquetto: None. **L. Oliveira:** None. **A. Takakura:** None. **T. Moreira:** None.

Poster

336. Neural Control of Respiration II

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Program#/Poster#: 336.15/RR14

Topic: E.08. Respiratory Regulation

Support: NIH Grant HL104101

CT dep of health grant150263

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Title: Differential noradrenegic modulation of retrotrapezoid nucleus in neonatal rats

Authors: ***F. KUO**[1,2], B. FALQUETTO[3], D. CHEN[2], A. C. TAKAKURA[3], T. S. MOREIRA[4], D. K. MULKEY[2];
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Abstract: CO2/H+ sensitive chemosensitive neurons in the retrotrapezoid nucleus (RTN) regulate breathing in response to changes in tissue CO2/H+, and serve as an integration center for other autonomic centers including noradrenergic neurons. Considering that application of norepinephrine (NE) into the RTN strongly modulates breathing and disruption of NE signaling has been implicated in a variety of breathing disorders, we sought to characterize the effects of NE on chemosensitive RTN neurons. Cell-attached voltage-clamp recordings (Vhold= -60 mV) from chemosensitive RTN neurons in slices from rat pups (7-12 days postnatal) show that bath application of NE (1 μM) increased activity by 1.3 ± 0.05 Hz in 79% of 86 cells , decreased activity by 1.0 ± 0.3 Hz in 7%, or had no effect on activity in 14% of cells tested. The excitatory effect of NE on RTN chemoreceptors was dose dependent (EC_{50} of 235 nM), retained during synaptic blockades and could be mimicked by an α1-receptor against (phenylephrine; 10 uM) and blocked with a α1-receptor antagonist (prazosin; 1 uM). A subset of NE-activated cells were inhibited by NE in the presence of prazosin, and the inhibitory effect revealed by prazosin can be eliminated by a α2-receptor antagonist (idazoxan; 1 μM). In the NE inhibited RTN chemoreceptors, the NE induced inhibition was retained in synaptic blockade but showed an excitatory response to NE in the presence of idazoxan. A third group of RTN chemoreceptors did not respond to NE under control conditions or during hypercapnia, and these cells also did not respond to phenylephrine. We also found that the excitatory effect of NE was partially (23%) blunted by a KCNQ channel blocker (XE991; 10 μM). However, since the majority of NE activation was retained during KCNQ blockade, made whole-cell voltage-clamp recordings (TTX) to identify other downstream effectors contributing to this response. We found that exposure to NE decreased outward current by ~14 pA but with little change in conductance, suggesting NE has offsetting effects on conductance of multiple channels. These results indicate that i) RTN chemoreceptors are intrinsically sensitive to NE; ii) NE can increase or decrease chemoreceptor activity by α1- and α2-receptor dependent mechanisms, respectively; iii) NE modulates discrete subsets of RTN chemoreceptors based on the differential modulation of α1-α2-receptors. iiii) NE sensitive current is comprised of multiple components.

Disclosures: F. Kuo: None. **B. Falquetto:** None. **D. Chen:** None. **A.C. Takakura:** None. **T.S. Moreira:** None. **D.K. Mulkey:** None.

Poster

336. Neural Control of Respiration II

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Topic: E.08. Respiratory Regulation

Support: Wellcome Trust

Title: Retrotrapezoid neurons control breathing during exercise and determine exercise capacity

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Abstract: Breathing is a vital physiological activity that continually adapts to ever-changing behavioural and environmental conditions to maintain constant levels of arterial and brain PO_2, PCO_2 and pH. To match ventilation to the metabolic demands of the body during exercise, respiratory output is controlled by several feed-forward and feedback mechanisms. Descending cortical projections increase ventilation in anticipation of, and during the initial stages of, exercise, whilst central/peripheral chemoreceptors and muscle afferents maintain enhanced respiratory activity during continued exercise. Detection of changes in PO_2 primarily occurs in the carotid bodies located at the bifurcation of the common carotid artery, whilst PCO_2/pH are primarily detected by the brainstem chemoreceptors. Neurones of the retrotrapezoid nucleus (RTN) in the rostral ventrolateral medulla oblongata are sensitive to changes in PCO_2/pH, and integrate chemosensory information from the periphery and other CNS structures. Therefore, we hypothesised that RTN neurones may play an important role in the control of breathing during exercise. In adult male Sprague-Dawley rats, RTN neurons were transduced by targeted microinjections of adeno-associated viral vectors to express the HM_4D (G_i-DREADD) receptor (HM_4DR) or a control transgene (eGFP). In awake behaving rats (n=6) an intraperitoneal injection of CNO (2mg/kg), to activate the HM_4DR and silence RTN neurons, reduces exercise capacity by 76% as assessed using an intensity controlled forced treadmill running paradigm. Exercise capacity of eGFP transfected rats (n=5) was unaffected by CNO treatment. Simulated exercise, i.e., sciatic nerve stimulation, in spontaneously breathing urethane-anaesthetised (1.2-1.7 g/kg) rats increased respiratory rate, tidal volume, minute ventilation, blood pressure and heart rate. In eGFP transfected rats, administration of CNO had no effect on cardiorespiratory responses elicited by simulated exercise (n = 7). However, inhibition of RTN neurons dramatically reduced exercise-induced increases in ventilation, without affecting the reflexive increases in heart rate and mean arterial blood pressure (n = 6). These data suggest that RTN

neurones are essential for the development of an appropriate respiratory response during exercise and, therefore, determine the exercise capacity.

Disclosures: R.T. Huckstepp: None. **A. Korsak:** None. **A. Machhada:** None. **S. Sheikhbahaei:** None. **A.V. Gourine:** None.

Poster

336. Neural Control of Respiration II

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Topic: E.08. Respiratory Regulation

Support: NIH HD071302

Title: Influence of developmental nicotine exposure on serotonergic control of breathing-related motor output

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Abstract: Prenatal nicotine exposure with continued exposure through breast milk over the first week of life (developmental nicotine exposure, DNE) alters the development of brainstem circuits that control breathing. Here, we test the hypothesis that DNE alters the breathing-related motor response to serotonin (5HT). Pregnant rats were exposed to nicotine or saline with osmotic minipumps, and brainstem-spinal cord preparations from 1-5 day old pups were studied in a split-bath configuration so drugs could be applied to the medulla, or the spinal cord. The outcome measures were the frequency and amplitude of the fourth cervical ventral nerve roots (C4VR), which contain axons of phrenic motoneurons. We applied 5HT alone; 5HT + the 5HT1A antagonist NAN-190; or 5HT + the 5HT2A antagonist ketanserin. The main findings include: 1) In control animals (N=14), 30-min of 5HT superfusion (20 uM) evoked a transient increase (2-5 min) followed by a sustained decrease in C4VR frequency. In DNE animals (N=10), 5HT caused a more sustained frequency increase (10 min), followed by a sustained decrease. In both groups, either Ketanserin or NAN-190 blocked the increase in C4VR frequency, with little effect on the sustained decrease. 5HT transiently increased C4VR burst amplitude (5 min), but this was followed by a 50% reduction, with both treatment groups responding identically. This effect was blocked by NAN-190, but not ketanserin. We also gave 5HT to the caudal chamber to activate phrenic motoneurons directly. This caused a 2-fold increase in tonic C4VR discharge in both groups, and ketanserin abolished this effect. NAN-190

reduced the 2-fold increase to a 1.3 fold increase. These data show that DNE alters the C4VR frequency response to 5HT, by actions on both 5HT1 and 5HT2A receptor subtypes in the medulla. DNE did not alter the phrenic motoneuron response to 5HT.

Disclosures: A.A. Hill: None. **R.F. Fregosi:** None.

Poster

336. Neural Control of Respiration II

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Title: Genetic mapping of developmental noradrenergic neuron subpopulations in respiratory homeostasis

Authors: *J. SUN[1], M. KEY[2], R. RAY[1];
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Abstract: Central noradrenergic (NA) neurons are a critical participant in the brainstem networks that maintain respiratory homeostasis. However, previous approaches to study the NA system have been limited in their lack of resolution, off-target effects, broad developmental perturbations, and the confounding effects of anesthesia and restraint. Thus, it is still not entirely clear how NA neurons are integrated into central respiratory circuits and how they may play a role in congenital respiratory pathophysiologies including Congenital Central Hypoventilation Syndrome and Sudden Infant Death Syndrome.
Previous studies suggest that proper partitioning of the developing hindbrain into transient, genetically-defined segments called rhombomeres (r) is required for normal respiratory development. As many features of neural circuits are determined during embryogenesis, we hypothesize that the genetic programs within individual rhombomeres play a role in functionally

patterning the adult NA system to integrate into the broader central respiratory circuitry. To test our hypothesis, we used acute, non-invasive, and reversible pharmaco-genetic DREADD receptors to either perturb (hM4D) or stimulate (hM3D) genetically-defined rhombomere specific NA subpopulations in the adult mouse brainstem. NA subsets were targeted with a series of established rhombomere-specific Cre drivers and our own set of NA FLP drivers combined with dual recombinase responsive DREADD alleles engineered in our lab. This intersectional pharmacogenetic approach enables high spatial resolution across widely dispersed developmental NA subpopulations and bypasses potential developmental confounds.
To measure respiration in conscious and unrestrained mice, we used whole-body barometric plethysmography under room air, hypercapnic (5% CO_2), and hypoxic (10% O_2) conditions. Our measurements of minute ventilation normalized to metabolic rate (V_E/V_{O2}) demonstrate that hM4D mediated perturbation of the whole NA system results in reduced hypercapnic and hypoxic responses. Our data also show that hM4D mediated perturbation of neurons derived from whole rhombomeres (r1, r2, r3&5, r4, and r7&8) results in a variety of respiratory phenotypes. Finally, preliminary data suggests that several developmental NA subpopulations are uniquely involved in the adult hypercapnic and hypoxic ventilatory responses. Cumulatively, our data supports the contribution of early embryonic patterning in defining the functional organization of the adult respiratory network.

Disclosures: **J. Sun:** None. **M. Key:** None. **R. Ray:** None.

Poster

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Topic: E.08. Respiratory Regulation

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Title: Cholinergic modulation of the parafacial respiratory group

Authors: *S. PAGLIARDINI, R. C. T. BOUTIN, Z. ALSAHAFI;
Physiol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Active inspiration and expiration are opposing respiratory phases generated by two separate oscillators in the brainstem; inspiration driven by a neuronal network located in the

preBötzinger Complex (preBötC) and expiration driven by a neuronal network located in the paraFacial Respiratory Group (pFRG). While continuous activity of the preBötC is necessary for maintaining ventilation, the pFRG behaves as a conditional expiratory oscillator, being silent in resting conditions and becoming rhythmically active in the presence of increased respiratory drive (e.g., hypoxia, hypercapnia, exercise, or through release of inhibition).
Recent evidence from our laboratory suggests that expiratory activity in the principal expiratory pump muscles, the abdominals, is modulated in a state-dependent fashion, frequently occurring during periods of REM sleep. We hypothesised that acetylcholine, a neurotransmitter released in wakefulness and REM sleep by mesopontine structures, contributes to the activation of pFRG neurons and thus acts to promote the recruitment of expiratory abdominal muscle activity. We investigated the stimulatory effect of cholinergic neurotransmission on pFRG activity and recruitment of active expiration *in vivo* under urethane anesthesia. We demonstrate that local application of the acetylcholinesterase inhibitor physostigmine into the pFRG potentiated expiratory abdominal activity. Furthermore, local application of the cholinomimetic carbachol into the pFRG activated late expiratory neurons and induced long lasting rhythmic active expiration. This effect was completely abolished by pre-application of the muscarinic antagonist scopolamine and was reversed after washout.
We conclude that cholinergic muscarinic transmission contributes to excitation of pFRG neurons and promotes both active recruitment of abdominal muscles and active expiratory flow.

Disclosures: S. Pagliardini: None. **R.C.T. Boutin:** None. **Z. Alsahafi:** None.

Poster

336. Neural Control of Respiration II

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Program#/Poster#: 336.20/RR19

Topic: E.08. Respiratory Regulation

Title: Neuronal morphology changes in the respiratory centers of the developing rat

Authors: *P. A. WILLIAMS[1], C. G. WILSON[2];
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Abstract: Autonomic control centers within the brainstem generate and modify breathing rhythm. Previous studies have shown that control of breathing changes during early development [Li et al., J Physiol, 577:957-770, 2006]. However, little is known about developmental changes in the morphology of neurons in the respiratory regions of the brainstem during the first three weeks following birth and how changes in morphology correlate with changes in breathing

pattern. We hypothesize that changes in the morphology of neurons in the preBötzinger Complex (pBC), the *nucleus tractus solitarii* (nTS), and the hypoglossal motor nucleus (XII) are correlated with changes in breathing pattern over postnatal days 0 through 21. To test this hypothesis, we used Golgi-Cox staining, a high-resolution bright-field stain, to examine the somata and dendritic arbor of developing neurons in pBC, nTS, and XII. At each of nine postnatal ages (P1, P3, P5, P7, P10, P12, P13, P17, & P21), we removed the brain (from C1 rostrally) from the animal, processed the tissue, sectioned the brain (150 micron thickness) using a vibratome, and performed Golgi-Cox staining to visualize the dendritic morphology of neurons in the pBC, nTS, and XII. Stack images were obtained at 2 micron steps in the z-axis for subsequent Sholl analysis (2D) and 3D reconstruction using Neuromantic [Myatt, et al., Front Neuroinform, 6(4):1-14, 2012]. The dendritic arbor of developing neurons becomes more complex from P1 through P13 as indicated by the maximum radii of intersections and the increased maximum number of intersections. There is a gradual increase in the maximum number of intersections from P1 to P12, but at P13 there is a marked increase in the number of intersections. Also, the maximum radius at which intersections occur increased from approximately 200 microns (P1-P5) then 300 microns (P7 and P10) increasing to about 400 microns at P12 and P13. The variance in the number of primary branches increased from P1 to P7 and then decreased at P13. The dendrite morphology appears to reflect a sensitive period as has been described for respiratory control [Li et al., J Physiol, 577:957-770, 2006]. These studies will provide a foundation for the development of high-resolution computational models of developing neurons in rats.

Disclosures: P.A. Williams: None. **C.G. Wilson:** None.

Poster

336. Neural Control of Respiration II

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Topic: E.08. Respiratory Regulation

Support: National Heart, Lung, and Blood Institute of the NIH RO1HL074011

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Title: Sciatic nerve stimulation activates presympathetic (C1) neurons and retrotrapezoid nucleus (RTN) chemoreceptors in anesthetized rats

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[1]Lebanese American Univ., Byblos, Lebanon; [2]Pharmacol., Univ. of Virginia, Charlottesville, VA

Abstract: Somatic afferent stimulation increases sympathetic tone and blood pressure by activating presympathetic neurons located in the rostral ventrolateral medulla (RVLM). Somatic afferent stimulation also increases breathing. Here we tested whether retrotrapezoid neurons (RTN), a cluster of central respiratory chemoreceptors, also located in the RVLM, is activated by somatic afferents. The sciatic nerve (SciN) was electrically stimulated in Inactin-anesthetized, bi-vagotomized, pharmacologically paralyzed, mechanically ventilated Sprague-Dawley rats. C1 and RTN were recorded extracellularly along with the phrenic nerve discharge (PND) and end-expiratory CO_2 ($eeCO_2$). SciN stimulation significantly increased blood pressure, heart rate, PND frequency and amplitude. SciN stimulation produced a similar pattern of activation in C1 and RTN neurons. Neuronal activation was in both cases triphasic, consisting of an early and late excitation, followed by reduced activity. However, C1 neurons were more strongly activated than RTN neurons. RTN neurons were more likely to be recruited by SciN stimulation when $eeCO_2$ was elevated than at lower $eeCO_2$ when these neurons were typically silent. SciN stimulation shifted the relation between RTN neuron firing rate and $eeCO_2$ upwards suggesting additivity between the effects of CO_2 and nerve stimulation. In short, activation of hindquarter somatic afferents increases the firing probability of both C1 and RTN neurons. These inputs likely contribute to the increased BP and breathing associated with exercise or nociception.

Disclosures: R. Kanbar: None. **P.G. Guyenet:** None.

Poster

336. Neural Control of Respiration II

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Topic: E.08. Respiratory Regulation

Title: A practical biomarker for obstructive apnea in potential sudden death in epilepsy (SUDEP) cases

Authors: *M. G. STEWART[1], R. KOLLMAR[2], K. NAKASE[1], J. SILVERMAN[3], K. SUNDARAM[3], R. ORMAN[1], J. LAZAR[4];
[1]Physiol. & Pharmacol., [2]Cell Biology, Otolaryngology, [3]Otolaryngology, [4]Med. (Division of Cardiology), State Univ. of New York Downstate Med. Ctr., Brooklyn, NY

Abstract: Sudden death in epilepsy (SUDEP) is the major cause of death among persons with epilepsy. The mechanism of SUDEP is, however, poorly understood, and no specific indicator of SUDEP events is known. Using a rat model, we demonstrated a sequence of events ending in death that begins with obstructive apnea caused by seizure-induced laryngospasm. Attempts to breathe during airway occlusion eventually cease (respiratory arrest, also referred to as the onset

of terminal apnea). In between the onset of obstructive apnea and respiratory arrest, the seizure stops and a bradyarrhythmia develops as a result of hypoxemia. We propose that the electrical artifact of the attempts to inspire during airway obstruction can be used as a practical biomarker of obstructive apnea. For controlled airway occlusion, a T-tube was inserted into the distal trachea of urethane-anesthetized rats. EEG, ECG, and inspiratory pressure at the sidearm of the T-tube were bandpass-filtered from 1 Hz to 1 kHz. The open port of the T-tube was occluded for 100 seconds or until respiratory arrest. Inspiration artifacts in the EEG and EEG records were isolated with a digital high-pass filter (corner frequency 367 Hz, rolloff -3 dB/octave) and quantified by full-wave rectification. Inspiration artifacts matched the inspiratory pressure extrema during airway occlusion. Correlations (r) of peak inspiratory pressure to artifact amplitude in a within-animal comparison were -0.88 (ECG) and -0.75 (EEG), suggesting that artifacts extracted from ECG records may be better than those derived from EEG records. The average correlation of artifact magnitude (ECG) with peak inspiratory pressure was -0.89 ± 0.04 (N=5 rats). Our results suggest that a sudden increase in the amplitude of the inspiratory artifact in EEG and ECG recordings indicates an occluded airway, and we observe a very high correlation of increasing inspiration artifact size with increasing inspiratory effort. This artifact pattern could serve as a biomarker in two important ways: First, to review existing records for the possible contribution of obstructive apnea to documented SUDEP cases. Second, to warn about obstructive apnea in patients being monitored in real time. The specificity of the biomarker would be further enhanced by marking decreases in seizure activity and heart rate. To maximize the sensitivity of the biomarker, EEG and ECG should be recorded at the highest bandwidth possible. The most attractive feature of this biomarker is that it can be derived from commonly-used measures in epilepsy-monitoring units and even potentially portable devices outside of the hospital.

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Poster

336. Neural Control of Respiration II

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Title: Anatomical arrangement of neurons and astrocytes in the phrenic nucleus of the rat

Authors: *Y. OKADA[1], S. YOKOTA[2], Y. SHINOZAKI[3], Y. YASUI[2];
[1]Murayama Med. Ctr., Tokyo, Japan; [2]Dept. of Anat. and Morphological Neurosci., Shimane Univ. Sch. of Med., Izumo, Japan; [3]Dept. of Orthopaedic Surgery, Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: To obtain histological basis to understand the mechanism of neuron-astrocyte interaction in the inspiratory neural output formation in the spinal cord, we analyzed distribution of neurons and astrocytes in the phrenic and scalene nuclei of which motor neurons innervate the diaphragm and scalene muscle, an accessory inspiratory muscle, respectively. First, using retrograde tracing by DiI in rats, we demonstrated that the phrenic and scalene motor neurons are located in the mid-ventral portion of the ventral horn corresponding to the central motor column through the C3-C5 segments and in the ventromedial portion of the ventral horn corresponding to the medial motor column through the C3-C8, respectively. Then, we analyzed the cervical spinal cord at the C4 level by Nissl stain and immunohistochemistry for neuronal nuclear antigen (NeuN) for neurons, choline acetyltransferase (ChAT) for motor neurons, glial fibrillary acidic protein (GFAP), and S100-protein β-subunit (S100) both for astrocytes. Nissl-staining showed diffuse distribution of cell bodies in the gray matter with high density in the ventral horn. ChAT-immunoreactive (ir) neurons were located in the most ventral layer of the ventral horn, composed of subpopulations; i.e., ventrolateral portion corresponding to the lateral motor column with large polygonal-shaped neurons, mid-ventral portion corresponding to the central motor column with multipolar neurons, and the ventromedial portion along the ventral funiculus with fusiform-shaped neurons. GFAP-immunoreactivity was strong in the white matter especially in the marginal layer, and GFAP-ir fibrous astrocytes were distributed in the whole gray matter. S100-ir cells were distributed almost equally in the gray and white matter, except for the marginal layer where S100-ir was particularly strong. In triple staining of cell markers, ChAT, NeuN, GFAP, S100 and DAPI, we could distinguish ChAT-ir motor neurons, ChAT-negative NeuN-ir interneurons, GFAP- or S100-ir astrocytes, and other cells which were stained solely with nuclear marker DAPI. In the medial and central motor columns, we found that GFAP-/S100-ir astrocytes were intermingled with ChAT-ir motor neurons, and GFAP-ir fibrillary processes extended from S100-ir cells were closely associated with ChAT-ir motor neurons. ChAT-ir neurons constituting medial and central motor columns were surrounded by a large number of ChAT-negative NeuN-ir interneurons. In summary, motor neurons and astrocytes are intermingled in the phrenic and scalene nuclei, indicating that astrocytes are coupled with inspiratory motor neurons and involved in the inspiratory motor output formation.

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Poster

336. Neural Control of Respiration II

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Program#/Poster#: 336.24/SS1

Topic: F.08. Biological Rhythms and Sleep

Title: Voluntary control of breathing engages multiple supratentorial brain areas and modulates sensory processing.

Authors: *J. L. HERRERO, A. ASHESH;
Neurosci., Cushing Neurosci. Inst., New York City, NY

Abstract: Breathing is a rhythmic behavior whose automatic control by the brainstem has been well studied. However, voluntary influences upon breathing often override the automatic breathing control system in a number of behaviors such as during speech, exercise, cognitive processing, eating, sniffing, and meditation practice. This voluntary control of breathing has received very little attention in the literature. We studied the neural circuits underlying the voluntary breathing at the level of the cortex. We recorded intracranial electroencephalogram (iEEG) simultaneously with the breathing patterns in eight subjects undergoing iEEG monitoring. We found that phase-locking of the neural activity to the breath (iEEG-breath coherence) occurs in specific grey matter regions, and this coherence is greater during voluntary breathing. These regions include the hippocampus, amygdala, premotor and anterior cingulate cortex, as well as in the insula, olfactory bulb and orbitofrontal cortex. Phase-amplitude coupling of the gamma band to the phase of the low frequency (1Hz) breath rate was demonstrated. Evidence for a neural source of these effects is further strengthened by the fact that electrodes located in the white matter and cerebrospinal fluid did not show these effects. Moreover, in certain areas, these effects were sensitive to interoceptive attention. When subjects attend to their breath, correctly reporting their breath counts, the iEEG-breath coherence and cross-frequency coupling are stronger in electrodes located in the insula and specific areas of the prefrontal cortex. Finally, we show the role of breathing in cortical information processing by measuring evoked potentials from visual and auditory cortices while subjects paired their inhalations to the appearance of a rhythmic auditory or visual stimulus. We found larger evoked potentials in this condition compared to a control natural breathing condition, suggesting enhanced sensory processing when breath is timed to a sensory stimulus. These findings demonstrate a cortical circuit involved in the control of breathing.

Disclosures: J.L. Herrero: None. **A. Ashesh:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

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Title: Neural correlates of responses to mechanical loading in *Aplysia* californica

Authors: *J. P. GILL[1], D. N. LYTTLE[1], M. J. CULLINS[1], P. J. THOMAS[2], H. J. CHIEL[3]; [1]Dept. of Biol., [2]Dept. of Mathematics, Applied Mathematics, and Statistics, [3]Departments of Biology, Neuroscience, and Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Animals are remarkable in their ability to flexibly and robustly respond to changing conditions in their environment. Understanding the neural mechanisms of this process could be of great value. Previous studies in the marine mollusk *Aplysia* have shown that its response to mechanical loading while feeding is complex (Hurwitz and Susswein 1992). When mechanical loads are small, the animal recruits a stronger muscular response to generate more force. When mechanical loads become large enough to potentially injure the feeding apparatus, the animal may cut food and release it. What are the neural correlates of this behavior? We have recently developed a suspended buccal mass preparation (McManus et al. 2012) in which it is possible to look at neural correlates during biting and swallowing while applying different mechanical loads. We have also developed a computational model of the feeding apparatus in which we observed that it compensates for load by pulling stronger and for longer during retraction (Shaw et al. 2015; Lyttle et al. in revision). As animals switch from attempts to grasp food (biting) to swallowing, we have shown that there is increased activity in specific motor neurons that aid in retraction (Lu et al. 2015). Our preliminary results suggest that during swallowing, a small increase in mechanical load may recruit additional motor neurons for jaw muscles, whereas a large increase may actually suppress activity in these neurons so the animal may release the food

and avoid injury. These results suggest a neural mechanism whereby animals can flexibly adjust the output of their motor system in response to mechanical load. The neural mechanisms for flexible adjustment may suggest general principles for nervous systems in many other animals.

Disclosures: J.P. Gill: None. **D.N. Lyttle:** None. **M.J. Cullins:** None. **P.J. Thomas:** None. **H.J. Chiel:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 337.02/SS3

Topic: F.01. Neuroethology

Title: Grasping by a muscular hydrostat: function of the radular surface in *Aplysia* californica.

Authors: *C. E. KEHL[1], D. M. NEUSTADTER[3], S. LU[2], H. J. CHIEL[2];
[1]Biol., [2]Case Western Reserve Univ., Cleveland, OH; [3]Calore Med. LTD, Or Akiva, Israel

Abstract: Biomechanics, which is the study of how the mechanics of the body affects behavior, is the complementary partner to the study of neural control. Understanding biomechanics is important because it clarifies both constraints and opportunities for neural control. The biomechanics of soft bodied organisms, such as worms, slugs and squid, are especially challenging. These creatures can also serve as the inspiration for soft bodied robots, which can grasp and move through spaces inaccessible to rigid systems. The feeding grasper of *Aplysia californica*, a soft bodied structure, has a long history as a model of neural control. Its surface undergoes conformational changes as it opens and closes, allowing it to grasp food of complex and differing shapes. The opening of the grasper had been attributed to the action of the I7 muscles, but experiments in our lab have shown that removing these muscles has no effect on opening in intact, behaving animals. We have, however, discovered fine muscular fibers underlying the tissues that produce openings in reduced preparations. Lesions of these tissues in intact animals greatly impair openings. A 3D model based on high resolution MRI data validates the role of these sub-radular fibers in radular opening. Understanding the details of the motor control of the grasper in *Aplysia* is likely to be of use for understanding the motor control of soft muscular structures in general, which could have implications for understanding structures such as tongues and the digestive system.

Disclosures: C.E. Kehl: None. **D.M. Neustadter:** None. **S. Lu:** None. **H.J. Chiel:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Program#/Poster#: 337.03/SS4

Topic: F.01. Neuroethology

Support: NSF Grant DGE-0951783

NSF Grant DMR-1306665

NIH Grant R01 AR063701

GAANN Grant P200A150316

Title: *Aplysia* californica as a source of actuators, scaffolds, and controllers for the development of biohybrid robots and living machines

Authors: *V. WEBSTER, K. J. CHAPIN, O. AKKUS, H. J. CHIEL, R. D. QUINN;
Case Western Reserve Univ., Cleveland, OH

Abstract: A biohybrid robot needs a skeleton (structure), muscles (actuators), and a controller. An appropriate source for these components should be both robust and capable of flexible use in a robot. The sea slug *Aplysia californica* is extremely robust, living in tidal regions whose temperature, osmotic balance, and turbulence vary. Furthermore, the animal's muscles respire slowly, and do not require a dedicated output system for wastes, because the animal relies on an open circulatory system, making them ideal for use in bio-bots. *Aplysia californica* has been investigated as a source of material for fabrication of biohybrid robots and living machines. *Aplysia* provides a source of material for development of completely organic scaffolds with the potential for use in cell culture and the fabrication of living machines in the form of collagen isolated from the skin. Collagen has been isolated and scaffolds have been fabricated by both electrocompaction and gelation. Such scaffolds have been mechanically tested and compared to mammalian collagen scaffolds fabricated from commercially available bovine collagen. Additionally, a complete neuromuscular model has been developed for the design of an inchworm-inspired biohybrid robot powered by the I2 muscle from *Aplysia californica*. The I2 muscle from the *Aplysia* feeding apparatus has been chosen as an actuator due to its thin structure, allowing nutrients to penetrate the muscle via diffusion, and the existing knowledge of the neural structures that control it. This muscle has been characterized using a deflected cantilever technique and the maximum force was found to be 58.5 mN with a maximum muscle strain of 12 ±3 %. Additionally, the muscle has durability, being capable of continuously contracting for up to 3 hours. Scaffolds for the biohybrid robots have been fabricated using a Formlabs 1+ SLA 3D printer. This printer is capable of printing flexible structures that the I2

muscle can deform when attached. Contraction of the muscle can be initiated by field stimulation or by using the ganglia that regulate its movements as controllers, applying carbachol to one of the two attached ganglia (the cerebral ganglion). Using these components, we have developed a locomoting biohybrid robot that uses frictional anisotropy and is driven by the I2 muscle.

Disclosures: V. Webster: None. **K.J. Chapin:** None. **O. Akkus:** None. **H.J. Chiel:** None. **R.D. Quinn:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 337.04/SS5

Topic: F.01. Neuroethology

Support: NIH Intramural Program

Title: A gravity-sensing cell in Trichoplax adhaerens, an early branching metazoan

Authors: *T. D. MAYOROVA[1], C. L. SMITH[2], N. B. PIVOVAROVA[1], T. S. REESE[1]; [1]Structural Biol., [2]Light Microscopy facility, NINDS, Bethesda, MD

Abstract: *Trichoplax adhaerens* is a small coin-shaped marine animal, a member of the small phylum, Placozoa. *Trichoplax* lacks electrical or chemical synapses, neurons, and muscles. Knowledge is emerging regarding the function of five out of its six cell types; ventral and dorsal epithelial cells, lipophil cells, gland cells, and fiber cells. However a role for the sixth cell type, the crystal cell, is lacking. These small (5-6 μm) spherical-shaped cells are arrayed around the perimeter of the animal and have a single birefringent crystal inside that flashes polarized light as it nutates. The nucleus of the crystal cell is cup shaped and localized closely to a sector of the crystal cell membrane. The cytoplasm is electron lucent and has few inclusions other than the ~1-2 μm crystal, which is surrounded by mitochondria. We apply laser confocal and electron microscopy and X-ray microanalysis to further characterize crystal cells. The cup-shaped nuclei of crystal cells are oriented with the cup opening toward the rim of the animal. The crystal is rhomboid from above but lenticular-like in its lateral aspect. As a crystal is in a constant movement and nutation inside a crystal cell, it does not have any particular orientation, and crystals in different crystal cells may not have the same orientation. X-ray microanalysis of single crystals revealed that they are calcite, highly enriched in calcium but lacking sulfur. Normally the crystal is positioned in the center of the cell or slightly eccentric, but when an animal is attached to a vertical surface, crystals are displaced in the direction of gravity while the orientation of the cup-shaped nucleus remains unchanged. Phalloidin staining reveals a cortex of

actin filaments covering the outer surface of the cup-shaped nucleus and strands of actin extending from the cortex to the crystal. The repositioning of
crystals with changes in the orientation of the animal raises the possibility that the crystals might be statoliths as they are in plants and some protists (Hemmersbach & Braun, Signal Transduction, 2006). It is reported that *Trichoplax* can right its self when turned over, but it remains to be seen whether they can sense the gravity. While our new observations point to the role of crystal cells in gravity sensing, their role in detecting light cannot be ruled out as many invertebrates utilize calcite or aragonite crystals to focus light on receptors.

Disclosures: T.D. Mayorova: None. **C.L. Smith:** None. **N.B. Pivovarova:** None. **T.S. Reese:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 337.05/SS6

Topic: F.01. Neuroethology

Support: NSERC

CFI

Title: *C. elegans* ASE sensory neurons differentially code NaCl information providing greater environmental resolution for successful navigation

Authors: *M. DESROCHERS, J. LEE, M. HENDRICKS;
McGill Univ., Montreal, QC, Canada

Abstract: C. elegans are capable of sensing and navigating in response to a variety of chemical compounds. The two main NaCl sensing neurons, ASER and ASEL, respond to different information about NaCl in the environment, with ASER responding as an “off” neuron and ASEL responding as an “on” neuron. It has been suggested that these opposing responses may underlie robust sensing of changes in stimulus concentration. While ASER is essential for efficient gradient navigation, we found that the fidelity and magnitude of ASER responses to step stimuli is not predictive of behavioral responses to stimulus steps. Instead, conditions that enhance ASEL sensitivity and reduce ASER responses contribute to robust behavioral response to salt steps. Thus, ASEL and ASER lateralization may reflect functional specialization for responding to distinct spatiotemporal features of a stimulus rather than acting together to optimize detection of either gradients or steps.

Disclosures: **M. Desrochers:** None. **J. Lee:** None. **M. Hendricks:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Program#/Poster#: 337.06/SS7

Topic: F.01. Neuroethology

Support: NIH Grant R01-NS086932

Title: Dissecting the function of acetylcholine in the *Caenorhabditis elegans* egg-laying behavior circuit

Authors: *R. KOPCHOCK, III, K. M. COLLINS;
Univ. of Miami, Coral Gables, FL

Abstract: Egg laying in the nematode *C. elegans* alternates between an active and inactive behavior state. The active phase is initiated by activity in the hermaphrodite specific, command motor neurons (HSN) which release serotonin onto postsynaptic vulval muscles to increase their excitability. The vulval and body wall muscles are also innervated by the cholinergic Ventral Type C (VC) motor neurons whose activity may trigger muscle contraction and egg release. Our research aims to dissect how acetylcholine signals within the egg-laying circuit. Acetylcholine has been shown to be the primary fast-acting neurotransmitter responsible for body wall muscle contractions and locomotion, but whether it has a shared function at the vulval muscles remains unclear. Cholinergic agonists stimulate egg-laying behavior, but mutants defective for acetylcholine synthesis instead show a hyperactive egg-laying phenotype, suggesting acetylcholine may inhibit egg laying and/or function as part of a negative-feedback loop. Our results show the HSNs have peak activity two seconds prior to each egg-laying event, after which the VC motor neurons fire and the vulval muscles contract to lay an egg. Optogenetic activation of the VCs fails to trigger egg laying but instead hypercontracts the body wall muscles. These results suggest that one function of VC acetylcholine is to slow locomotion when the vulval muscles are contracting for egg laying. Passage of eggs through the vulva mechanically activates the uv1 neuroendocrine cells. Recent work has identified a muscarinic receptor, GAR-2, expressed in the uv1s. GAR-2 is predicted to signal through $G\alpha_o$ to regulate release of tyramine and neuropeptides that inhibits HSN activity to terminate the active behavior state. By combining molecular genetics, optogenetics, and calcium imaging in behaving animals, we will test our hypothesis that acetylcholine signals through distinct receptors and cells to drive different motor events during egg laying. These results will allow us to better understand the dynamic ways in which acetylcholine can function through multiple cellular pathways and work

in concert with other neurotransmitters, such as serotonin, to regulate neural circuit activity and alternate behavior states.

Disclosures: **R. Kopchock:** None. **K.M. Collins:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 337.07/SS8

Topic: F.01. Neuroethology

Title: Behavioral characterization of magnetotaxis in the nematode *C. elegans*

Authors: ***C. BAINBRIDGE**, A. AHLERT, L. BARICKMAN, B. BRACHT, A. VIDAL-GADEA;
Biol. Sci., Illinois State Univ., Normal, IL

Abstract: The magnetic field of the earth provides many organisms with sufficient information to navigate through their environments. Despite emerging evidence supporting the widespread use of magnetic sensory information across animal taxa, unlike other orientation behaviors (such as chemotaxis, thermotaxis, and electrotaxis), it remains an understudied sensory modality. Magnetic orientation is thought to be accomplished through two distinct transduction pathways: a light-dependent mechanism using magnetically orienting radical pairs, and a light independent pathway using permanently magnetic particles. The adult nematode *C. elegans* is capable of orienting to magnetic fields light-independently in a satiation and polarity dependent manner. We are using in-depth behavioral analysis to characterize this unique behavioral strategy. We previously showed that a pair of sensory neurons (the AFDs) are necessary for magnetic orientation in *C. elegans*. Here we report that genes involved in the normal development of AFD villi are essential for normal magnetotactic behavior.. Consistent with the importance of AFD villi for magnetotaxis, we find that early larval stages (L1 through L3) of *C. elegans* which are known to possess fewer AFD villi, are unable to orient to magnetic fields. However, this was not the case for L4 larva and the alternative early life stage larva (dauers), which not only are known to have additional AFD villi, but also are capable of orienting to magnetic fields. Our findings suggest that the number and integrity of AFD sensory villi are important for magnetic orientation. The genetic and behavioral tractability of *C. elegans* makes it ideal for investigating strategies by which animals across taxa detect and orient to magnetic fields.

Disclosures: **C. Bainbridge:** None. **A. Ahlert:** None. **L. Barickman:** None. **B. Bracht:** None. **A. Vidal-Gadea:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 337.08/SS9

Topic: F.01. Neuroethology

Title: Investigations in to the neural deficits of Duchenne muscular dystrophy using *C. elegans*

Authors: *A. M. RODRIGUEZ[1], S. GOEL[2], A. SCHULER[2], L. BARICKMAN[2], M. CISNEROS[2], P. DEVRIES[2], B. RODEMOYER[2], A. VIDAL-GADEA[2];
[1]Biol. Sci., Illinois State Univ., Bloomington, IL; [2]Biol. Sci., Illinois State Univ., Normal, IL

Abstract: Duchenne muscular dystrophy (DMD) is one of the most common genetic disorders. It is characterized by muscle atrophy and developmental delays. Symptoms often manifest in early childhood and become more pronounced with age. DMD is caused by a mutation in the dystrophin gene, the third largest human gene. Dystrophin maintains cell integrity during muscle contraction, and its absence leads to muscle cell degeneration and eventually to death. Currently there is no cure for DMD. Dystrophin is expressed in nervous tissues as well as muscles, and DMD patients can display neurological impairments such as developmental delays, behavior disorders, and learning disabilities. While there have been many studies characterizing the muscular aspects of DMD, not much is known about its neural components. We have recently shown that the nematode *C. elegans* models DMD genetically, physiologically, and behaviorally. We are currently investigating if lack of the functional dystrophin protein produces any neurological deficiencies in *C. elegans* consistent with the effects of this disease seen in human patients. Understanding all aspects of DMD can lead to therapeutic methods that target muscular and neural components, greatly improving quality of life.

Disclosures: A.M. Rodriguez: None. **S. Goel:** None. **A. Schuler:** None. **L. Barickman:** None. **M. Cisneros:** None. **P. DeVries:** None. **B. Rodemoyer:** None. **A. Vidal-Gadea:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: NIH Grant R01-NS086932

NIH Grant 1R25GM076419-01

Title: Understanding how sex modulates the female nervous system to drive distinct reproductive behavior states

Authors: *L. M. NASSAR, A. BODE, K. M. COLLINS;
Dept. of Biol., Univ. of Miami, Coral Gables, FL

Abstract: We are interested in understanding how mating and reproductive behaviors are coordinated in the female nervous system using the nematode *Caenorhabditis elegans* as a model system. Specifically, we are identifying the neural signaling systems that drive two mutually exclusive vulval motor behaviors: mating with males or the release of progeny during egg laying. We hypothesize that circuit activity that drives egg laying is inhibited so that mating can take place. To investigate this hypothesis, we are using ratiometric calcium imaging to record circuit activity before and during mating with males, after successful insemination, and during the resumption of normal egg laying behavior. We have found that self-fertilizing hermaphrodites have increased locomotive behaviors near males and decreased mating compared with sperm-deficient females. During a mating event with successful sperm transfer, both fertile hermaphrodites and unfertilized females experience low vulval muscle activity before mating, uncoordinated but higher levels of activity during mating, and high activity after mating is complete that is phased with locomotion. These unfertilized females experience decreased vulval muscle and HSN motor neuron activity before mating when compared to fertile hermaphrodites during egg laying. We are now examining the activity of other cells in the egg-laying circuit during mating, including the female-specific cholinergic VC motor neurons and the tyraminergic uv1 neuroendocrine cells. The VC neurons slow locomotion and have high activity coincident with vulval muscle contraction during egg laying. As such, we expect VC activity to slow locomotion when the male spicules are inserted during mating. The uv1 cells are mechanically activated by passage of eggs through the vulva during egg laying. We anticipate uv1 will be similarly activated by male spicule insertion and sperm deposition. Because we observe sperm ejection from the vulva after prolonged mating and sperm deposition, mechanical stretch of the uterus may initiate features of the egg-laying active phase including muscle activity that releases the inserted spicules and resumption of normal locomotion. Together, our comparative analysis of *C. elegans* reprodutive behaviors will show how differential activity in the same neural circuit can be used to drive distinct egg-laying and mating behavior states.

Disclosures: L.M. Nassar: None. **A. Bode:** None. **K.M. Collins:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Program#/Poster#: 337.10/DP07 (Dynamic Poster)

Topic: F.01. Neuroethology

Support: R01 NS094403

Burroughs Wellcome Fund CAS I

Title: Pan-neuronal recording in the leech nervous system using dual-sided voltage sensitive dye imaging

Authors: *Y. TOMINA, D. A. WAGENAAR;
Univ. of Cincinnati, Cincinnati, OH

Abstract: Sensory processing and motor control are functions of intricately organized neuronal ensembles. Therefore, their study could greatly benefit from technologies that simultaneously record the activities of large numbers of individual neurons. We have developed a double-sided microscope for voltage-sensitive dye (VSD) imaging, and used it to record from the medicinal leech *Hirudo verbana* that is a classic experimental animal for comprehensive analysis of the function of neural circuits. This optical measurement technique realized neuronal recording from the majority of cell bodies located in both ventral and dorsal surfaces of a leech ganglion at high temporal resolution: The dye VoltageFluor VF2.1(OMe).H enabled recording of individual action potentials and excitatory/inhibitory synaptic potentials even in many small cells. By removing vibrational noise from the CCD cameras and algorithmically correcting motion artifacts and global trends, we were able to reconstruct stereotyped electrical activity associated with several leech behaviors. To demonstrate the applicability of these newly developed VSD imaging methods, we addressed the following two questions. (1) How does a leech ganglion discriminate the location of a pressure stimulus based on the population activity of neurons on both surfaces of the ganglion? Using principal component analysis, we demonstrate that neuronal population activity differs markedly depending on which sensory neuron (P cell) was stimulated. (2) To what extent are neural circuit components unique or shared between different behaviors? We simultaneously recorded from nearly all neurons in a ganglion during three fictive behaviors: swimming, crawling and local bending. Coherence analysis of rhythmic activity helped us improve our understanding of the involvement of all ganglionic neurons in each behavior. Using double-sided imaging allowed us, for the first time, to directly analyze functional relationships between neurons on the ventral and dorsal surfaces of the ganglion. Taken together, double-sided VSD imaging is a promising new method for observation of neuronal population dynamics for identification of individual neurons responsible for multiple behaviors, and for the analysis of functional relationships between those neurons.

Disclosures: **Y. Tomina:** None. **D.A. Wagenaar:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Program#/Poster#: 337.11/SS11

Topic: F.01. Neuroethology

Support: NIH/NINDS R01 NS094403

Burroughs Wellcome Fund CASI

Title: Visual responses of the S-cell system of the leech Hirudo verbana suggest complex integration mechanisms

Authors: ***D. A. WAGENAAR**, A. STOWASSER;
Biol. Sci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Vision requires complex integration mechanisms. Investigating those is generally difficult because of the large number of neurons involved and the challenge of identifying specific neurons. Among animals that express complex visual processing mechanisms, the leech *Hirudo verbana* is a rare example in which all sensory neurons can be readily identified. *Hirudo*'s previously documented ability to detect the direction of water waves demonstrated that its visual system has the ability to process spatiotemporal patterned visual stimuli. Thus far, however, little is known about the processing mechanisms of the leech's visual system, which is composed of several pigmented eyes in the head and photosensitive non-pigmented sensilla that are distributed across its entire body. While several interneurons are known to respond to visual stimuli, their response properties remain poorly understood. Among these, the S-cell system is especially intriguing because it is multimodal and spans the entire body of the leech. Thus it could potentially be involved in complex sensory integration.
Using a nearly intact leech preparation, and stimulation protocols that tightly controlled area and spectral content of the stimulus as well as background light conditions, we measured the sensitivity of the S-cell system to light stimuli, tested if it performs spatial integration, and whether adaptation is local or global. Two peaks were found in the spectral response of the S-cell system: in green and in UV. The response of the S-cell system was found to be strongly dependent on the size of the area of the leech body that is stimulated. Furthermore, adaptation was local and different areas of the leech's body contributed differentially to the response depending on their adaptation state.
A 'bleach experiment' confirmed the recent suggestion that at least two color channels contribute

to the response. Their contributions were found to be dependent on the adaptation to background illumination. Taken together, our results show that the response properties of the S-cell system are highly complex, making it an attractive target for future studies of high-level processing of complex visual stimuli in a model animal that is uncommonly amenable to electrophysiological methods.

Disclosures: D.A. Wagenaar: None. **A. Stowasser:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

Location: Halls B-H

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Program#/Poster#: 337.12/SS12

Topic: F.01. Neuroethology

Support: R01 NS094403

Burroughs-Wellcome Fund CASI

Title: S-cell responses to visual and mechanical water waves in the leech Hirudo verbana

Authors: *A. MUTHUSAMY[1], A. M. LEHMKUHL, II[2], D. A. WAGENAAR[1];
[1]Biol. Sci., [2]Psychology/Biological Sci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Sensitivity to water waves in aquatic predators greatly facilitates prey location. The behavioral response to visual and mechanical information from water waves is well documented in the leech *Hirudo verbana*: in response to low-amplitude water waves, they orient themselves and initiate swimming or crawling toward the source of the waves. Here, we begin to quantitatively characterize the neuronal response patterns of the leech to water waves in terms of visual and mechanoreceptive sensitivity. We recorded activity of the S cell, an interneuron that forms a syncytium connecting all midbody ganglia. The S cell is excited by mechanical and visual stimuli and could be critical to coordinating responses across the whole body. Although leeches are behaviorally capable of discerning the direction of water waves, we found that the magnitude of the S-cell response to mechanically cued water waves (in darkness) does not depend on the direction of waves relative to the leech's orientation: Mechanical waves presented toward the head, toward the tail, and laterally all evoked the same number of action potentials. Remarkably, regardless of wave direction, most action potentials propagated retrogradely (tail-to-head) along the nerve cord, but when waves approached a leech head-first, an initial burst of action potentials propagated anterogradely. The S cell responded to a wide range of wave frequencies, and its sensitivity profile contained multiple peaks. Leeches'

responses to water waves have been linked to hunger state, but here we found that feeding did not immediately impact the mechanosensory response of the S cell. However, sensitivity to high-amplitude, low-frequency waves increased in the weeks following feeding.
We explored the leech's visual response pathways by projecting visual waves in the absence of mechanical cues. In contrast to the high sensitivity to mechanical wave frequency, the magnitude of S-cell responses to bright visual waves was not strongly dependent on wave frequency, although waves with wavelengths longer than the leech's body evoked more sustained responses. For visual as well as mechanical waves, a majority of action potentials traveled tail to head. Initial bursts of anterograde spike propagation were occasionally seen, but for visual waves were found to be independent of stimulus direction. We are currently exploring the cause of the predominance of retrograde spike propagation in the S-cell system. Initial results indicate that retrograde propagation persists in the absence of the tail brain and even in the absence of most posterior ganglia.

Disclosures: **A. Muthusamy:** None. **A.M. Lehmkuhl:** None. **D.A. Wagenaar:** None.

Poster

337. Neuroethology of Sensory and Motor Systems: Invertebrates

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Topic: F.01. Neuroethology

Support: NSF OISE 1545803

NSF DBI-1337284

NIMHD 8G12-MD007600

NSF HRD-1137725.

Title: Localization of allatotropin-like immunoreactivity in the central nervous system of Biomphalaria glabrata, an intermediate host for intestinal schistosomiasis.

Authors: J. MALDONADO-ALERS[1], A. HERNÁNDEZ-VÁZQUEZ[1], S. ROLÓN-MARTÍNEZ[1,2], *M. W. MILLER[1,2];
[1]Inst. Neurobio., San Juan, PR; [2]Anat. & Neurobio., Univ. of Puerto Rico Med. Sci. Campus, San Juan, PR

Abstract: Approximately ten percent of the world's population lives at risk of contracting the parasitic disease, schistosomiasis, commonly known as "snail fever". The digenetic trematode

worm species *Schistosoma mansoni* that causes the most common form of intestinal schistosomiasis requires the freshwater snail *Biomphalaria glabrata* to serve as its primary intermediate host, where it proliferates and develops into its cercariae form that can infect humans. As infection of pulmonate snails by larval trematodes has been shown to alter neuropeptide gene expression, a neural transcriptomics approach was undertaken to determine precursor prohormones that could encode neuropeptides in *Biomphalaria*. A transcript (1616 nucleotides) was found to encode a putative precursor (316 aminoacids) that could liberate a single copy of *B. glabrata* allatotropin (GFRMNSASRVAHGYa). For this investigation, an antiserum (rabbit polyclonal) generated against Cys-GFRMNSASRVAHGY conjugated to BSA was used to localize allatotropin-like immunoreactivity in the central and peripheral nervous systems of *B. glabrata*. Allatotropin-like immunoreactivity was observed throughout the central nervous system (CNS) with distinct neurons and clusters on the ventral and dorsal surfaces of each major ganglion. Allatotropin-like cells of smaller diameter were present on the dorsal and ventral surfaces of the buccal ganglion (18.8 ± 2.8). In addition, dispersed clusters of small diameter cells were observed in the cerebral (14.7 ± 8.8) and pedal ganglia (25.4 ± 18.6). However, in the pleural ganglia no allatotropin-like neurons were present. Within the left parietal (4.0 ± 2.8) and visceral ganglia (2.4 ± 1.0), clusters of small cells were observed. These results suggest that allatotropin could regulate behaviors related to feeding and reproduction that are altered during the course of infection in this host-parasite system.

Disclosures: J. Maldonado-Alers: None. **A. Hernández-Vázquez:** None. **S. Rolón-Martínez:** None. **M.W. Miller:** None.

Poster

338. Parental Behavior

Location: Halls B-H

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Program#/Poster#: 338.01/SS14

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01 HD057962 to JS Lonstein

Title: Maternal aggression is impaired by prepartum serotonin-specific lesions of the midbrain dorsal raphe

Authors: *E. M. VITALE[1], M. A. HOLSCHBACH[2], J. S. LONSTEIN[1];
[1]Behavioral Neurosci. Program, Michigan State Univ., East Lansing, MI; [2]Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: The postpartum period in laboratory rats and other animals is characterized by increased maternal responsiveness, decreased anxiety, and increased aggression. Pharmacologically manipulating the serotoninergic system during the postpartum period alters all of these behaviors, and our lab recently found that lesioning serotonergic neurons in the dorsal raphe (DR; primary source of forebrain serotonin) after parturition decreases maternal aggression as well as pup licking in laboratory rats. This demonstrates serotonin's importance for these behaviors during the postpartum period, but no studies have evaluated the function of serotonin during pregnancy, a highly sensitive period when hormones and peptides alter neurochemistry to initiate maternal responsiveness. Given serotonin's role in hormone and neuropeptide release, DR serotoninergic activity beginning during pregnancy may be particularly important for the onset of postpartum changes in anxiety, maternal responsiveness, and maternal aggression. To test this hypothesis, we destroyed serotonergic cells with a neurotoxin targeting the serotonin transporter (anti-SERT-saporin; Advanced Targeting Systems) infused into the DR on pregnancy day 15. After parturition, we observed subjects' maternal caregiving behaviors, maternal motivation during retrieval tests, maternal aggression, and anxiety-like behaviors. We found that DR lesions during pregnancy greatly reduced maternal aggression towards an intruder, and that lesioned mothers also showed increased contact with pups immediately after disruption of the nest site during retrieval tests. Preliminary analysis of serotonin fiber innervation in several forebrain regions indicates tremendous reduction in serotonin fiber density in the amygdala and medial prefrontal cortex of lesioned subjects, but much less so in the medial preoptic area (MPOA). These findings demonstrate that prepartum serotonin-specific lesions of the DR affect particular maternal behaviors, especially aggression, and likely do so by reducing serotonergic innervation of the forebrain in a site-specific manner.

Disclosures: **E.M. Vitale:** None. **M.A. Holschbach:** None. **J.S. Lonstein:** None.

Poster

338. Parental Behavior

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 338.02/SS15

Topic: F.02. Behavioral Neuroendocrinology

Support: 2013 NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation

NICHD Grant HD073710

Title: Altered monoamines and associated metabolites across the postpartum period in the Wistar-Kyoto rat model of postpartum depression

Authors: *S. B. WINOKUR, Y. MOPARTHI, E. ELGUENAOUI, V. LEE, A. FARRAR, M. PEREIRA;
Psychological and Brain Sci., Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: Postpartum depression (PPD) is a serious psychiatric condition that has deleterious effects on the mother and poses a risk for the mother-infant relationship and ultimately the infant's development. Previous work suggests that alterations in monoamine levels in specific cortical and striatal structures can explain the pathophysiology of cognitive and motivational symptoms of depression. While many studies have characterized the symptomatology of PPD, relatively little is understood about the underlying neurobiological mechanisms by which parenting is disrupted in postpartum depression. The present study used Wistar-Kyoto (WKY) mother rats, an animal model of depression-like symptomatology which we have developed to examine the postpartum disorder (Pereira et al. 2012), to examine whether alterations in monoamine levels in discrete brain structures underlying cognitive and motivational deficits predict parenting disturbances in WKY mothers. To this aim, tissue samples were collected from the medial prefrontal cortex, the orbitofrontal cortex, the nucleus accumbens, and the medial preoptic area of early and late WKY and Sprague-Dawley (SD) postpartum females. Concentrations of norepinephrine, dopamine and serotonin, as well as their respective precursors and metabolites were assayed using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Initial results suggest that there are significant differences in intracellular monoamine concentrations between WKY and SD strains, and across the postpartum period. For example, early postpartum WKY rats have reduced tissue levels of dopamine in the medial preoptic area compared to SD mothers, which is consistent with the observed parenting disturbances in WKY mothers. Results are presented in the context of relating tissue levels of monoamines to maternal behavior. Planned microdialysis studies will provide additional insight into the neurochemical phenotype of WKY.

Disclosures: S.B. Winokur: None. **Y. Moparthi:** None. **E. Elguenaoui:** None. **V. Lee:** None. **A. Farrar:** None. **M. Pereira:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: 2013 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

NICHD grant HD073710

Title: Altered genetic expression in the maternal circuitry of Wistar-Kyoto rat model of postpartum depression across the postpartum period

Authors: *V. LEE, S. B. WINOKUR, A. M. FARRAR, M. PEREIRA;
Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Postpartum depression (PPD) is one of the most prevalent and severe mental illnesses affecting women, negatively shaping maternal affective, cognitive, and motivational functions and parenting capacities, posing a risk to the mother-infant relationship and ultimately infant development. Emerging evidence for genetic studies of depression highlights the need to understand the molecular mechanisms of depression-like symptomatology and associated parenting disturbances. This study used the Wistar-Kyoto (WKY) genetic rat model of depressive-like behavioral symptomatology to examine whether alteration in the expression of candidate genes within discrete structures involved in cognitive and motivational processes, are related to the observed deficits in parenting in WKY mothers. To this aim, quantitative polymerase chain reaction (qPCR) was utilized to profile the expression of candidate genes involved in DA neurotransmission, epigenetic mechanisms, and hormone signaling within the medial prefrontal cortex, the nucleus accumbens, and the medial preoptic area in virgin, as well as early and late postpartum WKY and control Sprague-Dawley (SD, an outbred reference strain) female rats. Preliminary analysis suggests a reduced expression of several genes, including oxytocin receptor (Oxtr), estrogen receptor 1 and 2 (Esr1 and Esr2), vesicular monoamine transporters (Slc18a2), monoamine oxidase B (MAOB), and DNA methyltransferase 3-alpha (Dnmt3a) in all regions examined in early postpartum WKY compared to SD mothers. In addition, there seem to be dynamic changes in the expression of these genes in all regions examined from early to late postpartum that are different between WKY and SD mothers. Together, these findings identified multiple genes that are differentially expressed in WKY rats across multiple regions during the process of motherhood, and likely contribute to the depressive-like phenotype of WKY mothers.

Disclosures: V. Lee: None. **S.B. Winokur:** None. **A.M. Farrar:** None. **M. Pereira:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH grant RO1 HD057962 to J.S. Lonstein

Title: Effects of oxytocin receptor knockdown in the dorsal raphe on maternal and anxiety like behaviors in postpartum rats

Authors: *Z. GRIEB, F. MANFREDSSON, J. LONSTEIN;
Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: Oxytocin (OT) is well-known for its role in mammalian maternal care. In ovarian hormone-primed female laboratory rats, administering OT facilitates the onset of mothering, while blocking central OT signaling interferes with caregiving. OT also influences other postpartum behaviors, including anxiety and aggression. OT acts in many brain sites to affect these postpartum behaviors, but midbrain sites sensitive to OT, such as the dorsal raphe (DR; source of most forebrain-projecting serotonergic neurons) are rarely studied. Recently, we found that on the day of parturition there is an ~60% increase in oxytocin receptor (OTR) binding in the DR compared to that found in diestrous nulliparous rats. This increase may be functionally relevant, as manipulating the serotonergic system directly in the DR or at its projection sites modifies many postpartum behaviors. In the present study we hypothesized that elevated OT signaling specifically in the DR influences the display of mothers' socioemotional behaviors. To test this hypothesis, we created an adeno-associated virus (AAV) expressing a short hairpin RNA (shRNA) targeted to OTR mRNA (AAV-OTRKO) or a scrambled shRNA control. The OTR shRNA was tested *in vitro* and produced >80% knockdown of OTR mRNA. *In vivo* DR AAV injections will be performed on pregnancy day 8 with maternal behaviors studied postpartum. We predict that mothers treated with the AAV-OTRKO will show impairments in some aspects of their mothering, increased anxiety-like behavior, and decreased maternal aggression compared to scrambled shRNA treated controls. These results would suggest that OTR signaling in the DR is necessary for the display of numerous maternal behaviors in laboratory rats. Such results would be an interesting contrast to recent findings in laboratory mice indicating that OTRs expressed specifically on serotonergic neurons do not influence maternal behaviors. Specifically, because ovarian hormones and OT are less important for the propensity to display pup caregiving behaviors in mice compared to rats, our data could indicate that OTR in the DR influences maternal behaviors in a species-specific manner.

Disclosures: Z. Grieb: None. **F. Manfredsson:** None. **J. Lonstein:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: IGO-103692

Title: Intensity of exercise and postpartum exposure to fluoxetine differentially affect behavior and hippocampal neurogenesis in a rat model of postpartum stress

Authors: *A. R. GOBINATH[1], R. J. RICHARDSON[2], C. CHOW[2], J. L. WORKMAN[2], S. E. LIEBLICH[2], A. M. BARR[3], L. A. M. GALEA[2];
[1]Neurosci., [2]Psychology, [3]Anesthesiology, Pharmacology, and Therapeut., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Postpartum depression (PPD) affects approximately 15% of mothers and imposes a lifelong burden of mental health concerns for women. Pharmacological antidepressants such as fluoxetine (Prozac) are commonly used to treat PPD. However, use of fluoxetine during the postpartum period remains controversial due to issues with efficacy as well as neonatal exposure to the drug. For this reason, non-pharmacological therapies such as exercise may be of interest as an alternative intervention. However, physiological changes typified by the postpartum period may alter the antidepressant potential of exercise. Unfortunately, it is unclear whether exercise is efficacious for treating PPD. To investigate this, we treated rat dams daily with high levels of corticosterone (40 mg/kg), to induce a depressive-like phenotype, or oil during the postpartum period. Within the oil and corticosterone conditions, four additional antidepressant groups were created: 1. Fluoxetine (Prozac; 10 mg/kg) + exercise (voluntary access to running wheel); 2. Fluoxetine + no exercise; 3. Saline (vehicle for fluoxetine) + exercise; 4. Saline + No exercise. Dams were tested for maternal behavior, anxiety-like behavior (novelty suppressed feeding), and depressive-like behavior (forced swim test). We also quantified serum corticosterone levels as well as doublecortin (marker of immature neurons) expression in dorsal and ventral dentate gyrus. Daily running activity was recorded and using a median split, dams were further categorized as “high-running” or “low-running.” Preliminary results reveal that maternal fluoxetine reversed corticosterone-induced disruptions in maternal care, especially in low-running but not high-running dams. However, maternal fluoxetine increased anxiety-like behavior in the novelty suppressed feeding task regardless of concurrent corticosterone or exercise exposure. Exercise also tended to decrease immobility (depressive-like behavior) in the forced swim test. The combination of exercise and fluoxetine attenuated stress-induced rises in serum corticosterone in comparison to fluoxetine alone. Finally, exercise bolstered doublecortin expression in ventral but not dorsal dentate gyrus in comparison to non-exercising dams. Of corticosterone-treated dams, the combination of high-running and fluoxetine increased doublecortin expression in ventral dentate gyrus in comparison to fluoxetine alone. Our findings will shed light on how the postpartum antidepressant treatments (Prozac, exercise) interact to differentially affect the well-being of the mother at the behavioral, endocrine, and hippocampal levels. Funded by CIHR to LAMG.

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Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH grant R21HD083791-A1

Title: Gestational stress effects on dopamine and oxytocin within the postpartum reward circuitry: implications for mood and mothering

Authors: *B. LEUNER[1,2,3], A. HAIM[1], C. ALBIN-BROOKS[1], D. JULIAN[1], B. SPRINGER[1], H. BROTHERS[1];
[1]Dept. of Psychology, [2]Dept. of Neurosci., [3]Behavioral Neuroendocrinology Group, Ohio State Univ., Columbus, OH

Abstract: Postpartum depression is a debilitating disorder affecting an estimated 20% of new mothers making it one of the most prevalent mental health conditions in women. In addition to depressive symptoms, postpartum depression is accompanied by disrupted caregiving which can have long-lasting deleterious effects on offspring development. Epidemiological studies have shown that chronic stress during pregnancy is a major risk factor for postpartum depression. Like humans, pregnant rodents exposed to chronic gestational stress exhibit increased depressive-like behaviors during the postpartum period along with deficits in maternal behavior. However, the neurobiological mechanisms underlying impaired maternal functioning associated with postpartum depression are largely unknown. Maternal care is a rewarding, motivated behavior that like other types of motivated behaviors, involves the mesolimbic dopamine (DA) system, particularly DA projections from the ventral tegmental area (VTA) to the nucleus accumbens. Further, there is evidence that the mesolimbic DA system is regulated by oxytocin (OT) inputs from the hypothalamus to the VTA which expresses oxytocin receptors (OTR). Given these links, we tested the extent to which the adverse effects of gestational stress on postpartum mood and maternal functioning would be accompanied by altered DA and OT. Our results show that gestational stress-induced depressive-like behavior and maternal care deficits during the postpartum period were accompanied by a reduction in accumbal DA levels and increased DA turnover. In addition, OT fiber density was reduced in the VTA of gestationally stressed mothers who also exhibited lower OTR mRNA expression in the VTA. In contrast, gestational stress had no effect on the number of OT-immunoreactive neurons in the hypothalamus or the number of TH-immunoreactive neurons in the VTA. Taken together, these data show that several aspects of the OT and DA systems within the postpartum reward circuit are affected by gestational stress and raise the possibility that a disruption in OT-DA interactions may underlie impaired caregiving in depressed mothers.

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Poster

338. Parental Behavior

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Program#/Poster#: 338.07/SS20

Topic: F.02. Behavioral Neuroendocrinology

Title: Maternal behavior in virgin forebrain oxytocin receptor knockout mice

Authors: *S. K. WITCHEY[1], H. K. CALDWELL[2];
[1]Biol. Sci., [2]Deparment of Biol. Sciences; Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: In female rodents, the oxytocin (Oxt) system is not only important to birth and milk ejection but also plays a role in the initiation of maternal behavior. Previous work from our lab has shown that Oxt receptor knockout (Oxtr-/-) mice and forebrain Oxt receptor knockout ($Oxtr^{FB/FB}$) mice are more likely to abandon their pups than wildtype (+/+) females; though, females that do initiate maternal behavior do not differ from +/+ controls. To determine if aversion to pups is restricted to the postpartum period, we examined maternal sensitization in virgin $Oxtr^{FB/FB}$ and Oxtr +/+ females following repeated exposure to foster pups. After behavioral testing we collected brains and processed them for immediate early gene activation (i.e. c-Fos immunocytochemistry). We hypothesized that there would be genotypic differences in the onset of maternal behaviors, with virgin $Oxtr^{FB/FB}$ females having a delay in the onset of maternal behaviors compared to controls. We also hypothesized that there would be genotypic differences in c-Fos immunoreactivity in brain areas known to be important in maternal care, such as the paraventricular nucleus, the supraoptic nucleus, the medial preoptic area, the bed nucleus of the stria terminalis, and the lateral septum. Specifically, we predicted that virgin $Oxtr^{FB/FB}$ females would show reduced c-Fos activation in some of these brain areas. While there were no significant differences in any maternal behaviors scored (i.e. nest building, licking pups, etc) across any days, there were significant genotypic differences in the latency to retrieve the first pup on day one and all pups retrieved on day one with virgin $Oxtr^{FB/FB}$ females retrieving pups more quickly. We also found a difference in c-Fos activation, with virgin $Oxtr^{FB/FB}$ females having decreased c-Fos immunoreactivity in the dorsal region of the lateral septum compared to +/+ controls.

Disclosures: **S.K. Witchey:** None. **H.K. Caldwell:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: MJ Murdock Charitable Trust Grant 2010181:JVZ:2/24/2011

MJ Murdock Charitable Trust Grant 2012224:JVZ:2/28/2013

Title: Arginine vasopressin increases maternal behavior in the biparental California mouse (Peromyscus californicus)

Authors: N. D. NG, M. F. CONLEY, G. E. MAMMARELLA, *J. K. BESTER-MEREDITH; Biol., Seattle Pacific Univ., Seattle, WA

Abstract: Arginine vasopressin (AVP) has been implicated in the regulation of social behavior in both male and female mammals. Although AVP promotes maternal care and aggression in rats, less is known about its role in regulating female social behavior in species with other types of complex social systems. Unlike most rodents, the California mouse (*Peromyscus californicus*) is monogamous and shows biparental care of offspring with males participating in all aspects of parental care other than nursing. Adult female California mice, regardless of reproductive state, also show high levels of territorial aggression in comparison to females of other rodent species. In the present study, we tested whether AVP and its antagonist (β-Mercapto-β,β-cyclopentamethylenepropionyl1, O-me-Tyr2, Arg8]-Vasopressin) alter maternal aggression and maternal behavior. Cannulae were implanted into the lateral ventricles of pregnant female California mice approximately one week prior to parturition. Beginning on the first day after the birth of pups, we administered daily injections of AVP, an AVP antagonist, or artificial cerebrospinal fluid into the lateral ventricles of these mice. Parental behavior was video recorded for a half hour on days two, four, six, and eight after the birth of pups. Aggression was tested on day five after the birth of pups using a resident-intruder paradigm with a female intruder. Although infusions of AVP, its antagonist, and artificial extracellular fluid did not alter aggression, we found a statistically significant interaction between infusion type and maternal behavior. Over the first eight days after the birth of pups, mothers receiving AVP infusions increased the amount of time spent nursing and huddling, whereas mothers receiving the AVP antagonist decreased the amount of time spent nursing and huddling. These results suggest that vasopressin may regulate maternal behavior in California mice.

Disclosures: N.D. Ng: None. **M.F. Conley:** None. **G.E. Mammarella:** None. **J.K. Bester-Meredith:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: DFG Grant BO1958/8-1 to OJB

Title: Activation of CRF receptor type 1 in the medial preoptic area severely impairs maternal behavior and increases anxiety-related behavior in lactating rats

Authors: *O. J. BOSCH[1], B. M. GAßNER[1], D. S. BAYERL[1], S. M. KLAMPFL[1,2];
[1]Univ. of Regensburg, Regensburg, Germany; [2]Dept. of Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The survival and proper development of the offspring is ensured by the adequate expression of maternal behavior. It is strongly disturbed by an elevated activity of the major stress neuropeptide system, the brain corticotropin-releasing factor (CRF) system, as shown for the lateral septum (D'Anna & Gammie 2009 *Behav Neurosci*) and the bed nucleus of the stria terminalis (Klampfl et al. 2014 *J Neurosci*, 2016 *PNEC*). In this line, the medial preoptic area (MPOA) is a promising brain area; it is a major maternal brain region and shows broad expression of most CRF family members. To test our hypothesis, we fitted pregnant Sprague-Dawley rats bilaterally with guide cannula targeting the MPOA. On lactation days (LD) 1 to 7, we acutely infused 0.5µl/side of the CRF-R1 agonist human/rat CRF, the CRF-R1 specific antagonist CP-154,526, the CRF-R2 specific agonist stresscopin, the CRF-R2 specific antagonist astressin-2B or vehicle bilaterally into the MPOA every other day and subsequently monitored maternal and anxiety-related behaviors. Under non-stress conditions (LD1), activation of CRF-R1 or -R2 immediately decreased the occurrence of arched back nursing and overall nursing; interestingly, the behavioral effect of CRF-R1 activation was stronger and longer-lasting (120 min versus 60 min). Under stress conditions (LD7), i.e. immediately after exposure to the maternal defense test, all groups showed an acute decrease in overall nursing. This decrease was even longer-lasting after CRF-R1 activation, which in turn increased licking/grooming the pups at the same time. During the maternal defense test (LD7), CRF-R1 inhibition more than doubled the number of attacks and the sum of aggressive behavior against a female intruder while CRF-R1 activation had no behavioral effect. CRF-R2 manipulations had no effect on maternal aggression. Maternal motivation to retrieve pups was not altered by any treatment (LD3). When testing anxiety-related behavior on the elevated plus-maze (LD5), CRF-R1, but not -R2, activation increased the percentage of time on and full entries into open arms whereas entries into closed arms were decreased. In summary, CRF-R1 in the MPOA are crucially involved in controlling maternal care, maternal aggression and anxiety. Interestingly, the latter two have

rarely been linked to the MPOA so far. On the contrary, the MPOA is well known for its prominent role in facilitating maternal motivation to retrieve pups in which the intra-MPOA CRF system is seemingly not involved. To conclude, we identified the CRF-R1 in the MPOA as an interesting and promising target with respect to treatment of maladaptations in maternal behavior.

Disclosures: O.J. Bosch: None. **B.M. Gaßner:** None. **D.S. Bayerl:** None. **S.M. Klampfl:** None.

Poster

338. Parental Behavior

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Narsad Young Investigator Award (AEA)

Title: Investigating neural circuits governing parental behavior

Authors: *A. E. AUTRY[1], Z. WU[2], B. MARIN-RODRIGUEZ[2], N. RUBINSTEIN[2], D. BAMBAH-MUKKU[2], J. KOHL[2], C. DULAC[2];
[2]Mol. and Cell. Biol., [1]Harvard Univ., Cambridge, MA

Abstract: Parental care is essential for the survival and well-being of young, though parents or other adults sometimes show neglect or aggressive behavior toward infants. While maternal behavior has been studied extensively, agonistic behavior toward infants remains poorly understood. In animal models of parental behavior, natural variation in levels of care is observable. In mice, virgin females are spontaneously maternal and show enhanced infant care during late pregnancy and lactation. However, virgin male mice ignore or attack pups but become paternal during a transient time period after mating coincident with the birth of their offspring. Our lab has recently demonstrated the power of combining molecular, genetic, and behavioral techniques to understand how galanin neurons in the medial preoptic area govern positive aspects of parental behavior (Wu et al., 2014). In the present study, we use an unbiased microarray based screen on laser-captured cells to molecularly define a population of hypothalamic neurons that are preferentially activated by agonistic pup-directed behavior. These neurons display a very restricted spatial pattern that overlaps with around 40% of neurons

showing enhanced *c-fos* levels following agonistic pup-directed behavior. We will present the functional impact of gain and loss-of-function studies on parental behavior in males and females using chemogenetic and neuronal ablation tools. The function of this putative pup-directed aggression circuit will be further interrogated by studying the pre- and post-synaptic targets of the neurons using tracing and modified rabies viruses.

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Poster

338. Parental Behavior

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NIH R21HD075021

UCR COR grant to Peter Hickmott

UGR Student Mini-Grant to Diane Luu

Title: Effects of fatherhood on synaptic, intrinsic, and morphological characteristics of neurons in the medial preoptic area of male California mice

Authors: *N. HORRELL[1], P. HICKMOTT[2], D. LUU[1], W. SALTZMAN[3];
[2]Psychology, [3]Biol., [1]Univ. of California Riverside, Riverside, CA

Abstract: Parental care by fathers enhances offspring survival and development in numerous species. In biparental mammals, the onset of paternal behavior is associated with changes in the responsiveness of specific brain regions. However, virtually nothing is known about effects of fatherhood on properties of neurons. The brain region most strongly implicated in parental behavior in both mothers and fathers is the medial preoptic area of the hypothalamus (MPOA). The California mouse, *Peromyscus californicus*, offers an excellent model system for paternal care. Behavioral plasticity is seen during the transition into fatherhood: adult virgin males often exhibit aggressive or indifferent responses when exposed to pups, whereas fathers engage in extensive paternal care toward both their offspring and unrelated pups. Differences in neuronal activity in the MPOA between virgins and fathers may account for the differences in paternal behavior. We evaluated synaptic, intrinsic, and morphological properties of MPOA in neurons in

adult male California mice that were either first-time fathers or virgins. We used standard whole-cell recordings in a novel *in vitro* slice preparation. Excitatory and inhibitory post-synaptic currents (EPSCs and IPSCs) from MPOA neurons were recorded in response to local electrical stimulation, and input/output curves were constructed for each. Responses to trains of stimuli were also examined. We quantified intrinsic excitability by measuring voltage changes in response to square-pulse injections of both depolarizing and hyperpolarizing current. Finally, biocytin was injected into neurons during recording. Laser-scanning confocal microscopy was used to reconstruct neuronal morphology. Z-stacks were collapsed to create a 2D representation of neuronal morphology for Sholl's analysis and other morphometric analyses. Preliminary data revealed trends for increased maximal EPSC and decreased maximal IPSC in MPOA neurons of fathers compared to virgins. In response to suprathreshold injections of current, neurons exhibited a variety of spiking phenotypes, including bursting, fast-spiking, and regular-spiking. Preliminary data showed a trend for fast-spiking cells to have an increased maximum number of action potentials in fathers compared to virgins. Fathers also had significantly more dendrites leaving the soma and significantly increased length of the longest dendrite. These findings suggest that the onset of fatherhood in California mice is associated with neuronal plasticity in the MPOA.

Disclosures: **N. Horrell:** None. **P. Hickmott:** None. **D. Luu:** None. **W. Saltzman:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: UNAM-DGAPA-PAPIIT-IN202315

CONACYT Scholarship

Title: Influence of parental interaction and prolactin treatment diminishing kainic acid-induced neurodegeneration in the hippocampus of male mice

Authors: I. ANAGNOSTOU[1], *T. MORALES[2];
[1]Inst. for Neurobio. UNAM, Queretaro, Mexico; [2]Intitute for Neurobio. UNAM, Queretaro, Mexico

Abstract: It has been shown that the hippocampus (HP) of lactating female dams is less sensitive to excitotoxic damage by kainic acid (KA) than that of virgin rats (Cabrera et al, 2013).

Part of this effect is attributed to the pituitary hormone prolactin (PRL) which diminishes KA-induced neurodegeneration when administered to virgin female rats (Tejadilla et al., 2010). However, we know neither the effects of exogenously administered PRL on neuroprotection in males, nor if paternity itself can provide such a protection through changes in the hormonal milieu, especially via PRL. To address these questions, male virgin adult mice CD-1 were paired with female virgin adult mice and co-housed throughout pregnancy. On the day of parturition (P0) the animals were randomly assigned to two groups: a) the pregnancy group (Pr), in which the males were removed from the home-cage where they were co-housed with the female until parturition, placed in an individual cage and injected with 100 ng KA / 1 µl saline 0.9% (SAL) or with 1 µl SAL i.c.v. on day P1, and b) the paternity group (P), in which the males were allowed to stay in the home-cage, interact with the lactating female and the pups until day P7, and underwent the i.c.v. injection with KA or SAL on day P8, shortly after the evaluation of parental behavior. PRL effects were analyzed in male virgin adult mice by injecting a daily dose of 8 µg of ovine PRL/100 µl of SAL or 100µl of SAL s.c. for 7 consecutive days, followed by i.c.v. injection of KA or SAL. The control group consisted of male virgin adult mice housed individually, subjected to KA or SAL i.c.v. injections. All animals were sacrificed 48 h after the i.c.v. injection and the cerebral tissue was processed for histology to measure neurodegeneration by Nissl and FluoroJade-C in the CA1, CA3 and CA4 subfields of the HP. Male rats treated with PRL showed less neurodegeneration in the hippocampal regions analyzed compared to SAL controls. Additionally, both Pr and P groups had diminished levels of cell death after KA-lesioning. These results indicate that PRL has a neuroprotective effect on the HP of male mice subjected to excitotoxic lesion and that experiencing the environment of pregnancy and paternity can show similar protective effects.

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Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Title: Esr1 expressing neurons in the medial preoptic area mediate maternal behaviors

Authors: *Y.-Y. FANG, D. LIN;
Inst. of Neurosci., NYU Langone Med. Ctr., New York, NY

Abstract: Female mice exhibit a repertoire of stereotypical actions aimed at caring and protecting the pups. Classical mapping experiments identified the medial preoptic areas (MPA)

as an essential region in mediating maternal behaviors but how cells in the MPA encodes maternal information and drives the behavior remain unclear. Here, we demonstrated that estrogen receptor alpha (Esr1) expressing cells in the MPA are essential for maternal behaviors. Reversible suppression of the cells impaired the behavior whereas optogenetic activation of the cells induced immediate maternal behaviors. *In vivo* electrophysiological recording demonstrated that cells in the MPA carry information regarding maternal state, and moment-to-moment maternal action. Importantly, Esr1 expressing cells are maximally active during displaying certain aspects of maternal behavior. Thus, MPA Esr1 neurons emerge as an essential mediator of maternal behavior, and are subjective to modulation by experience and reproduction state.

Disclosures: Y. Fang: None. **D. Lin:** None.

Poster

338. Parental Behavior

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 338.14/TT1

Topic: F.02. Behavioral Neuroendocrinology

Support: Eric L. and Lila D. Nelson Chair in Neuropharmacology

International Education IIE-SRF fellowship

Title: melanin concentrating hormone modulates maternal behavior

Authors: *A. ALACHKAR[1], L. ALHASSEN[2], Z. WANG[2], O. CIVELLI[2];
[1]Pharmacol., Univ. of California Irvine, Irvine, CA; [2]Univ. of California irvine, irvine, CA

Abstract: In order to prepare the mother for the demands of pregnancy and lactation, the maternal brain is subjected to a number of adaptations. Maternal behaviors are regulated by complex neuronal interactions. Here, we show that the melanin concentrating hormone (MCH) system is an important regulator of maternal behaviors. We report that melanin concentrating hormone receptor 1 knockout (MCHR1 KO) mice display a disruption of maternal behavior. Early postpartum MCHR1 KO females exhibit poor nesting, deficits in pup retrieval and maternal aggression. Ablation of MCH receptors results in decreased milk production and prolactin mRNA levels. These results are in line with those obtained in wild type mice (WT) treated with the specific MCHR1 antagonist GW803430. Furthermore, following pups retrieval, MCHR1 KO mice display a lower level of Fos expression than WT mice in the ventral tegmental area, and nucleus accumbens. With the progression of the lactation period, however, the MCHR1 KO mice improve maternal care towards their pups. Of particular interest is the finding that

genetic ablation and pharmacological blockade of MCHR1 caused reduction in postpartum depression-like behavior in the MCHR1 KO mice in the late lactation period but not in early lactation period or in virgin female mice. In conclusion, we show that the MCH system plays a significant role in the initiation of maternal behavior. **Key Words:** Melanin concentrating hormone, Maternal Behaviors, circuits, KO, Antagonist

Disclosures: **A. Alachkar:** None. **L. Alhassen:** None. **Z. Wang:** None. **O. Civelli:** None.

Poster

338. Parental Behavior

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 338.15/TT2

Topic: F.02. Behavioral Neuroendocrinology

Title: Experience-dependent alterations in maternal behavior are associated with gene expression changes in maternal neural pathways

Authors: *H. S. MAYER[1], D. S. STOLZENBERG[2];
[1]Psychology, [2]Univ. of California Davis, Davis, CA

Abstract: Prior to giving birth most mammalian females ignore or even avoid infants. However, at the time of birth a rapid behavioral transition from the non-maternal state occurs. For example, newly parturient female rodents build a nest to keep pups warm, retrieve pups to this nest and crouch over pups. The level of responsiveness toward pups is high; females will search a novel T-maze to locate and retrieve pups to a nest location. In rodents, this behavioral transition is regulated by the relative activation of two neural pathways within the maternal brain. Thus, the transition into the maternal state involves an attenuation of the pathway regulating infant avoidance (medial amygdala, anterior hypothalamus, periaqueductal gray) and an amplification of the pathway regulating infant approach (medial preoptic area, ventral tegmental area, nucleus accumbens, ventral pallidum). Whereas the hormonal events of late pregnancy and birth facilitate the transition into motherhood, this transition does not depend on hormonal exposure. Virgin female mice will display “spontaneous” caregiving behaviors towards pups placed in their home cage within 15-30 minutes. However, repeated pup exposure (2 hours/4 days) is required to induce pup retrieval in a novel T-maze. We hypothesize that the experience-dependent transition to the maternal state is mediated by alterations in gene expression that induce plastic changes within the approach and avoidance pathways. In support of this idea, administration of sodium butyrate, a drug that enhances experience-induced histone acetylation and gene expression, induced pup retrieval on the T-maze in mice with sub-threshold maternal experience (2 hours/2 days). In the present study, we quantified the expression of several genes associated with

synaptic plasticity throughout the approach and avoidance pathways. We are ultimately interested in how gene expression within these pathways corresponds to pup approach and pup avoidance behaviors, thus virgin mice with sub-threshold pup experience were exposed to the home cage (a context in which pup approach behavior occurs) and a novel T-maze (a context in which pup avoidance behavior occurs). Given that sodium butyrate induced pup approach on the T-maze in virgin mice with sub-threshold experience, the effects of histone deacetylation on gene expression in these two contexts were also examined. Finally, the extent to which experience-induced alterations in gene expression that mediate pup approach behaviors mimic hormone-induced alterations approach and avoidance pathways was examined.

Disclosures: H.S. Mayer: None. **D.S. Stolzenberg:** None.

Poster

338. Parental Behavior

Location: Halls B-H

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Topic: F.02. Behavioral Neuroendocrinology

Support: Longwood University PRISM Grant

Longwood University Faculty Research Grant

Longwood University Cook-Cole College Fund

Cormier Honors College Fund

Title: Maternal behavior and neuronal activation differs in lactating rats depending on ratio of own pups present

Authors: K. A. UNROE[1], T. C. FRUCHTERMAN[1], M. L. GRIMES[1], A. O. RIPLEY[1], C. L. FRANSSEN[2], *A. FRANSSEN[1];
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Abstract: Mother rats have been demonstrated to care for both their own (OWN) and alien pups in communal nests (e.g., Beach and Jaynes 1956; Branchi et al. 2006) and the evolutionary value of this behavior examined (e.g., Hayes 2000). Our lab has been investigating the decision making processes of mother Sprague-Dawley rats (*Rattus norvegicus*) presented with a litter of pups that contains either her own pups, a combination of her own and alien pups, or exclusively alien pups. Behavioral data suggests that mother rats will more rapidly retrieve and care equally for pups from a litter that contains more than 25% of her own pups than from a litter that contains

25% or fewer of her own pups. Using c-fos immunoreactivity as a marker, we have identified the frontal cortex, amygdala, insula, and hypothalamus as regions with differential levels of expression. These findings may suggest areas of the brain responsible for first identifying the presence of OWN pups and then deciding to care for the litter as a whole. Evolutionarily, the benefits to the mother rat may be significantly lessened once the ratio of OWN pups drops below this level. In clinical trials or other experiments with maternal rats, our findings may indicate that maintaining ratios of over 25% OWN will ensure that the behavior observed is a response to the independent variable rather than the litter of pups itself.

Disclosures: K.A. Unroe: None. **T.C. Fruchterman:** None. **M.L. Grimes:** None. **A.O. Ripley:** None. **C.L. Franssen:** None. **A. Franssen:** None.

Poster

338. Parental Behavior

Location: Halls B-H

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Program#/Poster#: 338.17/TT4

Topic: F.02. Behavioral Neuroendocrinology

Support: Center for Research on Families Graduate Student Research Grant, 2015-2016, University of Massachusetts, Amherst

NIEHS Grant K22ES025811

Title: Exposure to bisphenol-S during pregnancy and lactation alters maternal brain and behavior in CD-1 mice

Authors: *M. C. CATANESE[1], L. N. VANDENBERG[2];
[1]Neurosci. and Behavior Program, [2]Dept. of Envrn. Hlth. Sci., Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: Maternal care is critical for the survival and long-term success of offspring; poor maternal care can have profound effects on health that can last into adulthood. Classical studies investigating the neural and hormonal basis underlying maternal behavior in the rat model have demonstrated the central role of estradiol (E2) in the onset of maternal behavior via ERα signaling in the medial preoptic area (MPOA) of the forebrain. While the display of maternal behavior in mice is considered to be independent of estrogen, recent evidence using conditional knockouts indicate that ERα is necessary for the display of maternal care in mice.
The potential effects of xenoestrogens on maternal behavior and brain are poorly understood. Endocrine disrupting chemicals (EDC) such as BPA have been shown to interfere with

endogenous estrogen signaling through binding to classical and membrane- associated receptors. Currently, the potential endocrine disrupting effects of the BPA substitute, bisphenol S (BPS), remain largely unknown, although there is growing evidence that this compound acts as an estrogen. BPA has been previously shown to disrupt maternal behavior, however no studies have concurrently examined effects on the regions of the brain important for these behaviors.
We hypothesized that low doses of BPS would disrupt maternal behavior assessed through traditional assays such as pup retrieval, nest building and nesting behaviors, as well as lead to alterations in ERα expression in the MPOA. Further, we postulate that the plasticity of maternal behavior and the dynamic changes of the neuroendocrine state at parturition suggest that the onset of maternal behavior may be associated with neurogenesis in the MPOA.
We present preliminary evidence that low doses of BPS interfere with the expression of some, but not all, components of maternal behavior. Furthermore, BPS exposures induced changes in ERα expression in the MPOA in dams at lactational day 21. Finally, proliferation assays conducted during the early postpartum period with BrdU labeling to detect dividing cells provide evidence of actively dividing cells in this brain region.
The results of our study will allow us to better understand the impact of environmental chemicals on the maternal behavior and brain. To the best of our knowledge, there have been no studies analyzing effects of xenoestrogens on the maternal brain in conjunction with assessments of maternal behavior. We also know of no studies of neurogenesis in the MPOA in relation to maternal behavior or exposures to xenoestrogens. These results will offer insight into potential mechanisms underpinning behavioral changes.

Disclosures: M.C. Catanese: None. **L.N. Vandenberg:** None.

Poster

338. Parental Behavior

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 338.18/TT5

Topic: F.02. Behavioral Neuroendocrinology

Title: Perinatal exposure to a commercial formulation of glyphosate alters maternal behavior and neurobehavioral development in infant rats

Authors: *E. L. RICCI[1], M. O. RIBEIRO[2], M. M. BERNARDI[3], H. S. SPINOSA[4];
[1]Ctr. de Ciências Biológicas e da Saúde, Presbyterian Mackenzie Univ., osasco, Brazil; [2]Ctr. de Ciências Biológicas e da Saúde, Presbyterian Mackenzie Univ., São Paulo, Brazil; [3]Grad. Program of Envrn. and Exptl. Pathology and Grad. Program of Dent., Paulista Univ., São Paulo,

Brazil; [4]Dept. of Pathology, Sch. of Vet. Med. and Animal Sci., Univ. of São Paulo, São Paulo, Brazil

Abstract: Glyphosate is a non-selective herbicide registered for use on many food and non-food crops as well as non-crop areas where total vegetation control is desired. When applied at lower rates, it serves as a plant growth regulator. The most common uses include control of broadleaf weeds and grasses in: hay/pasture, soybeans, field corn; ornamentals, lawns, turf, forest plantings, greenhouses, rights-of-way. In Brazil glyphosate is marketed under several names, including Roundup ®. Ingredients considered inert are added to these products in order to allow the penetration of glyphosate across the plasma membrane of the plant, increasing its action, as well as providing greater stability and bioaccumulation potential. Considering that the presence of certain chemicals in commercial formulations of glyphosate may increase its toxicity and that there are insufficient data on the toxicology of development using these formulations, the present study investigated the effects of perinatal exposure of rats to Roundup®. Thus, the maternal behavior (MB) and neurochemistry, as well as the offspring physical and behavioral development from birth to adulthood were assessed. For that, pregnant rats received 50, 100 and 150 mg/kg from the 15th gestation day to the 7th lactation day. The results showed that glyphosate-Roundup® reduced the number of pups born in all herbicide doses. In rats exposed to glyphosate-Roundup® it was possible to observe a reduction in general activity in the open field at the highest dose, impairment in MB and maternal aggressive behavior (MAB), as well as in the hypothalamus there was an increase in the dopaminergic system activity and a reduction of the serotonergic system activity, whereas the hippocampus and striatum showed decreased activity of the dopaminergic system. These findings suggest that the increased activity of the hypothalamic dopaminergic system by reducing the release of prolactin, an essential hormone for the expression of MB, damaged the manifestation of this behavior. In offspring, the behavior of play fighting showed reduction only in the pinning from both male and female rats, without changing other parameters of this behavior. The duration and frequency of pinning are considered parameters that express the beginning of the social behavior of the rats. In the general activity, only punctual changes were observed in male and female offspring. It was suggested that perinatal exposure to all Roundup® doses during the perinatal period impaired the pups survival during pregnancy and had toxic effects in offspring.

Disclosures: E.L. Ricci: None. **M.O. Ribeiro:** None. **M.M. Bernardi:** None. **H.S. Spinosa:** None.

Poster

338. Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Title: Electroencephalographic correlation in biological & adoptive mothers while listening to a baby crying

Authors: *M. PÉREZ-HERNÁNDEZ, R. M. HIDALGO-AGUIRRE, M. HERNÁNDEZ-GONZÁLEZ, C. AMEZCUA, M. A. GUEVARA;
Correlación Electroencefalográfica y Conducta, Inst. De Neurociencias, Guadalajara, Mexico

Abstract: Maternity does not refer exclusively to a blood relation, for it also exists in cases of adoption. While, obviously, adoptive mothers do not experience the physiological changes of pregnancy, like biological mothers they care for, protect and educate their babies and show maternal behaviors in response to the stimuli they emit. Given that the prefrontal, temporal and parietal cortices all participate in processing relevant stimuli, this work analyzed the degree of electroencephalographic correlation (rEEG) among these cortices in biological (BM) and adoptive mothers (AM) while listening to a recording of a baby crying and white noise. A third group, consisting of non-mothers (NM), served as a control. BM and AM showed greater rEEG than NM in the alpha2 band between parietal areas (P3-P4) in crying condition. While listening to the stimulus emitted by the baby, BM showed a decreased rEEG in the fast frequencies (*i.e.*, alpha2, beta and gamma) between frontal (F3-F4) and fronto-temporal areas (F3-T3; F4-T4), but an increased rEEG between temporal areas (T3-T4) in beta1. In AM, crying elicited a higher rEEG between frontal areas (F3-F4) in beta2 and gamma; between temporal areas (T3-T4) in theta and alpha1; and between parietal areas (P3-P4) in theta. NM, in contrast, only showed a lower rEEG between frontal areas (F3-F4) in alpha1 while listening the cry recording. It is probable that the changes in the degree of coupling among the cortices are associated with plastic cerebral changes that could be necessary for BM and AM to process and respond adequately to the crying baby. These results show that the degree of functional coupling between cortices is modulated by the type of motherhood (biological *vs*. adoptive), and provide information on functional changes in the brain associated with the experience of motherhood and the processing of stimuli emitted by babies.

Disclosures: M. Pérez-Hernández: None. **R.M. Hidalgo-Aguirre:** None. **M. Hernández-González:** None. **C. Amezcua:** None. **M.A. Guevara:** None.

Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

Support: HRC Grant 12/670

CNPq Grant 232700/2014-1

Title: Role of rostral periventricular area of the third ventricle (RP3V) GABAergic neurons in generating the preovulatory luteinizing hormone surge in female mouse.

Authors: *B. KALIL, T. MCLENNAN, R. PIET, A. E. HERBISON;
Dept. of Physiol., Univ. of Otago, Dunedin, New Zealand

Abstract: The mid-cycle preovulatory luteinizing hormone surge (LH) surge is triggered by a massive discharge of gonadotropin releasing hormone (GnRH) into the hypophyseal portal blood. Recent studies demonstrated that gamma aminobutyric acid (GABA) neurons within the rostral periventricular area of the third ventricle (RP3V) innervate GnRH neurons to control the GnRH/LH surge generation. At present, the effects of RP3V GABA neurons on GnRH neurons and LH secretion in vivo are unknown. Herein, we examined the effect of optogenetic activation of RP3V GABAergic neurons in relation to the LH surge *in vivo*. For this, adult female VGAT-Cre mice were ovariectomized and channelrhodopsin-2 (ChR2) targeted to RP3V GABA neurons by the injection of a Cre-dependent adeno-associated virus vector (AAV). Three weeks later, mice were treated with an estradiol regimen that evokes the LH surge. On the day of the surge, an optic fiber was stereotaxically implanted into the RP3V of isoflurane anesthesized mice. ChR2-expressing GABA neurons were activated with pulses of blue light delivered at 2 and 10Hz for 15 min just prior to the predicted time of the LH surge. Blood samples were collected at different times before, during and after the stimulations via tail tip bleeding, and were later assayed for LH concentrations by ELISA. Stimulation of RP3V GABA neurons at 10 Hz, but not 2 Hz, for 15 min evoked a surge-like increment in plasma LH levels that lasted for 1 hour (n=6). Control animals (n=4), in which AAV injection and/or the optic fiber implantation missed the RP3V, did not exhibit any variation in LH plasma levels in response to optogenetic stimulation. These results demonstrate that selective activation of RP3V GABAergic neurons can drive the activity of GnRH neurons at the time of the preovulatory LH surge.

Disclosures: **B. Kalil:** None. **T. McLennan:** None. **R. Piet:** None. **A.E. Herbison:** None.

Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

Support: Health Research Council of New Zealand grant 11/404

Title: Defining subpopulations of arcuate nucleus GABA neurons in male, female and prenatally androgenized female mice: A role for GABAergic NPY neurons in regulating fertility?

Authors: *C. J. MARSHALL, E. DESROZIERS, R. E. CAMPBELL;
Univ. of Otago, Dunedin, New Zealand

Abstract: Fertility is controlled by gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus. Steroid hormones control GnRH neurons via an upstream neural network, essential for mediating GnRH neuron activity. A recently defined neural circuit between steroid hormone-sensitive GABAergic neurons in the arcuate nucleus (ARN) and GnRH neurons is enhanced in prenatally androgenized (PNA) mice that model polycystic ovarian syndrome (PCOS), implicating them in the steroid hormone mediated regulation of fertility. To determine the functional relevance of ARN GABA neurons, it is essential to define the neurotransmitters co-released from ARN GABA neurons. The initial aim of this study was to define the phenotypic subpopulations of ARN GABAergic neurons. The neurotransmitters produced by ARN GABA neurons were defined in fixed brain sections from male (n= 4-6), female (n = 4-6) and PNA-treated (n=4-6) VGAT-Cre;floxed-stop-tdTomato mice. Immunofluorescence for kisspeptin (KP), β-endorphin (β-end), neuropeptide Y (NPY), tyrosine hydroxylase (TH) and neuronal nitric oxide synthase (nNOS), along with tdTomato reporter was detected by confocal microscopy and co-localization was quantified throughout the ARN. GABA neurons were rarely identified co-localized with KP (<2%) or β-end (<1%). The largest proportion of GABA neurons co-localized with NPY in all groups throughout the ARN (20-40%), while TH and nNOS labeling was also detected in subsets of ARN GABA neurons, 5-12% and 5-15% respectively. This large NPY/GABA subpopulation was further defined in male, female and PNA-treated AgRP-Cre;τGFP-reporter mice. GFP expression in ARN NPY/GABA somata and fibre processes was coupled with immunofluorescent detection of progesterone receptor (PR) or GnRH to assess ARN NPY/GABA progesterone sensitivity, and innervation of GnRH neurons. To date, confocal analysis has revealed that ARN NPY neurons heavily innervate GnRH neuron somata, proximal and more distal dendrites in female mice (n=3). Fewer PR-positive cells were detected in the ARN of PNA female mice (108.4 ± 6.2 cells/section, n=10) compared with control females (137.2 ± 5.8 cells/section; n=8, $p < 0.01$). However, in ARN NPY neurons specifically, there was a near complete lack of PR immunoreactivity in any group (< 0.5%). These data suggest that NPY/GABA composes a significant subpopulation of ARN GABA neurons that project heavily to GnRH neurons. While it remains to be determined whether ARN NPY/GABA projections to GnRH neurons are perturbed in PCOS model mice, they do not express PR, and are likely not the same population of ARN GABA neurons with a loss in progesterone sensitivity in PNA mice.

Disclosures: C.J. Marshall: None. **E. Desroziers:** None. **R.E. Campbell:** None.

Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

Support: NIH grant UO1 HD 066435

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RO1 HD 068777

Title: Arcuate kisspeptin neurons connect to hypothalamic histamine, GABA and oxytocin neurons

Authors: *G. E. HOFFMAN[1], K. J. MURPHY[1], A. WOLFE[2], H. NOVAIRA[3], M. KOBAN[1], S. RADOVICK[3];
[1]Biol., Morgan State Univ., Baltimore, MD; [2]Pediatric Endocrinol., Johns Hopkins Univ., Baltimore, MD; [3]Pediatrics, Rutgers Univ., New Brunswick, NJ

Abstract: The anatomical and physiological basis of how LHRH/GnRH neurons are controlled was enlightened by the discovery of kisspeptin, a neurotransmitter critical for LHRH/GnRH expression and release. There are 2 populations of kisspeptin neurons: one in the anteroventral periventricular preoptic area (AVPV) that directly stimulates LHRH/GnRH neurons in females to control ovulation in females and a second population, located in the arcuate nucleus (Arc) whose role appears broader than the preoptic area cells but is less well understood. Arc Kiss neurons express kisspeptin and 2 additional transmitters: neurokinin B (NKB) and dynorphin (Dy). The neurons are termed “KNDy” (pronounced “candy”). All 3 transmitters are regulated by E in similar ways - mRNA expression is up-regulated when E levels decline and expression goes down as E levels rise. KNDy projections are implicated in not only GnRH control but also autonomic functions that vary with E status. Projections of the KNDy system are described for nuclei of the hypothalamus but little is known of the targets of the neurons.. This study examined KNDy projections in male and female mice bearing GFP under the GAD promoter, and rats and their relationship to histamine neurons, oxytocin, dopamine, and GABA neurons. Sections from estrous or ovariectomized female rats and intact and castrated male rats were double-labeled using ABC immunoperoxidase methods. Proestrous, estrous and gonadectomized female mice and intact male GAD GFP mice were examined. NKB was used to identify axons of the KNDy neurons. Targets were stained with GFP or calbindin (to identify the diencephalic GABA neurons), histamine or histidine decarboxylase, tyrosine hydroxylase, and oxytocin. The results showed that approximately 1/3 of the histamine neurons were contacted by KNDy NKB axons. In the PVN, oxytocin neurons were targeted by KNDy neurons. In the arcuate nucleus,

subparaventricular zone and medial preoptic area (lateral to AVPV), KNDy neurons sent heavy projections to GABA neurons. Interestingly in the median preoptic nucleus, few KNDY axons reached GABA neurons. Rare inputs were seen to the AvPv kisspeptin/dopamine cells. The results suggest that when gonadal steroid levels are low, and sleep is disrupted, the histamine system may mediate how sleep and reproductive functions are linked. The relationship of the KNDy neurons to oxytocin cells is consistent with the role KNDy neurons play in lactation. The general abundance of KNDy projections to GABA neurons supports a role for KNDy neurons in the regulation of inhibitory tone within multiple hypothalamic systems.

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Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

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NIGMS/NIH K12GM068524 (MLR)

NIH F32 HD076606 (KPT)

Title: Effects of selective deletion of tyrosine hydroxylase from kisspeptin neurons on puberty and reproduction

Authors: *S. B. Z. STEPHENS, M. L. ROUSE, K. P. TOLSON, R. A. PARRA, N. CHAHAL, A. S. KAUFFMAN;
Reproductive Med., UCSD, La Jolla, CA

Abstract: Kisspeptin, encoded by the *Kiss1* gene, stimulates GnRH release and is necessary for reproduction. In the hypothalamus, *Kiss1* neurons reside in two primary regions: the anteroventral periventricular (AVPV) and arcuate (ARC) nuclei. *Kiss1* neurons in the AVPV are thought to be involved in estrogen (E_2)-positive feedback (the LH surge in females) because

these neurons express ERα, E_2 increases AVPV *Kiss1* expression, and AVPV *Kiss1* expression is greater in females than males. In addition to the sexually dimorphic *Kiss1* neurons, the AVPV also contains a sexually dimorphic population of neurons expressing tyrosine hydroxylase (TH, the rate-limiting enzyme in catecholamine synthesis; in this case, dopamine), with females having more TH neurons than males. In the AVPV of female mice, the large majority (>80%) of *Kiss1* cells co-express TH, but the function of dopamine in these *Kiss1* neurons is unknown. Because AVPV *Kiss1* neurons are well-known to target and stimulate GnRH neurons, it suggests that AVPV dopamine, like kisspeptin, may influence reproduction and E_2-mediated positive feedback in females. To assess this possibility, we examined the pubertal and reproductive consequences of selectively knocking out TH specifically from *Kiss1* neurons using Cre-Lox technology. Mice lacking TH only in *Kiss1* neurons were produced by mating KissCre+ mice with TH fl/fl mice to eventually generate KissCre+ TH fl/fl (Kiss THKO) and littermate controls, KissCre- TH fl/fl (WT) mice. *Kiss1* neurons in the ARC reportedly lack TH, and thus, it is hypothesized that any reproductive effect(s) of removing TH from *Kiss1* neurons is attributable to AVPV *Kiss1* neurons. Ages at vaginal opening and first estrus (females) and preputial separation (males) were similar between Kiss THKO and WT mice, indicating that TH in *Kiss1* neurons is not required for normal puberty onset. Surprisingly, data obtained thus far suggest that there are no significant differences in reproductive measures between Kiss THKO and WT mice of either sex, suggesting that despite its high prevalence in *Kiss1* neurons, TH (and hence, dopamine) in *Kiss1* neurons is not required for normal fertility, including E_2-positive feedback in females. Thus, our preliminary data so far indicate that despite the high degree of colocalization of *Kiss1* and TH in the AVPV, TH in *Kiss1* neurons is not required for normal puberty or fertility in mice of either sex, and the function of dopamine in AVPV kisspeptin neurons remains to be determined.

Disclosures: **S.B.Z. Stephens:** None. **M.L. Rouse:** None. **K.P. Tolson:** None. **R.A. Parra:** None. **N. Chahal:** None. **A.S. Kauffman:** None.

Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

Support: NIH RO1 HD039916

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Title: Stimulatory effect of neuromedin U on pulsatile LH secretion in ewes is dependent on melanocortin MC_4 receptor signaling

Authors: *P. GRACHEV, R. B. MCCOSH, M. N. BEDENBAUGH, M. VALENT, S. L. HARDY, J. M. CONNORS, S. M. HILEMAN, R. L. GOODMAN;
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Abstract: The neuropeptide, neuromedin U (NMU), signals within the hypothalamus to suppress appetite and feeding behavior. In rats, intracerebroventricular administration of NMU induces anorexia and increases expression of mRNAs encoding proopiomelanocortin (POMC) in the arcuate nucleus and corticotropin-releasing hormone (CRH) in the paraventricular nucleus. Moreover, NMU-knockout mice are obese and deficient in hypothalamic POMC and CRH mRNA. Energy status potently influences reproductive function, and products of POMC cleavage, as well as CRH, are strongly implicated in the regulation of LH secretion. We have recently demonstrated a stimulatory effect of central administration of synthetic NMU on pulsatile LH secretion in anestrous ewes and detected prominent expression of NMU receptor within neurons of the arcuate and paraventricular nuclei. These discoveries prompted the hypothesis that NMU targets POMC and CRH neurons and exerts its stimulatory effect on pulsatile LH secretion through increased melanocortin and CRH receptor signaling. To address this hypothesis, anestrous ewes fitted with chronically indwelling third cerebral ventricle cannulae for drug delivery were subjected to frequent serial sampling of jugular blood following pretreatment with JKC363 (a selective melanocortin MC_4 receptor antagonist), alpha-helical CRH (a non-selective CRH receptor antagonist) or vehicle, and subsequent administration of synthetic NMU. The amplitude of LH pulses was increased by NMU administration, and this effect was attenuated ($P > 0.05$) by alpha-helical CRH and blocked ($P < 0.05$) by JKC363 pretreatment. These data suggest that the NMU 'satiety signal' requires melanocortin signaling through the MC_4 receptor to acutely augment pulsatile LH secretion in anestrous ewes.

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Poster

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Topic: F.03. Neuroendocrine Processes

Support: France-Berkeley Fund

ANR grant Pherosex

Title: Seasonal gating of chemosensory processing in the male Syrian hamster

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Abstract: Males of many species rely heavily on chemosensory information for social communication. In Syrian hamsters (*Mesocricetus auratus*), as in many rodent species, female chemosignals potently increase testosterone (T) and stimulate male sexual behavior. During winter-like, short-day photoperiods Syrian hamsters are reproductively quiescent and these same female chemosignals fail to elicit behavioral or hormonal responses. It is currently unknown where in the brain chemosensory processing is gated to ensure that reproductive responses are only displayed during the appropriate breeding season. To explore this question, adult male Syrian hamsters housed under either long-day (reproductively active) or short-day (reproductively inactive) photoperiods were exposed either to female hamster vaginal secretions (FHVS) diluted in mineral oil or to vehicle. Additionally, because expression of male sexual behavior requires a threshold concentration of circulating T, a third cohort of animals housed under short-day photoperiods was implanted with Silastic capsules containing T whereas all other animals received empty capsules. Brains were collected one hour following FHVS or vehicle exposure and cFOS expression was examined. The main and accessory olfactory systems exhibit similar responses to FHVS across photoperiods. Additionally, seasonal differences were not observed at the level of the medial amygdala, where chemosensory and sex steroid information are integrated. Unexpectedly, downstream hypothalamic targets associated with the expression of male sexual behavior, such as the preoptic area, also respond to FHVS similarly across photoperiods. These findings suggest that regional analysis of cFOS activity might mask seasonal differences within specific cell phenotypes, prompting examination of several reproductively-relevant neuropeptidergic systems. As a result, kisspeptin, RFamide-related peptide, and gonadotropin-releasing hormone neuronal activation are presently being assessed. Given the pronounced differences in reproductive axis activation in response to FHVS, it is likely that gating of this chemosensory signal occurs at the level of one or more of these systems.

Disclosures: K.J. Jennings: None. **M. Chasles:** None. **J. Cho:** None. **M. Keller:** None. **L.J. Kriegsfeld:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

Support: Supported by UNAM, DGAPA, PAPIIT: IN306216

Title: Restraint-stress-induced effects or administration of corticosterone on novel object recognition in rats

Authors: *N. L. GARCIA SALDIVAR[1], M. R. GONZÁLEZ LÓPEZ[2], G. CASTILLO ROBERTO[2], G. A. BARRIOS DE LA CRUZ[2], S. E. CRUZ MORALES[2];
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Abstract: The effects of the stress on memory are dependent on the kind of memory evaluated and the stressor, given that each type of stressor activates different neural circuits. Adrenal hormones modulate memory; however, there are controversies on whether the concentration of glucocorticoids in plasma is indicative of high levels of stress and if they are responsible for the effects on memory. Corticosterone (CORT) is released in rodents, and the concentration in plasma is considered as an index of the response to different stressors. Glucocorticoid administration improves memory or decreases it. The type of memory evaluated and the doses of CORT administered could explain such differences. In rodents, the novel object recognition (NOR) is a task used to measure declarative memory, attention, anxiety, and preference for novelty. In addition, the restriction of movement (R) is a severe traumatic stressor. The aims of present study were to evaluate the effects of exposure to 15 min restraint or the administration of 5 mg of corticosterone before training on NOR and to measure the plasma corticosterone levels. Male Wistar rats were assigned to 5 groups: intact (I), NOR (C), restraint (R), and two groups that were exposed before the training of NOR task to R (R+T) or 5 mg/kg of CORT (CORT+T). In training session rats were exposed for 5 min to 2 identical objects (O). During the test session, 24 h later, the animals were exposed to a familiar object (FO) and to a novel object (NO); the exploration time to every object (TN and TF) was registered. After the test, the subjects were sacrificed and the corticosterone levels were quantified in plasma by ELISA. Subjects exposed to R or injected with CORT before training in NOR presented less frequency and time exploring the NO than in the control group, this was related with a lower motor activity and an increased activation of the HPA axis. It is likely that the observed changes in NOR, relates to the largest concentration of CORT. In previous studies we showed that R exposure before training impaired memory, but had no effect post training (Argote et al., 2015). Present results are consistent with other studies where impairment on other memory tasks occurs by the previous exposure to acute stress (Cordero et al., 2003; Park et al., 2016). The results show that the [CORT] in plasma was

higher in the R and CORT groups compared with the control group. Both groups treated presented impairment in the NOR and reduced motor activity. Other studies have reported similar results with acute stress in other memory tasks. The results suggest that the restriction for 15 min and corticosterone administration impair declarative memory and trigger the release of corticosterone.

Disclosures: **N.L. Garcia Saldivar:** None. **M.R. González López:** None. **G. Castillo Roberto:** None. **G.A. Barrios de la Cruz:** None. **S.E. Cruz Morales:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

Support: UNAM DGAPA-PAPIIT IN217016

Title: Acute effects of blocking beta adrenergic receptors in ovaries on ovulation and steroidogenesis in rats with polycystic ovary syndrome

Authors: ***B. VENEGAS MENESES**, L. Y. DE LEÓN GORDILLO, J. A. ESPINOZA MORENO, L. MORALES-LEDESMA;
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Abstract: Injecting estradiol valerate (EV) to pre-pubertal results in effects similar to those observed in women with polycystic ovarian syndrome (PCOS). One of the mechanisms involved in PCOS development is the hyperactivity of the sympathetic nervous system due to an increase in the release and content of noradrenaline (NA) as well as a decrease in number of receptors β-adrenergic receptors in the ovarian compartment receiving catecholaminergic innervation. The aim of present study was analyzing the β-adrenergic receptors blocking in ovaries of rats with PCOS. Ten-day old rats were injected with EV dissolved in corn oil. At 60-days of age, on estrus day, control and EV rats were injected with propranolol [10^{-4}M] or vehicle into the ovarian bursas. The animals were sacrificed at 68 days of age, when they presented vaginal estrous preceded by a pro-estrus and were measured the ovulation rate, the number of ova shed and the concentration of progesterone, testosterone and estradiol in serum. In the control group, the blocking β-adrenergic receptors in the ovaries does not change progesterone levels, mean while the same treatment increased testosterone levels when the right ovary were injected. In animals with PCOS and blocking the β-adrenergic receptors in the ovaries were increased the number of the ova shed. On the other hand, the treatment resulted in the increased of progesterone levels

without changes in testosterone levels. Estradiol concentration increases when the β-adrenergic receptors are blocked in the right ovary with PCOS. In conclusion our results suggest that in animals with PCOS, the NA regulates the inhibitory feedback effects of estrogen and progesterone secretion and which causes a deregulation in ovulatory response.
This study was supported by UNAM DGAPA-PAPIIT program with ID IN217016. The author Berenice Venegas was supported by the Postdoctoral fellowship program UNAM 2016-2017.

Disclosures: B. Venegas Meneses: None. **L.Y. De León Gordillo:** None. **J.A. Espinoza Moreno:** None. **L. Morales-Ledesma:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

Support: R00HD071970

Title: The effect of PACAP on fertility is relayed through a subset of hypothalamic leptin receptor expressing neurons in the female mouse.

Authors: R. A. ROSS[1,2,3], S. LEÓN[4,3], C. A. MAGUIRE[4,3], J. C. MADARA[1,3], A. M. J. VERSTEGEN[1,3], U. KAISER[4,3], B. B. LOWELL[1,3], *V. M. NAVARRO[5,3];
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Abstract: Nutrient status and metabolism play a critical permissive role for reproduction, but the mechanisms by which this occurs are not yet understood. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuromodulator implicated in human anxiety, metabolism and reproductive behavior. PACAP whole body knockout mice display decreased fertility and PACAP itself stimulates LH release. However, the origin of the neuropeptide, and its role at the hypothalamic level, remain unknown. Centrally, high expression of PACAP (*Adcyap1*) is found in hypothalamic regions known to be involved in leptin-related control of puberty and fertility, though PACAP functionality has not been investigated there. To investigate the role that central PACAP plays in leptin-driven metabolism and reproduction we created $PACAP^{fl/fl}$ mice that possess loxP sites flanking the 2nd exon of the PACAP gene, allowing for deletion of functional PACAP in the presence of *cre*-recombinase. The $PACAP^{fl/fl}$ mice were bred with LepRb-*cre* mice, which express *cre*-recombinase in all sites where the long form of the leptin receptor is

expressed. Female mice were found to have significantly delayed puberty onset, prolonged estrous cycle, and decreased litter size when compared to littermate controls ($PACAP^{fl/fl}$). Furthermore, after overnight fast, these animals show 30% less induction of LH expression after treatment with leptin, showing that PACAP is likely required for a part of leptin-dependent activation of the gonadotrophic axis. There were no equivalent changes in males. There was no difference in body weight on standard chow, but female conditional knock out animals gained less weight on high fat diet. Dual-fluorescent immunohistochemistry and *in situ* hybridization revealed strong co-localization of LepR activity with PACAP expression in the ventral premammillary nucleus (PMV). Therefore, we repeated this study deleting PACAP from the PMV neurons only in adult $PACAP^{fl/fl}$ female mice, which showed the same results. Using a cre-dependent adeno-associated virus carrying synaptophysin, we traced projections from the PMV_{PACAP} neurons in PACAP-i-cre mice to the two regions that contain kisspeptin neurons (arcuate and AVPV nuclei), and used channelrhodopsin assisted circuit mapping to show the direct connection. Based on these findings, we propose a new (sex specific) role for the PACAP-expressing, glutamatergic neurons of the PMV: they transform nutritional state to regulate GnRH release by modulating the pulse generating kisspeptin neuronal population, thereby coordinating reproductive function.

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Poster

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant HD039916

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Title: Blockade of somatostatin receptor 2 stimulates episodic LH secretion, but not surge LH secretion, in ewes

Authors: *R. MCCOSH, M. N. BEDENBAUGH, J. A. LOPEZ, S. M. HILEMAN,, M. VALENT, P. GRACHEV, R. L. GOODMAN;
Physiol. & Pharmacol., West Virginia Univ., Morgantown, WV

Abstract: The neuropeptide somatostatin (SST) produced within the hypothalamus is best known for its role in suppressing growth hormone secretion, but accumulating evidence supports a role for SST in regulation of GnRH and LH secretion. ICV infusion of SST suppressed episodic LH secretion in ewes, and a somatostatin receptor 2 (SST2R) agonist suppressed episodic and surge LH secretion in humans and rodents, respectively. Using an antagonist to SST2R, we tested the hypothesis that endogenous SST suppresses episodic and surge LH secretion in sheep. Ovariectomized ewes received a 1cm-long estradiol (E) implant and a permanent cannula into the 3rd cerebral ventricle at least 1 week before any experimental treatments. An LH surge was induced in a follicular phase model: 2 progesterone containing CIDRs were inserted and removed 8 days later, the next day four 3 cm-long E implants were inserted and left in place for 2 days. A SST2R antagonist, CYN154806, (n=4) or saline (n=3) was injected ICV every 6 hrs for 24 hrs, beginning at the time of 3 cm-long E implant insertion. Jugular blood samples were collected at 2 hr intervals for 36 hrs and at 4 hr intervals for 12 additional hrs. CYN154806 treatment did not significantly alter LH surge amplitude (control: 35.6 ± 10.8 ng/mL; CYN154806: 43.8 ± 4.4 ng/mL) or time to surge peak (control: 31.1 ± 3.7 hrs; CYN154806: 26.0 ± 1.6 hrs). To test the effect of this SST2R antagonist on pulsatile secretion, similar ewes were injected with CYN154806 (n=5) or saline (n=6) on the 6th day of CIDR treatment. Jugular blood samples were collected at 12 min intervals for 24mins before and 4hrs following injections. CYN154806 induced an LH pulse within 24 mins in 100% of ewes that received CYN154806, whereas only 33% of control ewes had a pulse during the same time. CYN154806 also increased mean LH concentrations during the first 2 hrs after injection from 2.0 ± 0.1 ng/mL to 3.4 ± 0.1 ng/mL, while saline did not alter mean LH concentrations (pre: 1.9 ± 0.1 ng/mL; post: 1.8 ± 0.1 ng/mL). These data demonstrate that CYN154806, a selective SST2R antagonist, stimulates pulsatile LH secretion, but does not alter surge secretion of LH. These results indicate a role for endogenous SST acting through SST2R to suppress pulsatile LH secretion. In contrast, no effect of CYN154806 on surge secretion of LH was observed; this may be due to lack of a role for endogenous SST secretion during the LH surge, or that redundant systems compensate for any potential effects of SST during the LH surge.

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Poster

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Topic: F.03. Neuroendocrine Processes

Support: USDA 2013-00896

NIH P20GM103434

Title: Neurokinin B, but not dynorphin, acts in the arcuate nucleus of prepubertal female sheep to control LH secretion

Authors: *M. BEDENBAUGH[1], C. A. RAINEY[2], R. B. MCCOSH[3], J. A. LOPEZ[3], R. L. GOODMAN[3], S. M. HILEMAN[3];
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Abstract: Increased gonadotropin-releasing hormone (GnRH) secretion is critical for puberty onset. However, the neural mechanisms that underlie this increase are not completely understood. Neurokinin B (NKB) and dynorphin may play a role in regulating GnRH secretion during pubertal development, as senktide, an NKB receptor (NK3R) agonist, and nor-BNI, a kappa-opioid receptor antagonist, stimulate luteinizing hormone (LH) secretion in prepubertal ewes. However, where these effects occur within the hypothalamus is unknown. Our first study examined whether senktide or nor-BNI would increase LH secretion when placed in the arcuate nucleus (ARC). Following guide cannula placement in ovary-intact prepubertal females, ewes received microimplants that were empty (n=5), contained senktide (n=6), or contained nor-BNI (n=5). Blood samples were taken every 12 min for 4 h before and 4 h after microimplant insertion. Senktide significantly increased mean LH concentrations (8.0±0.5 ng/ml) vs. empty microimplants (1.9±0.2 ng/ml) but did not alter LH pulse frequency or amplitude; nor-BNI had no effect on any measure of LH secretion. Therefore, NKB, but not dynorphin, acts within the ARC to influence LH secretion prepubertally. We next tested whether changes in response to NKB may also play a role by determining whether estradiol (E) would decrease NK3R expression in several hypothalamic areas. Prepubertal female sheep were ovariectomized (OVX; n=4) or ovariectomized and given a 1-cm E implant (OVX+E; n=4). Two weeks later, sheep were bled for 4 h to assess LH secretion, then euthanized immediately. NK3R expression was assessed by immunocytochemistry. Although LH secretion was decreased by E, numbers of NK3R-positive cells were similar in both groups for the preoptic area, paraventricular nucleus, retrochiasmatic area, and ARC. We then further determined if E influenced NK3R expression that was associated with NKB- vs. non-NKB cells (Nissl stained) in the ARC. Triple label ICC and confocal analysis was used to visualize NKB, NK3R, and non-NKB cells (10 NKB and 10 non-NKB cells/animal). A greater percentage of NKB cells (96.4±1.3) was associated with NK3R than non-NKB cells (78.4±4.3). NKB cells also exhibited a higher number of NK3R-positive associations (4.4±0.5) than non-NKB cells (2.3±0.5). No influence of E was noted. Hence, although percentages are high for both cell types, more NKB than non-NKB cells are associated with NK3R in prepubertal female sheep.

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Poster

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Topic: F.03. Neuroendocrine Processes

Support: HD042645

HD007228

Title: Rapid activation of classical progesterone receptor in kisspeptin neurons

Authors: *M. A. MITTELMAN-SMITH, A. K. SCOTT, A. M. WONG, P. E. MICEVYCH; Neurobio., UCLA, Los Angeles, CA

Abstract: The luteinizing hormone (LH) surge is triggered by rising levels of estradiol (E2) in the female rodent. Exogenous replacement of E2 in an OVX animal will reinstate the surge. However, this LH surge is magnified by additional, subsequent replacement of progesterone (P4). Hypothalamic astrocyte-derived P4 (neuroP) appears to be the critical source of P4 for the LH surge: local infusions of aminoglutethimide (AGT, inhibits steroidogenesis) arrest the estrous cycle and preclude the LH surge. Kisspeptin-expressing neurons in the hypothalamic RP3V are positively affected by E2 and trigger GnRH neurons to cause the LH surge. While the stimulatory effect of E2 has been well characterized in both kisspeptin neurons and LH release, the neural targets of P4 have not been established. We hypothesize that RP3V kisspeptin neurons integrate E2 and P4 signals to trigger the LH surge. We use the mHypoA51 cell line as an in vitro model of RP3V kisspeptin neurons. In these cells, just as in their in vivo counterparts, E2 stimulates the expression of kisspeptin and classical progesterone receptor (PR). To investigate the integration of E2 and P4 signaling, we examined whether astrocyte-derived neuroP augmented E2 effects in mHypoA51 kisspeptin neurons. We demonstrate that mHypoA51 neurons express multiple P4 receptors: non-classical membrane receptors (mPRα and mPRβ), and classical PR localized to the membrane. In E2-primed mHypoA51 neurons, R5020 (selective PR agonist) induced MAPK phosphorylation, which was prevented by blocking Src, a non-receptor tyrosine kinase associated with classical steroid receptors at the membrane. Further, using an ELISA to measure kisspeptin release into the media, we found that both P4 and a Src agonist caused release of kisspeptin, showing that P4 and Src elicit functional changes in the kisspeptin pathway involved in E2 positive feedback. In a co-culture, we found that neuroP augmented the effects of E2 on mHypoA51 neurons. Kisspeptin protein expression was doubled by the presence of primary astrocytes, compared to E2 treatment alone. This increase was prevented by AGT, indicating that steroidogenesis is necessary for this kisspeptin induction. Similarly, E2-stimulated astrocyte-conditioned media caused an increase in kisspeptin mRNA. This was prevented by astrocyte siRNA knockdown of 3βHSD1, which converts pregnenolone to

progesterone. Together, these data suggest that locally-synthesized neuroP plays an important role in kisspeptin expression and release, in addition to that induced by rising levels of E2 observed on proestrus.
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Disclosures: M.A. Mittelman-Smith: None. **A.K. Scott:** None. **A.M. Wong:** None. **P.E. Micevych:** None.

Poster

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RYC-2012-11873

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Title: Sexually dimorphic Kisspeptin neurons in the RP3V regulate testosterone synthesis in male mice.

Authors: *E. SANZ[1], A. QUINTANA[1], A. URPI[1], G. MCKNIGHT[2];
[1]Univ. Autonoma de Barcelona, Bellaterra, Spain; [2]Univ. of Washington, Seattle, WA

Abstract: Male and female brains present significant anatomic and physiological differences, including different number of cells and gene expression profiles in certain neuronal populations. These differences are thought to be responsible for the sex differences observed in several aspects of reproduction, such as the timing of puberty onset or the ability to generate a pre-ovulatory LH surge. In males, a very low number of kisspeptin-expressing cells has been detected in the rostral periventricular area of the third ventricle (RP3V) using standard gene expression techniques, leading to the hypothesis that the sexual dimorphism for this particular population may be responsible for the ability of females, but not males, to generate preovulatory LH surges. Our data using mouse genetics and highly sensitive cell-specific gene expression

analysis shows that although the male RP3V contains less Kisspeptin cells than its female counterpart, the number of Kisspeptin cells is quite significant and not as residual as initially thought. To gain further insight on the role of RP3V kisspeptin neurons in the male mice, we have functionally manipulated this population using a combination of mouse genetics and adeno-associated viral delivery of Cre-dependent pharmacosynthetic tools. Activation of kisspeptin neurons in the RP3V of the male mouse significantly increased serum Testosterone levels. These results suggest that this subset of Kisspeptin neurons may have active role in the regulation of the reproductive function in the male mouse.

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Poster

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Support: FAPESP

CNPq

Title: Chemically-induced periestropause is associated with changes in kisspeptin/gonadotrophin-releasing hormone/luteinizing hormone cascade of female rats

Authors: *C. M. LEITE[1], N. P. OLIVEIRA[2], E. T. UCHOA[2,4], J. ANTUNES-RODRIGUES[2], L. L. K. ELIAS[2], J. A. ANSELMO-FRANCI[3];
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Abstract: Perimenopause is a period of transition from reproductive to non-reproductive life in women, usually characterized by neuroendocrine, metabolic and behavioral changes. Studies of the mechanisms involved in these changes have been facilitated by an animal model of perimenopause chemically-induced by 4-vinylcyclohexene diepoxide (VCD). The effect of VCD is characterized by the acceleration of the natural process of follicular atresia with a progressive depletion of primordial and primary follicles in female rodents, thus allowing studies on the transition period to estropause named here as periestropause. The aim of this study was to investigate during periestropause, the changes on estradiol (E), progesterone (P) and luteinizing hormone (LH) plasma levels as well as on the mRNA expression of gonadotrophin-releasing hormone (GnRH), kisspeptin (kiss) and kisspeptin receptor (KissR) in the preoptic area (POA) of

female rats. Female rats (28 days) were daily injected with VCD (160mg/Kg) or oil for 15 days. Approximately 80 days after the first injection the rats were decapitated in the morning (9h) of diestrus. Trunk blood and brain tissue were collected for E, P and LH measurement, and for determination of relative mRNA expression of POA GnRH, kiss and KissR by real time PCR. VCD-treated rats showed a significant reduction ($P<0.05$) in plasma progesterone and LH levels, as well as a significant decrease ($P<0.05$) on GnRH and kisspeptin mRNA expression in the POA, with no changes on plasma estradiol concentrations and KissR mRNA expression in the POA. These results indicate that ovarian impairment caused by VCD induces a reduction on plasma progesterone and LH levels, which is associated with a decrease on POA GnRH and kisspeptin mRNA expression in the diestrus. These data suggest that VCD-induced changes in kisspeptin/GnRH/LH cascade may be involved in the reproductive impairments observed during periestropause.

Disclosures: **C.M. Leite:** None. **N.P. Oliveira:** None. **E.T. Uchoa:** None. **J. Antunes-Rodrigues:** None. **L.L.K. Elias:** None. **J.A. Anselmo-Franci:** None.

Poster

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Title: Kiss1 expression is modulated by estrogen and endocrine disruptors in immortalized female AVPV- and arcuate-specific neuronal kisspeptin cell lines.

Authors: D. C. JACOBS[1], R. E. VEITCH[2], *P. E. CHAPPELL[3];
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Abstract: Ovulation requires preovulatory surges of gonadotropin-releasing hormone (GnRH) from preoptic hypothalamic neurons, initiated by elevated ovarian estradiol (E_2). Rising E_2 activates a subset of sexually dimorphic kisspeptin (Kiss-1) neurons in the female, located in the anteroventral periventricular nuclei (AVPV). Conversely, estradiol negative feedback on GnRH secretion is mediated by a neuroanatomically separate population of Kiss-1 neurons in the arcuate nuclei. Kisspeptin stimulates GnRH expression and secretion *in vivo*, and the development of this system is critical for the initiation of puberty. To elucidate how phenotypically similar Kiss-1 neuronal populations react differentially to E_2 exposure, we have generated two immortalized Kiss-1 cell lines from *kiss1*-GFP post-pubertal female mice. These

cell models recapitulate *in vivo* differential responsiveness to E_2, with KTaV-3 (AVPV-derived) demonstrating ~6-fold increases in *kiss1* expression under higher E_2 doses (5pM - 50pM E_2), while *kiss1* expression in KTaR-1 cells is suppressed up to 80% under lower E_2 concentrations (2pM - 10pM). Further, we have found that baseline expression of estrogen receptor α (ERα/*esr1*) is significantly different between these lines, with KTaR-1 cells exhibiting a 5-fold higher expression of *esr1* relative to KTaV-1, whereas estrogen receptor β (ERβ/*esr2*) is not differentially expressed. Additionally, we are exploring the impact of endocrine disrupting class of perfluorinated alkyl substances (PFASs) on these neurons, with preliminary results illustrating *kiss1*, *esr1*, and *esr2* transcriptional activation and/or repression at relevant doses of perfluorooctanoic acid, perfluorooctanesulfonic acid, and perfluorohexanoic acid in the two lines. Finally, we are probing temporal patterns of *kiss1* and core clock gene expression in these lines in response to estradiol, and find distinct antiphasic patterns of *bmal1* and *per2* in KTaV-3 cells irrespective of E_2 exposure. Treatment of KTaV-3 cells with 25pM E_2, however, elicited distinct patterns of *kiss1* expression over time in contrast to vehicle, suggesting differential coupling of intracellular oscillators to *kiss1* transcriptional activity in the presence of E_2. Ongoing delineation of responsiveness to E_2 in these lines could reveal novel molecular mechanisms underlying differential expression patterns demonstrated *in vivo* between these neuronal populations. Furthermore, investigating the impact of select PFASs on transcriptional activity of *kiss1*, *esr1*, and *esr2* between these two cell lines could elucidate the consequence of estrogen mimicry during sex-steroid sensitive developmental phases.

Disclosures: **D.C. Jacobs:** None. **R.E. Veitch:** None. **P.E. Chappell:** None.

Poster

339. HPG Axis II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 339.16/UU8

Topic: F.03. Neuroendocrine Processes

Support: VIEP-BUAP 0867

Title: Effects of unilateral orchidectomy to immature rats on dendritic arborization of pyramidal neurons in the hippocampus

Authors: N. B. SANTOS TENORIO[1], N. P. CORDERO FLORES[1], F. M. GONZÁLEZ CARRERA[1], G. LEÓN LÓPEZ[1], G. FLORES ALONSO[2], R. REYES LUNA[1], U. QUIRÓZ LÓPEZ[1], *C. MORAN[3];
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Abstract: There are neural connections between testicles and central nervous system. The role of neural information in the control of spermatogenesis is unclear. It has been previously shown that sensorial denervation decrease the total number, the motility and viability of the sperm and provokes an asymmetrical response in the dendritic morphology of CA3 hippocampal nucleus. The aim of the present study was to analyze the effects of the unilateral orchidectomy on the dendritic morphology in the neurons of hippocampus (CA3). Groups of male rats of 21 days old strain CIIZ-V were assigned to one of the following treatments: left orchidectomy (LO), right orchidectomy (RO), left sham (LS) or right sham (RS). The animals were sacrificed at 90 days of age. Dendritic morphology and characteristics were measured by using the Golgi-Cox procedure followed by Sholl analysis. Dendritic parameters were not significantly modified by the left or right orchidectomy compared with their corresponding sham group. However in the group RO was observed an increased dendritic arborization in the right CA3 pyramidal-neurons of the hippocampus compared to the either group. It is likely to have been presented plasticity mechanisms in CA3 pyramidal neurons. Our results support the existence of an asymmetric response in the nucleus CA3. This asymmetric response could contribute to the understanding of the neural mechanisms involved in the regulation of spermatogenesis in mammals. Most studies have been performed with unilaterally castrated rats to correlate with asymmetrical response of the testes in spermatogenesis.

Disclosures: **N.B. Santos Tenorio:** None. **N.P. Cordero Flores:** None. **F.M. González Carrera:** None. **G. León López:** None. **G. Flores Alonso:** None. **R. Reyes Luna:** None. **U. Quiróz López:** None. **C. Moran:** None.

Poster

339. HPG Axis II

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Program#/Poster#: 339.17/UU9

Topic: F.03. Neuroendocrine Processes

Support: Grant-in-Aid for Scientific Research from Japanese Society for the Promotion of Science 26893106

Title: Utility of anti-rabphilin-3A antibodies in the diagnosis of lymphocytic infundibulo-neurohypophysitis in pediatric patients

Authors: ***S. IWAMA**[1], Y. SUGIMURA[2], N. IWATA[2], Y. YASUDA[2], H. ARIMA[2];
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Abstract: Autoimmune hypophysitis (AH) is a chronic inflammatory disease in the pituitary gland. Hypophysitis is basically classified into 3 forms, lymphocytic adeno-hypophysitis (LAH), lymphocytic infundibulo-neurohypophysitis (LINH), and lymphocytic pan-hypophysitis (LPH), according to the location of the hematopoietic infiltrate. LINH, in which lymphocytes infiltrate the hypothalamic infundibulum and neurohypophysis, causes central diabetes insipidus (CDI) and a swelling of posterior pituitary and/or stalk. Clinically, it is quite difficult to differentially diagnose it from other diseases that cause CDI, such as sellar or suprasellar tumors (eg, germinoma), because of the similar clinical presentation and radiographic appearance. It has been reported that LINH is involved in the pathogenesis of idiopathic CDI. We recently reported rabphilin-3A as an autoantigen in LINH by mass spectrometry on immunoprecipitates obtained from patient sera incubated with posterior pituitary protein lysate and the presence of anti-rabphilin-3A antibodies in patients with LINH (J Clin Endocrinol Metab. 2015). In the present study, we analyzed the utility of the presence of anti-rabphilin-3A antibodies for diagnosis of LINH in children. We used the sera from 10 patients with LINH (biopsy-proven [n = 4], clinical diagnosed [n = 6]), 9 patients as control group (germinoma [n = 3], post-traumatic hypopituitarism [n = 1], pan-hypopituitarism [n = 2], LAH [n =3]), or 3 patients with idiopathic CDI and tested the presence of anti-rabphilin-3A antibodies by Western blotting. Cerebrospinal fluid (CSF) samples (2 from biopsy-proven LINH, 1 from germinoma) were also tested for the antibodies. Anti-rabphilin-3A antibodies were positive in 6 of 10 in LINH or 1 (germinoma) of 9 in control. Furthermore, all 3 patients showed positive in idiopathic CDI, suggesting the involvement of autoimmunity in the pathogenesis. All CSF samples showed negative. Since anti-rabphilin-3A antibodies were positive in one germinoma patient, histopathological analysis is required to clarify the presence of infiltration with hematopoietic cells around the tumor. Our results suggest that anti-rabphilin-3A antibodies could be useful in the diagnosis of LINH and that LINH is involved in the pathogenesis of idiopathic CDI at a higher rate in pediatric patients.

Disclosures: **S. Iwama:** None. **Y. Sugimura:** None. **N. Iwata:** None. **Y. Yasuda:** None. **H. Arima:** None.

Poster

339. HPG Axis II

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Topic: F.03. Neuroendocrine Processes

Support: Grant-in-Aid for Scientific Research from Japanese Society for the Promotion of Science 26893106

Title: Autoantibodies against corticotrophs as a biomarker for IgG4-related hypophysitis

Authors: ***N. IWATA**[1], S. IWAMA[2], Y. SUGIMURA[1], Y. YASUDA[1], H. ARIMA[1]; [1]Endocrinol. and Diabetes, [2]Res. Ctr. of Health, Physical Fitness and Sports, Nagoya Univ., Nagoya-Shi, Japan

Abstract: IgG4-related disease (IgG4-RD) is characterized by the elevated serum IgG4 levels and histopathological infiltration of IgG4 positive plasma cells in multiple organs. Autoimmunity is thought to be involved in the pathogenesis of IgG4-RD. Pituitary gland can be involved, which is called IgG4-related hypophysitis (IgG4-RH). IgG4-RH is featured with the swelling of pituitary gland and/or stalk, clinical manifestations with headache, visual disturbances, and various degrees of hypopituitarism. Since these clinical features are similar to other pituitary diseases, including autoimmune hypophysitis and sellar/suprasellar tumors, the differential diagnosis can be difficult. Although pituitary biopsy is necessary for definite diagnosis, it is often difficult for the invasive procedures. We recently reported an appropriate way to evaluate anti-pituitary antibodies (APA), which is an indication of autoimmunity (J Clin Endocrinol Metab. 2014). The presence of anti-hypothalamus antibodies (AHA) in patients with hypothalamo-pituitary diseases has been also reported. However, the presence of APA and AHA in patients with IgG4-RH and the involvement of IgG4 in the pathogenesis are unclear. In this study, we tested the presence of APA/AHA and compared the targeted cells by the antibodies in IgG4-RH with those in control diseases showing similar clinical symptoms. We assessed APA or AHA by indirect immunofluorescence using human pituitary or monkey hypothalamus as substrates. This study included serum samples from 17 patients with IgG4-RH, 3 patients with lymphocytic infundibulo-neurohypophysitis (LINH), 2 patients with craniopharyngioma, 3 patients with germinoma and 9 healthy controls. To clarify the targeted cells by the APA, double immunofluorescence with each patient serum and anti-pituitary hormone (GH, ACTH, PRL, TSHβ, or LHβ) antibodies was examined in APA positive samples. Furthermore, the IgG subclass of APA was evaluated by using a secondary antibodies specific for IgG1, IgG2, IgG3 or IgG4. APA were detected in 5 of 17 samples from patients with IgG4-RH, but not in any control samples. AHA were negative in all samples. The APA of 5 IgG4-RH patients targeted only corticotrophs. Furthermore anti-pro-opiomelanocortin (POMC) antibodies were detected in 2 IgG4-RH patients. The IgG subclass of APA was commonly IgG1 but not IgG4 in all 5 cases, suggesting elevated IgG4 is not directly involved in the pathogenesis. These findings suggest that APA against corticotrophs may become a diagnostic marker in IgG4-RH, autoimmunity is involved in the pathogenesis of IgG4-RH, and that autoantigens including POMC exist within corticotrophs.

Disclosures: **N. Iwata:** None. **S. Iwama:** None. **Y. Sugimura:** None. **Y. Yasuda:** None. **H. Arima:** None.

Poster

339. HPG Axis II

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Program#/Poster#: 339.19/UU11

Topic: F.03. Neuroendocrine Processes

Support: CB 169861 CONACYT, MEXICO

Title: Viability after permeabilization and introduction of trehalose in intracytoplasmic sperm porcine compartment

Authors: *M. BARRIENTOS, E. JACOME-SOSA, M. ORTEGA CASTRO, B. DOMINGUEZ MANCERA, P. CERVANTES ACOSTA, A. HERNANDEZ BELTRAN, D. ROMERO SALAS;
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Abstract: Streptolysin O (SLO) is a cytolysin has the particularity to form pores in the plasma membrane (Hotze, 2012). Permeabilization with SLO has been widely used to introduce extraneous molecules (Brito et al., 2008). Cryopreservation protocols produce damage in sperm (Cordova et al., 2005). Trehalose, has special features for cryopreserve cells (Barbas y Mascarenhas 2008), and it has been introduced oocytes and improving the viability post-thaw (Erougle et al 2003). The aim of this work was to expose pig sperm to a solution with SLO and trehalose, and evaluate their effects on intracellular compartment penetration and viability after thawing. 15 ejaculates were obtained from 5 boars. Inclusion criteria were ejaculate mass motility values ≥ 3 and ≥ 70% motility individually. The samples were subjected to two experiments. Experiment 1. The sample were incubated on solution with a SLO (Sigma-Aldrich) at 0.6 IU / ml and trehalose at 200 µM concentration for 5, 15 and 30 min (T_1, T_2 and T_3) at 37 ° C; after these times, pore were sealed with fetal bovine serum at 5%. A control group (treatment 7), was incubated without SLO and trehalosa. All treatments were cryopreserved as Westerdof in 1975. Experiment 2. A Sample each ejaculate were incubated on solution with SLO at 0.6 IU / ml and trehalosa at 100, 200 y 400 µM (T_4, T_5, and T_6), for 5 min at 37°C, and were cryopreserved too. Both experiments had a control group. The semen was evaluated after thawing. For this were used, eosine-nigrosine (EN), Host-Comasiee, and the chlortetracycline technique. For evaluate the trehalose penetration, Alcian blue staining was used. The dates obtained were analyzed with STATISTICA V7.01. Results. Experiment 1 The motility results showed that the control group had the largest decrease, at 4°c ($P< 0.05$). Resulted of EN, showed that the treatmentsT_3 and control were different (32.49% vs 24.32%)($P<0.05$). In the case for the acrosome, T_3 (24.09%) was different to the rest of treatment ($P<0.05$). The functional status of the membrane, was different between control group and the others ($P<0.05$). Experiment Results 2. The Trehalose concentration did have effect on membrane integrity between T_4 and control

group (23.32%vs 34.59%) (P<0.05). The coomassie blue staining, showed that the control group was similar to T_5 (33.45% vs 28.16%). The Alcian blue staining showed that T5 was better for introduce trehalose Intracytoplasmic with 65,27%, T_4 48,41%, T_6 47,03% and Control 14,61% (P<0.05). Conclusions: The treatments trehalose 200 µm and 5 minutes at 37 °, in a medium with 0.6 ui SLO, allow pores opening and introduce trehalose, but the sugar did not improve the results of viability with respect to the control group.

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Poster

339. HPG Axis II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 339.20/UU12

Topic: F.03. Neuroendocrine Processes

Support: CONACyT Grant 237334

Title: Emergence of folliculo-stellate cells in the postnatal pituitary gland development in wistar rats

Authors: *E. GÓMEZ DOMÍNGUEZ[1], C. SOLANO-AGAMA[1], E. VERA-AGUILAR[2], J. CAMACHO[2], M. E. MENDOZA-GARRIDO[1];
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Abstract: The pituitary is an endocrine gland located in the Sella Turcica formed by the neurohypophysis (posterior pituitary) and adenohypophysis (anterior pituitary). The parenchyma of the adenohypophysis is composed of secreting cells and non-secretory cells (as the Folliculo-Stellate cells, FS). The FS expressed the S100β and GFAP proteins. This allows identifying the tissue adenohypophysis. Recently Horiguchi and collaborators identified the FS rat through GFP under the control of promoter S100β and observing the presence of S100β-GFP cells from postnatal day 5 in the parenchyma of the anterior pituitary and expression of messenger at 3 postnatal days. On the other hand, Shannon and collaborators suggest that the FS engages in the gestational stage and matures after birth. Our hypothesis is that the FS are found from the first postnatal day in rats. In this work we analyze the time when the FS cells with cell markers are identified as S100β and GFAP. We observed by Western Blot to S100β protein from first day of postnatal development and were being increased during development of adult rats observed in a

wide expression of this protein. By analyzing the expression of GFAP we are found this greatly increased in newborn rats and low expression in adult rats. With respect to expression messenger S100β, observed by RT-PCR is the same behavior observed with protein. The messenger of this protein is present in newborn rats and increases with age. These results suggest that the FS are mature from the first day of postnatal development and express their two cell markers, suggesting that are committed to the FS phenotype before birth.

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Poster

339. HPG Axis II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: F.03. Neuroendocrine Processes

Support: Lundbeck Foundation

Aarhus University

Title: The anatomy and neurohistology of the minipig pituitary

Authors: L. TVILLING[1], A. GLUD[2], D. ORLOWSKI[2], J. JAKOBSEN[3], M. PETERSEN[4], K. ETTRUP[2], M. WEST[2], D. BENDER[3], C. BJARKAM[5], *J. SORENSEN[6];
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Abstract: Diseases in and around the pituitary gland are related to homeostatic changes due to the secretion of global and local hormones, and local affection of compressed nearby structures including cranial nerves. Accordingly, imbalance in the pituitary gland induces substantial disturbances with an array of different symptoms, for instance infertility and growth disorders. This study will illuminate the cytoarchitectonic biochemical composition as well as the fiber connections from the normal Göttingen minipig (GM) pituitary with the purpose of enabling translational enhancement and improvement of pharmacological, endocrinological, and neurosurgical models for pituitary disorders and treatment initiatives.
We will describe fiber connections from subcortical structures to the pituitary gland with MR-

tractography, and perform immunohistological evaluation and stereological non-biased quantification of the sub-components of the GM pituitary. In addition, an HPLC analysis will be conducted to examine the chemical composition of the neuro- and adenohypophysis.
A minipig model of pituitary deficiency will enable preclinical studies with gene therapy, stem cells, and drug candidate testing.

Disclosures: **L. Tvilling:** None. **A. Glud:** None. **D. Orlowski:** None. **J. Jakobsen:** None. **M. Petersen:** None. **K. Ettrup:** None. **M. West:** None. **D. Bender:** None. **C. Bjarkam:** None. **J. Sorensen:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: University of Maryland College of Behavioral and Social Sciences Dean's Research Initiative Award (LRD)

University of Maryland Research and Scholars Award (LRD)

University of Maryland MRI Dean's Research Initiative (TR, LRD)

University of Maryland ADVANCE Program for Inclusive Excellence Award (LRD, TR)

Title: Hippocampal resting-state connectivity: longitudinal and concurrent associations with parenting and cortisol reactivity during childhood

Authors: *S. L. BLANKENSHIP[1], T. RIGGINS[2,1], L. R. DOUGHERTY[2,1];
[1]Neurosci. and Cognitive Sci., [2]Psychology, Univ. of Maryland, College Park, MD

Abstract: Decades of rodent research has demonstrated a link between the early parenting environment and offspring neural development. Evidence suggests that early caregiving behaviors epigenetically program the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased reactivity to stress and functional changes within the hippocampus, a medial temporal lobe structure implicated in episodic memory and the pathophysiology of depressive disorders. Despite evidence of these associations in rodents, no research has examined these effects in young human offspring. The present study aims to fill this gap and translate the rodent literature by examining the associations between parenting and cortisol reactivity on hippocampal resting-

state functional connectivity (rs-fcMRI) in a longitudinal sample of children (*n*=54). Observational measures of parenting and children's salivary cortisol responses to a laboratory stressor were assessed at 3-5 years (Time 1; T1) and 5-10 years (Time 2; T2) of age. Rs-fcMRI was collected at T2. Analyses examined main effects of T1 and T2 parenting (positive, negative) and cortisol reactivity (AUCg, AUCi) on whole-brain anterior (i.e., head) and posterior hippocampal functional connectivity, as well as, explored the mediating role of cortisol reactivity. Greater T2 positive parenting was associated with (1) greater left head connectivity with the left medial prefrontal cortex and the left posterior cingulate cortex (2) greater left posterior connectivity with left medial prefrontal cortex; and (3) greater right posterior connectivity with right cerebellum. Main effects of cortisol reactivity revealed that greater T1 AUCg (i.e., total cortisol secretion), was associated with (1) greater connectivity between left head and right posterior cingulate cortex; (2) greater connectivity between right head and right dorsomedial prefrontal cortex; and (3) greater connectivity between right posterior hippocampus and right dorsomedial prefrontal cortex, right posterior cingulate cortex, right precuneus, and right visual association cortex. There were no main effects of T1 parenting or T2 cortisol reactivity. Significant mediation was not observed. These results demonstrate possible timing-dependent mechanisms through which early experiences may shape developmental outcomes, with early cortisol reactivity and concurrent parenting predicting hippocampal networks. In light of evidence that parenting and offspring cortisol reactivity are affected by maternal depression, these results provide insight into the early mechanisms of the intergenerational transmission depression risk.

Disclosures: S.L. Blankenship: None. **T. Riggins:** None. **L.R. Dougherty:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.02/VV1

Topic: F.04. Stress and the Brain

Support: R01-HD055689

Title: Reliability of SES effects on hippocampal and frontal brain structure in children and youth: A systematic review

Authors: L. T. WITTMAN[1], A. J. WINKELMAN[1], G. M. LAWSON[1], *M. J. FARAH[2]; [1]Univ. of Penn, Philadelphia, PA; [2]Univ. Pennsylvania, Philadelphia, PA

Abstract: A growing literature documents differences in brain structure between children of lower and higher socioeconomic status [SES]. The most commonly reported differences are in hippocampal and frontal cortex, which accords with observed SES differences in childhood memory and executive function. How reliable are these findings? We systematically searched for published findings on SES (income [INC] and/or parental education [PE]) and brain structure in children and youth between infancy and 22 yo (mostly 4-18 yo) and identified: 9 articles reporting analyses of 9 unique data sets on the relation between SES and hippocampal volume [VOL] and 12 articles reporting analyses of 8 unique data sets on SES and frontal structure. We found a robust positive effect of SES on hippocampal VOL, with 7/9 studies finding reliable hippocampal differences (L hippocampal in 7/7 studies, R in 5/7 studies for which L and R were separately reported). Both INC and PE were found to predict hippocampal VOL in different studies, with slightly stronger evidence for INC influence. Of articles on frontal structure, 3 reported whole brain analyses of VOL, surface area [SA] and/or cortical thickness [CT], with 2 showing extensive frontal differences and one, confined to CT, showing differences in a small area of R lateral frontal, all positive relations. In 2 other articles, overall frontal cortical volume was measured and found to be positively related to INC. R-L differences and effects of PE were not reported. More specific frontal ROIs were examined in 5 publications, but few examined the same regions and results were mixed. Our own ROI re-analysis of the largest dataset, previously the basis for a published whole brain analysis, failed to resolve these existing inconsistencies. The small number of studies, focused on different dimensions of brain structure and using incommensurate analytic approaches (whole brain, differing ROIs, differing covariates) make it difficult to draw firm or specific conclusions concerning regional frontal differences. In sum, childhood SES has fairly reliable correlates in hippocampal volume and overall frontal size, but consistent regional differences within frontal cortex have yet to be demonstrated.

Disclosures: **L.T. Wittman:** None. **A.J. Winkelman:** None. **G.M. Lawson:** None. **M.J. Farah:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.03/VV2

Topic: F.04. Stress and the Brain

Support: NSF grant IOS 1146853

Tulane Bridge Fund

Title: Acute predator odor exposure rapidly activates the CREB turn-off pathway in the hippocampus of adult male Wistar rats.

Authors: *D. R. HOMIACK[1], E. O'CINNEIDE[1], S. HAJMURAD[1], M. STANLEY[1,3], B. BARRILEAUX[2], L. SCHRADER[1,2];
[1]Neurosci., [2]Cell and Mol. Biol., Tulane Univ., New Orleans, LA; [3]Infectious Dis., Broad Insitute, Cambridge, MA

Abstract: Exposure to acute stress impairs performance on hippocampus-dependent spatial memory tasks in male rodents. However, the underlying molecular plasticity induced by acute stress which modulates spatial memory is not fully understood. Previous studies have suggested that both acute and chronic stress can reduce phosphorylation of cyclic-AMP response element binding protein (CREB) in the hippocampal formation of male rodents. However, the mechanism by which acute stress reduces CREB phosphorylation remains largely unknown. In this investigation, we assessed activation of the CREB turn-off pathway in the hippocampus at the cessation of a 30 minute home-cage exposure to the synthetic Grüneberg ganglion agonist, 2-4-5 trimethylthiazole (TMT). A single exposure to TMT elicits robust activation of the HPA axis as well as immobility and avoidance behaviors in male Wistar rats. At the cessation of the 30 minute TMT exposure, we observed reduced levels of phosphorylated CREB, with no alterations in total CREB expression. To further investigate the surprising finding that acute stress exposure reduces expression of phosphorylated CREB within 30 minutes, we assayed markers associated with the previously described CREB turn-off pathway. We found reduced levels of phosphorylated extracellular signal regulated kinase (ERK) in a fraction enriched in cytosolic proteins, but increased levels of phosphorylated ERK in a nuclear-enriched fraction. Moreover, we observed increased expression of the non-phosphorylated form of the synapto-dendritic protein Jacob in the nuclear fraction of the hippocampus of rodents exposed to TMT. *In vitro* studies have previously associated nuclear accumulation of non-phosphorylated Jacob with reductions in phosphorylated CREB. Nuclear translocation of non-phosphorylated Jacob is thought to occur downstream of extrasynaptic NMDA receptors. Activation of extrasynaptic NMDARs is tightly controlled by glial uptake and clearance of synaptic glutamate by the excitatory amino acid transporter glutamate type 1 transporter (GLT-1/EAAT2) which mediates the majority of glutamate uptake in the adult hippocampus. Acute TMT exposure rapidly reduces expression of glial fibrillary acidic protein (GFAP) as well as GLT-1/EAAT2, but not GLAST/EAAT1 in hippocampal synaptosomes. These results suggest that acute predator odor stress can rapidly affect glutamate uptake and glial cell function in the hippocampus and may contribute to stress effects on memory formation.

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Poster

340. Stress: Hippocampus

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Program#/Poster#: 340.04/VV3

Topic: F.04. Stress and the Brain

Support: DA08259

Title: Effect of chronic stress on gene expression in the hippocampus of female and male rats

Authors: M. RANDESI[1], Y. ZHOU[1], S. MAZID[3], S. C. ODELL[3], B. S. MCEWEN[2], *T. A. MILNER[3,2], M. KREEK[1];
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Abstract: Opioid peptides and their receptors re-organize in the hippocampus of female, but not male, rats following chronic stress in a manner that promotes drug-related learning. This study was conducted to determine if there are also baseline sex differences in gene expression in the hippocampus following chronic stress. Female and male rats were subjected to chronic immobilization stress (CIS) for 10 days, 30 minutes per day. Control rats were handled but not subjected to CIS. On the 11th day animals were euthanized, the brains were harvested and the medial (dentate gyrus/CA1) and lateral (CA2/CA3) dorsal hippocampus were isolated. Following total RNA isolation, cDNA was prepared for gene expression analysis using a RT^2 Profiler PCR expression array (Qiagen). This custom designed PCR expression array contained genes for opioid peptides and receptors as well as candidate genes involved in synaptic plasticity including those upregulated following oxycodone self-administration in mice, and genes involved in stress-responses. Male and female CIS rats compared to non-stressed controls had significant down regulation of the same three genes in the lateral hippocampus: neurotrophic tyrosine kinase receptor type 2 (*Ntrk2*), beta arrestin 1 (*Arrb1*) and thymona viral proto-oncogene 1 (*Akt1*). Male rats had two additional genes significantly down-regulated following CIS: activity regulated cytoskeletal-associated protein (*Arc*) and arginine vasopressin receptor 1a (*Avpr1a*). In the medial hippocampus, 11 genes were significantly down regulated in CIS rats compared to non-stressed controls; however, the genes that were down-regulated were distinct depending on the sex. In females, CIS significant down-regulated the following genes: delta opioid receptor (*Oprd1*), proviral integration site 1 (*Pim1*; a member of the Ca2+/calmodulin-dependent protein kinase family), cadherin 2 (*Cdh2;* a Ca2+ dependent adhesion transmembrane protein), *Avpr1a*, mitogen activated protein kinase 1 (*Mapk1*) and beta arrestin 2 (*Arrb2*). In males, CIS down-regulated four of the same genes that were also down-regulated in the lateral hippocampus: *Ntrk2, Arrb1*, *Akt1* and *Arc*. Additionally, CIS in males compared to controls down-regulated FK506-binding protein 51 (*Fkbp5*), a co-chaperone that regulates glucocorticoid sensitivity, in

the medial hippocampus. In conclusion, modest sex- and regional-differences are seen in expression of the delta opioid receptor gene as well as genes involved in plasticity and stress responses in the hippocampus following chronic stress.

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Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.05/VV4

Topic: F.04. Stress and the Brain

Support: NPY RO1

Title: Functionally distinct ca1 npy interneurons regulate npy release in schaffer collateral and temporammonic feedforward pathways

Authors: ***Q. LI**, A. BARTLEY, L. DOBRUNZ;
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Abstract: The anxiolytic peptide Neuropeptide Y (NPY) is abundantly expressed in hippocampus. NPY has been implicated in stress-related disorders, and artificially increasing NPY levels has beneficial effects in animal models of anxiety. However, the underlying mechanisms that regulate NPY release, and NPY's effects on CA1 synaptic function, are not fully understood. Both bath applied NPY and NPY released in response to optogenetically-induced high frequency firing of NPY cells reduce synaptic responses from the two excitatory inputs to CA1 pyramidal cells, the temporammonic (TA) and Schaffer Collateral (SC) pathways, with effects being largest at SC synapses. In addition, we developed a novel assay to detect effects of NPY release CA1 in response to physiologically-derived Natural Stimulus Trains (NSTs) in these two feedforward pathways. TA stimulation with NSTs causes NPY release from neurogliaform cells located in s. lacunosum-moleculare, reducing short-term facilitation of TA synapses onto CA1 pyramidal cells. In contrast, NPY release from Ivy cells in s. radiatum, which reduces short-term facilitation of SC synapses, requires integration of both SC and TA inputs in response to the NST. Surprisingly, the effects are largest at TA synapses onto pyramidal cells. Neurogliaform and Ivy cells show distinct properties in short-term plasticity of their inputs, synaptic-evoked spiking, and intrinsic excitability, potentially explaining the differential release of NPY in the two feedforward pathways. Additionally, SC stimulation onto Ivy cells elicits two forms of short-term plasticity. While the majority of Ivy cells express paired-pulse facilitation

(PPF), a subset have paired-pulse depression (PPD). Ivy-PPF and Ivy-PPD cells also have different properties of intrinsic excitability and synaptically-evoked spiking, suggesting that they are distinct subtypes of Ivy cells. Interestingly, TA inputs onto Ivy cells only express short-term facilitation, which would allow for increased NPY release. Finally, we show that release of endogenous NPY in response to TA stimulation with NSTs is abolished in the Predator Scent Stress model of anxiety, resulting in altered short-term plasticity and therefore circuit function. This reduction in NPY release could be an important mechanism by which stress alters CA1 circuit function and leads to enhanced anxiety. Together, our results show that the three subtypes of NPY+ cells studied have different properties that likely contribute to the differences in NPY release observed in the two feedforward pathways to CA1.

Disclosures: Q. Li: None. **A. Bartley:** None. **L. Dobrunz:** None.

Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: BYU Mentoring Environment Grant

Title: Daily running exercise mitigates the negative consequences of increased corticosterone due to stress on hippocampal ltp

Authors: *R. M. MILLER, D. MARRIOTT, T. HAMMOND, D. LYMAN, J. TROTTER, T. CALL, Z. BADURA, J. G. EDWARDS;
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Abstract: In the hippocampus, learning and memory are mediated at the molecular level by synaptic plasticity, known as long-term potentiation (LTP). It is well established that stress decreases LTP and memory performance while exercise enhances them. What is not known is whether exercise in association with stress can mitigate the negative impact stress has on memory. We examined the effect exercise had on stress in the hippocampus of C57BL/6 male mice. We employed four groups: sedentary no stress (control), exercise no stress, exercise with stress, and sedentary with stress. Field electrophysiology confirmed that stress alone significantly ($P < 0.05$) reduced CA1 hippocampal LTP compared to controls and that exercise alone significantly increased LTP compared to controls. Exercise with stress mice exhibited LTP that was significantly greater than mice undergoing stress alone, but were not different from controls. A corticosterone ELISA was performed on blood samples from all groups and we confirmed that

our stress methodology increased corticosterone in sedentary with stress mice compared to control mice, and that levels were not different between control and exercise with stress mice and decreased in exercise no stress mice. In addition, quantitative PCR revealed significant differences in hippocampal mRNA expression. In general, exercise without stress demonstrated enhanced expression of many targets involved in hippocampal plasticity such as NMDA receptor subunits NR2A/2B, and PSD-95, as well as in elements of the stress-exercise pathway including BDNF, p70s6k, TrkB, glucocorticoid, mineralocorticoid, and dopamine 5 receptors. Based on these results, corticosterone levels and BDNF expression seem to be some of, but not the only, factors for changes seen in hippocampal LTP. To determine whether differences in LTP were associated with memory behaviorally, we used the radial arm maze to examine behavior differences. The two sedentary groups and the two exercise groups performed similarly to each other. Trends were seen between the exercise groups and the sedentary groups on time to complete a trial, total distance traveled, and working memory errors, but only reference memory errors showed a significant difference. Exercised mice groups made fewer errors in week 1, suggesting exercise as a suitable treatment to counteract some of the negative effects of stress on hippocampal memory. Collectively, our data suggest exercise can mitigate some of the negative impact stress has on memory and some of the potential neural pathways involved.

Disclosures: **R.M. Miller:** None. **D. Marriott:** None. **T. Hammond:** None. **D. Lyman:** None. **J. Trotter:** None. **T. Call:** None. **Z. Badura:** None. **J.G. Edwards:** None.

Poster

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Program#/Poster#: 340.07/VV6

Topic: F.04. Stress and the Brain

Support: KAKENHI 25116526

KAKENHI 24111546

KAKENHI 26780421

Title: Repeated restraint stress causes alteration in neuronal maturation makers in the dentate gyrus in BALB/c mice

Authors: *H. SHOJI[1], H. HAGIHARA[1], T. MIYAKAWA[1,2];
[1]Fujita Hlth. Univ., Inst. for Comprehensive Med. Sci., Toyoake, Japan; [2]Ctr. for Genet. Analysis of Behavior, Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Stress is considered to be a predisposing environmental factor in psychiatric disorders, including depression and schizophrenia. We previously found “immature dentate gyrus (iDG)”, in which almost all the granule cells in the hippocampal dentate gyrus (DG) fail to mature (Yamasaki et al., Mol. Brain, 2008; Hagihara et al. Cell Reports, 2016), in several genetic mouse models that display behavioral abnormalities related to psychiatric disorders. Additionally, chronic administration of fluoxetine, selective serotonin reuptake inhibitor, which is used to treat depression, induces “dematuration” of DG neurons: mature DG neurons go back to pseudo-immature state (Kobayashi et al., PNAS, 2010). However, whether there is any relationship between stress and maturation of DG remains to be understood. Here, we examined the effects of repeated restraint stress on maturation of DG neurons in C57BL/6J mice and BALB/c mice, which are known to be more susceptible to stress and more anxious than C57BL/6J mice. Animals were subjected to repeated restraint stress for 3 or 6 weeks. The gene and protein expressions of maturation markers of DG neurons were analyzed by real-time PCR and immunohistochemical methods, and anxiety-like and depression-like behaviors were assessed. Repeated stress downregulated expressions of maturation marker genes (desmoplakin, tryptophan 2, 3-dioxygenase, and calbindin) and upregulated an expression of immature marker gene (BDNF) in BALB/c mice. No such changes of the markers were detected in C57BL/6J mice. Repeated restraint stress also led to decreased protein expressions of calbindin and glucocorticoid receptor in the ventral DG of BALB/c mice. Additionally, repeated stress induced hyperlocomotor activity and increased depression-like behaviors. These results suggest that repeated restraint stress alters maturation state of the DG neurons in BALB/c mice, which may underlie some of the stress-induced behavioral changes.

Disclosures: **H. Shoji:** None. **H. Hagihara:** None. **T. Miyakawa:** None.

Poster

340. Stress: Hippocampus

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Title: Klf9 regulates dendritic spines to protect against chronic stress induced maladaptive fear responses

Authors: *A. SAHAY[1], T. LANGBERG[1], S. LEVINSON[1], D. CHU[1], K. SCOBIE[2], R. HEN[2], E. LEONARDO[2], A. BESNARD[3];
[1]MGH Psychiatry, HMS, HSCI, Ctr. For Regenerative Med., Boston, MA; [2]Neurosci. and Psychiatry, Columbia Univ., New York, NY; [3]Ctr. for Regenerative Medicine, Dept. of Psychiatry, Massachusetts Gen. Hospital, Harvard Stem Cell Institute, Harvard Med. Sch., Boston, MA

Abstract: There is growing appreciation that psychiatric disorders such as depression and post-traumatic stress disorder arise from inefficient adaptation to risk factors such as stress. Understanding how transcriptional programs respond to stress to confer vulnerability or resilience through their actions on neural circuits will inform strategies to promote adaptive responses in neural circuits to stress. Hippocampal circuit homeostasis is key for calibrating adaptive responses to a variety of different stressors. We recently identified Kruppel-like factor 9 (Klf9) as a potent transcriptional negative regulator of dendritic spines in the hippocampus. Klf9 expression is upregulated by acute restraint stress and glucocorticoids, but is downregulated in response to chronic restraint stress. Based on these observations, we hypothesized that inducible silencing of Klf9 protects against stress-induced changes in hippocampal dendritic spines to promote behavioral resilience. To test our hypothesis, we developed novel genetic tools to robustly and reversibly silence Klf9 in forebrain excitatory neurons of adult mice at baseline and prior to exposure to chronic restraint stress or chronic administration of glucocorticoids. Inducible silencing of Klf9 in forebrain excitatory neurons in adulthood did not affect contextual fear processing at baseline, but prevented chronic restraint stress-induced enhancement of contextual fear memory. Additionally, Klf9 downregulation blunted the corticosterone response to an acute stressor challenge. Importantly, inducible silencing Klf9 in forebrain excitatory neurons in adulthood prevented corticosterone-induced overgeneralization of fear in safe, neutral contexts. Using triple transgenic mice to link changes in hippocampal circuitry with behavior, we genetically visualized dendritic spines in hippocampal subpopulations of neurons following adult forebrain silencing of Klf9 and these different stressors. The protective effects of inducible Klf9 silencing on stress-induced maladaptive fear responses were mirrored by reversal of chronic stress (restraint stress and CORT) induced structural changes in dendritic spines in dentate gyrus and CA1. Our studies begin to uncover a novel transcriptional mechanism that regulates behavioral and circuit resilience to stress-induced maladaptive fear responses. Because Klf9 expression is upregulated in the hippocampus of patients with major depressive disorder and by glucocorticoids, our results suggest that targeting Klf9 dependent circuit changes may confer resilience to stress induced maladaptive fear responses.

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Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: Kaken-hi (15H05569)

Keken-hi (15H01417)

Title: Reorganization of hippocampal spatial maps during adaptive behavior in an aversive situation

Authors: *S. OKADA, H. IGATA, T. SASAKI, Y. IKEGAYA;
The Univ. of Tokyo, Tokyo, Japan

Abstract: Organisms evaluate how much an external event influences their living activity and makes a decision about whether they should avoid it. There is, however, even a case where animals need to actively accept an aversive situation depending on the balance between the amount of expected benefit and the strength of negative emotion. The hippocampus plays a central role in processing episodic experiences and thus has been implicated in the planning of adaptive behavior related to emotional valence. In addition to spatial factors, firing of hippocampal cells is affected by non-spatial contexts, such as colors, objects, or task demands, which is assumed to be a mechanism by which the hippocampus encodes information of individual experiences onto a neuronal spatial framework. It remains unknown how the reorganization of hippocampal activity is associated with adaptive behavior and decision-making process driven by a combination of positive and negative environments. To address this issue, we designed a behavioral paradigm that imposes an acceptance/aversion conflict on rats in a continuous version of the T-maze alternation task. In the maze, an electrical shock pulse was incorporated onto a specific zone before reward area only along a right-turned trajectory (shock trajectory). The intensity of the pulse was adjusted so that the rats could actively accept the shock of their own will motivated by a predictable reward. The moderate stimulus induced weak signs of avoidance behavior, including extended time before entering into the shock zone and decreased correct task performance. In these conditions, we performed multiunit recordings from hippocampal CA1 neurons and found that addition of the shock stimulus altered firing rates of a certain population of neurons that originally showed trajectory-specific firing before adding the stimulus. This alteration occurred both shock and no-shock trajectories and remained for several laps after the stimulus was removed. The trajectory-dependent firing patterns on a moment-by-moment basis tended to correlate with task performance. The results will add to an accumulating body of evidence for the nature of the hippocampal signals involved in adaptive behavior depending on contexts.

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Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Title: The results of forced swim test in Wistar rats are negatively correlated with the degree of neurogenesis in the subventricular zone and dentate gyrus

Authors: ***K. KIN**, T. YASUHARA, M. KAMEDA, M. UMAKOSHI, I. KIN, K. KUWAHARA, J. MORIMOTO, M. OKAZAKI, H. TAKEUCHI, A. TOYOSHIMA, T. SASAKI, T. AGARI, I. DATE;
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Abstract: [Introduction and Purpose]
Depression is one of the most common and burdensome health problems worldwide. Treatment-resistant forms of psychiatric disorders still remain unsolved despite advances in medication. Animal depression models and behavioral tests are useful to explore effective treatments for them. It is generally accepted that depression is related to neurogenesis. In the present study, we evaluated the correlation between the results of behavior tests (sucrose preference test: SPT, open field test: OFT and forced swim test: FST) and the degree of neurogenesis in the subventricular zone (SVZ) and dentate gyrus (DG).
[Material and Method]
Adult male Wistar rats (5-7 weeks old: n=30) were divided into control and behavior tests groups and acclimated to our animal facility for 5 days before the start of the behavior tests. SPT (Day 6), OFT (Day 6) and FST (Day 7 and 8) were performed to rats in behavior tests group. All animals received injection of BrdU (50mg/kg, i.p.) every 12 hours over the last 3 days to label proliferative cells.
[Result and Discussion]
Immunohistochemical results revealed that behavior tests did not affect the number of BrdU/Doublecortin (Dcx) positive cells in the SVZ and DG. There was a negative correlation of immobility time in FST at Day 2 with the number of BrdU/Dcx positive cells in the SVZ (r = -0.447, p=0.048) and DG (r = -0.504, p=0.023). Results of other behavior tests did not correlate with the number of BrdU or BrdU/Dcx positive cells. Immobility time in FST at Day 2 is one of the most common behavior tests to assess depression model rats. This test is usually used to evaluate *learned helplessness* caused by unescapable stress of FST at Day 1. In this study, there

was a negative correlation of immobility time with the number of BrdU/Dcx positive cells in the SVZ and DG. That is, rats with less BrdU/Dcx positive neural precursors showed longer immobility time than those with more neural precursors. The degree of neurogenesis in the SVZ and DG might correlate with stress resistance of Wistar rats.

Disclosures: **K. Kin:** None. **T. Yasuhara:** None. **M. Kameda:** None. **M. Umakoshi:** None. **I. Kin:** None. **K. Kuwahara:** None. **J. Morimoto:** None. **M. Okazaki:** None. **H. Takeuchi:** None. **A. Toyoshima:** None. **T. Sasaki:** None. **T. Agari:** None. **I. Date:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.11/VV10

Topic: F.04. Stress and the Brain

Title: Social buffering prevents psychosocial stress-decreased neurogenesis in mouse dentate gyrus

Authors: *C.-Y. WANG[1], L. YU[2];
[1]Natl. Cheng Kung Univ., Tainan, Taiwan; [2]Inst. of Behavioral Medicine, Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Previous studies indicate that a psychosocial stress may induce decreases in neurogenesis in dentate gyrus (DG). We find that social buffering prevents physical stress-induced decreases in DG neurogenesis. This study is undertaken to study whether social buffering may prevent a psychosocial stress-produced decrease in DG neurogenesis. Social defeat is used to provoke the psychosocial stress in male Balb/c mice. Two groups of mice receive daily social defeat stress or no stress for 10 consecutive days. These groups are further divided into two groups with one group being housed alone, while the other group being housed with 3 companions, serving as social buffering. Social defeat-provoked stress does decrease DG neurogenesis. Moreover, the social defeat-provoked stress increases the wet weight of spleen but decreases the pH value of gastric lavage samples. In contrast, social buffering prevents these social defeat-produced changes in DG neurogenesis, spleen weight and gastric pH value. **Key words:** social defeat, social support, neurogenesis

Disclosures: **C. Wang:** None. **L. Yu:** None.

Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: NS28912

MH73136

NS045260

Title: Endogenous CRH enhances excitatory synaptic transmission and intrinsic hippocampal network activity.

Authors: *B. G. GUNN, C. D. COX, Y. CHEN, C. M. GALL, G. LYNCH, T. Z. BARAM; Univ. of California Irvine, Irvine, CA

Abstract: Rationale: In addition to regulating the stress response directly via release from neuroendocrine cells in the hypothalamic paraventricular nucleus, CRH is expressed in a number of brain regions including hippocampus. In this region, the peptide is synthesized and released from interneurons (Chen et al., 2001) and its receptors (CRHR1 and CRHR2) reside in discrete subcellular domains on pyramidal cells. Stress provokes the release of hippocampal CRH, which may contribute to the established effects of stress upon hippocampal function and structure (Chen et al., 2004; Chen et al., 2012; Maras & Baram., 2012). Previous studies have demonstrated that infusions of CRH increase the excitability of hippocampal pyramidal cells but data are lacking on the role(s) played by release of *endogenous* peptide in modulating physiology. The present study investigated this question using selective antagonists of CRHR1, the primary hippocampal CRH receptor.
Methods: C57BL/6 mice (P21-P40 and adult) were decapitated, the brains dissected and horizontal brain slices (300-400 μm) prepared using standard procedures for patch-clamp and extra-cellular recordings. The whole-cell voltage-clamp configuration was applied to record excitatory and inhibitory postsynaptic currents (EPSC and IPSC respectively) from CA3 pyramidal cells. The presence of an endogenous CRH tone was determined by the bath application of two structurally distinct CRHR1 antagonists. Extracellular recordings of sharp waves (SPWs) were made from the apical dendrites of CA3 pyramidal cells and the effect of inhibiting CRHR1 upon this intrinsic network activity assessed.
Results: Inhibition of CRHR1 caused a rapid reduction in the frequency and an increase in the decay kinetics (τ) of spontaneous EPSCs in field CA3 of hippocampal slices, results indicative of a decrease and de-synchronization of excitatory input. Surprisingly, the antagonists also altered the frequency but not waveform of miniature EPSCs. The inhibition of CRHR1 had little or no

effect upon $GABA_AR$-mediated inhibition within these cells. These effects were reflected in the decreased incidence of the memory-related SPWs generated by autonomous activity of intra-hippocampal networks. Ongoing modeling work will investigate the relationship between excitatory transmission and the incidence of SPWs.
Conclusions: These findings describe a novel mechanism whereby the secretion of CRH can modulate synaptic transmission and intrinsic network activity within the hippocampus CA3. These observations provide a potential mechanism by which stress-induced release of CRH exerts its effect(s) on memory processing by the hippocampus.

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Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: KBSI to HS

Title: Temporal analysis of hippocampal glucocorticoid receptor activity in the therapeutic action of fluoxetine

Authors: *S. HER[1], M. JEONG[2], J. JUNG[2];
[2]Bio-Imaging Ctr., [1]KBSI, Chunchen, Korea, Republic of

Abstract: Previous studies have shown inconsistent results regarding the actions of antidepressants on glucocorticoid receptor (GR) signalling. To resolve these inconsistencies, we used a lentiviral-based reporter system to directly monitor rat hippocampal GR activity during stress adaptation. Acute stress significantly induced not only GR activity but also its intra-individual variability, showing a temporal GR activation. However, these increases were dampened by exposure to chronic stress, which were partly restored by fluoxetine treatment without affecting glucocorticoid secretion. Immobility in the forced swim test was negatively correlated with the intra-individual variability, but was not correlated with the quantitative GR activity during fluoxetine therapy; this highlights the temporal variability in the neurobiological links between GR signalling and the therapeutic action of fluoxetine. Collectively, these results suggest a neurobiological mechanism by which fluoxetine treatment confers resilience to the chronic stress-mediated attenuation of hypothalamic-pituitary-adrenal axis activity.

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Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: Ryoushokukenkyukai 2014

Title: Folate deficiency-induced depression-like behavior and abnormal neuronal matulation in adult hippocampus in mice.

Authors: ***S. NISHIDA**[1], Y. HIRAKI[1], M. TSUBOI[2], Y. NAKAMURA[2], R. ARAKI[1], T. YABE[1];
[1]Setsunan Univ., Hirakata, Osaka, Japan; [2]Meiji Co., Ltd., Odawara, Kanagawa, Japan

Abstract: Since folate serves to transfer one-carbon units in various biosynthesis reactions such as methylation, folate is an essential nutrition in development and maintenance of biological function. Previous epidemiological and clinical studies have implied that folate deficiency is one of risk factors for depression, suggesting the impact of folate on development and maintenance in psychological function. However, the mechanism for the effect is still unknown. In the present study, we examined behaviors and adult hippocampal neurogenesis in folate deficiency diet-fed mice. Folate deficiency diet for 6 weeks decreased remarkably the serum folate level. Folate deficiency diet-fed mice showed increased immobility in the forced swim test. In contrast, there are no differences between control and folate deficiency diet-fed mice in locomotor activity, social behavior, latency to fall from a rotating rod and time spent in open arms in the elevated plus maze. DNA methylation levels were decreased in the hippocampus, especially the dentate gyrus, of folate deficiency diet-fed mice. Moreover, the number of newborn cells and of doublecortin (an immature neuron marker) immunoreactive cells was increased and the number of NeuN (a neuron marker) immunoreactive newborn cells was decreased in the dentate gyrus of folate decifiency diet-fed mice. These results suggest that folate deficiency-induced reduction of DNA methyation and abnormal neuronal maturation may be implicated in depression symptoms.

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Poster

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Topic: F.04. Stress and the Brain

Support: MH102065

Hope for Depression Research Foundation

Title: CA3 neurons of BDNF-Val66Met mice exhibit a unique translational profile in response to stress

Authors: *J. KOGAN[1], J. D. GRAY[1], T. G. RUBIN[1,3], E. F. SCHMIDT[2], N. HEINTZ[2], B. S. MCEWEN[1];
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Abstract: Stress in the environment has been linked to the exacerbation of mood disorders and several genetic loci have been associated with increased risk for developing a mood disorder. The hippocampus has been identified as a brain region that is highly susceptible to the effects of stress. In this study, the effects of stress on gene expression in the CA3 pyramidal neurons of the hippocampus were examined in a genetic mouse model of mood disorders susceptibility (BDNF-Val66Met) after exposure to both acute and chronic stress. Transgenic mice expressing an EGFP fused to the L10a ribosomal subunit that is under the control of a cell-type specific promoter (Gprin3) were used to isolate the in vivo translating RNA fractions from a genetically homogenous population of CA3 pyramidal neurons. These reporter mice were crossed with BDNF-Val66Met allele carriers to generate double transgenic animals that were subjected to either an acute forced swim stress or 21d of chronic restraint stress. Mice were rapidly decapitated and the hippocampus was dissected for RNA isolation by Translating Ribosomal Affinity Purification (TRAP). TRAP immunoprecipitated and unbound fractions were subjected to RNA-sequencing using an Illumina Hi-Seq 2500 to collect 100bp reads at a sequencing depth of 30M reads/sample. Results were aligned against the mouse genome (mm10) and the numbers of reads for each transcript were normalized against total reads to obtain relative expression levels. Strand software was used to perform statistical analysis to identify differentially expressed genes, which were grouped into pathways using the DAVID tool. $BDNF^{Met/+}$ mice exhibited 1,420 differentially expressed genes at baseline, with the majority in pathways associated with cellular homeostasis and ion transport. After both an acute and chronic stress exposure, $BDNF^{Met/+}$ mice showed highly distinct gene expression profiles from strain-matched WT mice exposed to the same stress, with less than 2% overlap in acute and 8% overlap in chronic. These results demonstrate how a genetic susceptibility to mood disorders can

significantly alter the in vivo translational response to stress of CA3 neurons. Further, these profiles will facilitate the identification of novel molecular mechanisms underlying stress susceptibility in these cell.

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Poster

340. Stress: Hippocampus

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Topic: F.04. Stress and the Brain

Support: IBS-R015-D1

Title: *In vitro* study of chronic stress effect on the adult neurogenesis

Authors: ***J. WOO**[1,2], H. RYU[1,2], C. HEO[1], M. SUH[1,2];
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Abstract: Chronic stress in the adult brain can induce decreases in cognitive function and neurogenic ability. However, the mechanism on the neurogenic changes under stress has not been fully understood. Neural stem cells (NSCs) in the subventricular zone (SVZ) is one of the largest stem cell reservoirs in the adult brain. We focused on the proliferation and differentiation changes of the adult NSCs and the neurogenic changes in whole brain regions in the stressed brain. We extracted NSCs from SVZ in 11-week-old mice following the restraint stress for 6 hours every day for 3 weeks. To verify the stress effect on mice, we performed the elevated plus-maze (EPM) test, observed body weight changes, and analyzed plasma with ELISA. Primary NSCs were passaged every week and performed neurosphere formation assay between control and restraint stress groups. We found that the population of primary NSCs in stress group was higher than control group. However, the neurosphere formation ability in period of 4^{th} passages of stressed animal was reduced compared to the control group. Also, the differentiation pattern of neurons and glial cells was changed under stress. Moreover, we confirmed that the neurogenic regions of whole brain were also changed under the chronic stressed condition. These results suggest that the chronic stress can alter neurogenic capability in NSC and also specific region of the adult brain. Thus, these changes can induce chronic neurodegeneration and cognitive malfunction.

Disclosures: **J. Woo:** None. **H. Ryu:** None. **C. Heo:** None. **M. Suh:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.17/VV16

Topic: F.04. Stress and the Brain

Support: College of Liberal Arts and Sciences

Title: ANA12 prevents hippocampal CA3 apical dendritic arbors from becoming more complex in the weeks after chronic stress ends

Authors: ***P. PAODE**, K. NISHIMURA, J. M. ANGLIN, J. M. JUDD, S. KEMMOU, B. Q. LE, A. FLEGENHEIMER, J. B. ORTIZ, C. D. CONRAD;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Chronic stress leads to hippocampal-mediated spatial learning and memory deficits and hippocampal CA3 apical dendritic pruning. When chronic stress ends and a post-stress recovery period ensues, spatial ability improves and CA3 apical dendritic arbors increase in complexity (termed "recovery"). Brain-derived neurotrophic factor (BDNF) is a critical mediator of the improvement in spatial ability and CA3 dendritic remodeling during recovery from chronic stress (Ortiz et al., 2014, 2015). In addition, we presented data last year in which ANA12, which antagonizes the BDNF TrkB receptor, was administered during the post-stress recovery period. ANA12 prevented the recovery of spatial ability and CA3 apical dendritic complexity in a subset of neurons (Anglin et al. 2015). Here, we present a complete morphological analysis of CA3 dendritic arbors from the full cohort of rats and include the two predominate CA3 pyramidal subtypes, short-shaft and long-shaft neurons. Male Sprague-Dawley rats were chronically stressed by restraint (STR) for 6hr/d/21d and then given a 21-day post-stress recovery period (REC) before behavioral testing. Rats were injected daily with ANA12 (A, 0.5 mg/kg) or vehicle (V, saline, i.p.) during the post-stress recovery period, or an equivalent amount of time in controls (CON). A chronic stress group without recovery was injected with vehicle (STR-IMM-V) and used as a positive control for the immediate effects of chronic stress on the brain and behavior. The procedures led to the following groups: CON-V, CON-A, STR-REC-V, STR-REC-A, and STR-IMM-V. Using Golgi Stain procedures and a light microscope with a camera lucida drawing tube, at least four neurons (at least two of each subtype) were traced for each animal (n=7-8 rats/group). In short-shaft neurons, chronic stress (STR-IMM-V) reduced the number of apical dendritic intersections as measured by Sholl analysis and total dendritic branch points, but dendritic complexity recovered in rats given a post-stress recovery

period (STR-REC-V > STR-IMM-V). Importantly, ANA12 prevented the restoration of short-shaft apical dendritic complexity when administered during the post-stress recovery period (STR-REC-A < STR-REC-V). The effect of ANA-12 was less robust in long-shaft versus short-shaft neurons, although long-shaft neurons from STR-REC-V had more complex apical dendritic arbors than did STR-REC-A. In both neuronal subtypes, basal dendritic arbors were unaffected by chronic stress or ANA12. These data reveal the importance of BDNF and its TrkB receptor for active hippocampal dendritic remodeling that occurs during weeks following the aftermath of chronic stress exposure.

Disclosures: **P. Paode:** None. **K. Nishimura:** None. **J.M. Anglin:** None. **J.M. Judd:** None. **S. Kemmou:** None. **B.Q. Le:** None. **A. Flegenheimer:** None. **J.B. Ortiz:** None. **C.D. Conrad:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 340.18/VV17

Topic: F.04. Stress and the Brain

Support: NWO VENI grant to PA

Title: Hippocampal endocannabinoid signalling mediates the residual effects of early life stress on fear memory

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Abstract: Early life stress (ELS), such as childhood neglect, creates life-long vulnerability to stress-related anxiety disorders such as post-traumatic stress-disorder (PTSD). Recent evidence has underscored the endocannabinoid dysregulations in PTSD; nevertheless, the endocannabinoid system has received very little attention in the context of ELS. We examined the delayed effects of ELS on the endocannabinoid system in limbic brain areas in male rats. We employed a limited-nesting paradigm (postnatal day 2 to 9) that induced robust disturbances in maternal care and stress in pups. We found that ELS persistently reduced the 2-arachidonoylglycerol (2-AG) levels in the hippocampus and amygdala but augmented the anandamide levels in the amygdala and prefrontal cortex (PFC) in adult animals. We did not

observe any profound effects in the hippocampus. Endocannabinoid system is essential in stress response, thus we further investigated the possible residual effects of ELS on the endocannabinoid system by exposing adult animals to an acute swim stress challenge for 15 minutes and subsequently determining the endocannabinoid levels. As expected, acute stress significantly reduced the anandamide levels in the amygdala and PFC in all animals. Moreover, stress dramatically increased the 2-AG levels in the hippocampus; strikingly, this increase was absent in rats with ELS history. Previously we reported that stress or corticosterone recruit the 2-AG signalling in the dorsal hippocampus to supress fear memory recall. Thus, we further tested the functional consequence of the insufficient recruitment of hippocampal 2-AG signalling by glucocorticoids on the fear memory suppression in animals with ELS history. Strikingly, we found that corticosterone (3mg/kg s.c.) failed to suppress the fear memory retrieval in rats with ELS history. We predicted that this failure of fear suppression in animals with ELS history resulted from the insufficient recruitment of the 2-AG signaling in the hippocampus. Consistently, we found that the activation of the 2-AG signalling with MAG Lipase inhibitor KML29 (0.2 μg/side) within the dorsal hippocampus induced suppression of fear recall. However, blocking CB1 receptors with AM251 (0.35 ng/side) within the hippocampus prevented the 2-AG effects on fear memory. To the best of our knowledge, this is the first study that demonstrates an involvement of the endocannabinoid signalling in the persistent and residual effects of ELS. These findings suggest that ELS induces dysfunctioning of the glucocorticoid-endocannabinoid loop that might have important implications for how ELS predisposes individuals to stress-related anxiety disorders.

Disclosures: P. Atsak: None. **M. Morena:** None. **C. Schoenmaker:** None. **M.N. HIll:** None. **B. Roozendaal:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: NIH DA029989

NIH MD007592

Title: Early life stress alters the levels of proteostasis markers in the rat hippocampus

Authors: *J. A. SIERRA FONSECA, J. N. HAMDAN, G. A. LODOZA, S. SAUCEDO, Jr., K. L. GOSSELINK;
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Abstract: Neurodegenerative diseases are characterized by a progressive loss of neuronal structure and function, and some include a pathological accumulation of aggregated proteins in specific neuronal populations. While early life stress has emerged as a factor that may contribute to the development of neurodegenerative disorders, very little is known regarding its effects on the mechanisms controlling abnormal protein aggregation and clearance. We hypothesized that early life stress can influence the protein degradation machinery, specifically the autophagic and proteasomal systems, in adulthood. We employed a maternal separation (MS) paradigm in Wistar rats, and evaluated hippocampal proteins in males and females at 3 months of age. Western blotting analysis was employed to assess the levels of proteostasis markers (LC3 and 20S proteasome) and disease markers (Tau, phospho-Tau, and α-synuclein). Levels of the autophagy marker LC3 were significantly increased ($p<0.001$) in the hippocampus of rats subjected to MS stress as neonates, compared to controls, with both males and females displaying elevated LC3 after MS. In contrast, the expression of the 20S proteasome subunit tended to decrease (non-significant) after MS. Interestingly, sex differences were observed, as MS males displayed significant decreases ($p=0.019$) in their levels of 20S proteasome, while females did not show changes in this proteasomal marker. Levels of phosphorylated Tau (pSer262, a neurotoxic variant of Tau) were not found to change significantly after MS stress. Total levels of α-synuclein, however, were also assessed, with male rats subjected to MS displaying a significant decrease ($p=0.010$) in the levels of this protein. Taken together, our results indicate that early life stress can exert deleterious effects on the protein degradation machinery of the brain, which could contribute to the development of neurodegenerative diseases in later life.

Disclosures: J.A. Sierra Fonseca: None. **J.N. Hamdan:** None. **G.A. Lodoza:** None. **S. Saucedo:** None. **K.L. Gosselink:** None.

Poster

340. Stress: Hippocampus

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: F.04. Stress and the Brain

Support: Graduate Student Organization at the University of Louisiana at Lafayette

Brain Behavior Research Foundation Young Investigator Award

National Institute of Mental Health (K01MH087845)

Ray P. Authement College of Sciences at the University of Louisiana at Lafayette

Undergraduate Research Mini Grant from the University of Louisiana at Lafayette

Title: Effects of stress on fibroblast growth factor receptor 1 expression in the tgFgfr1-EGFP BAC transgenic mouse line

Authors: *J. COLLETTE, H. M. TORRES, K. M. SMITH;
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Abstract: Fibroblast Growth Factor Receptor 1 (FGFR1) is one of four fibroblast growth factor receptors that act as a receptor for a family of fibroblast growth factor (FGF) ligands. FGFRs and their ligands are thought to be involved in a multitude of CNS developmental and maturational functions including radial glial proliferation in the cortex and hippocampus, oligodendrocyte proliferation and regeneration, cerebellar development, and midline glia morphology. Furthermore, deviations in the expression of Fgfr1 and its predominate ligand, Fgf2, have been observed in the prefrontal cortex, hippocampus, and other limbic system regions of patients with major depressive disorder (MDD). Fgf2 has also been implicated in HPA axis regulation. Congruent with previous findings, we have observed that in the BAC transgenic mouse line, tgFgfr1-EGFP, Fgfr1 driven expression of GFP is present in the same regions as previously published in situ hybridization studies of Fgfr1 expression. Additionally, we have also observed Fgfr1 expression in cell types that are consistent with known roles of Fgfr1 in development including hippocampal stem cells and CA neurons, DCX+ neuronal progenitors, OLIG2+ oligodendrocytes, and the majority of GFAP+ astrocytes. However, an important question that remains to be answered is whether the tgFGFR1-EGFP model will sufficiently match GFP levels to the endogenous levels of FGFR1, and whether it can be used to model the effects of stress or antidepressant treatment on FGFR1 expression. It is known that exposing mice to chronic stress induces anhedonia, a defining symptom of MDD, therefore, this is a suitable model for studying the mechanisms of stress and depression. We confirmed that the presence of the tgFgfr1-EGFP transgene did not alter baseline locomotor behavior, nor did it alter performance on the elevated plus maze test. We have exposed additional groups of tgFgfr1-EGFP mice to either an acute stress or a chronic unpredictable stress paradigm in order to validate previous studies that have observed a decreased in expression of Fgf2 and Fgfr1 via in situ hybridization, and to validate that the tgFgfr1-EGFP model is suitable for tracking endogenous Fgfr1 expression via GFP levels. We did this by comparing the GFP levels in stressed, both chronically and acutely, and non-stressed control mice. Future studies will investigate whether the known antidepressant effects of FGF2 can be used to recover Fgfr1 expression in vivo and in vitro cell cultures derived from the tgFgfr1-EGFP line.

Disclosures: **J. Collette:** None. **H.M. Torres:** None. **K.M. Smith:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.01/VV20

Topic: F.07. Autonomic Regulation

Title: Effect of hypo- and hyper-perfusion on neurovascular coupling

Authors: *D. KAIN[1,2], P. BLINDER[2];
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Abstract: Proper brain function relays on adequate blood flow, as the localized nature of neuronal activity requires constant reallocation of nutrients and oxygen on a spatio-temporal need-basis; a phenomena termed neurovascular coupling. Aging is accompanied by an increased risk of vascular and neuronal related pathologies as well as dementia. Among these, hypertension leads to the loss of vascular reactivity and has been demonstrated to delay neurovascular hemodynamic response. Under these conditions, a decoupling between neuronal activity and blood flow can lead to ischemic conditions, increased reactive oxidative stress and delayed protein synthesis, accumulation of Amyloid-β and eventually leading to a complete loss of cognitive function. In this research we focus on understanding the cascade of cellular events and changes in neuronal activity associated with pathological changes in blood flow.
Results: we established a combined transverse aortic constriction (TAC) model with dual craniotomy procedure to investigate -on the same individual over 4 weeks- the effect of both hypo and hyper-perfusion, using longitudinal, aweak two-photon imaging. Our results show a significant elevation in both diameter and red blood cells (RBC) velocity in the right hemisphere and an opposite effect in the left hemisphere ($p<0.01$), following aortic constriction. We also observe an apparent hippocampal and cortical neuronal death one month post TAC. In addition, we will show novel transgenic lines where we targeted a calcium reporter to contractile elements of the vascular wall (smooth muscle and endothelial cells). We expect this novel approach and transgenic tools to provide an insight on the acute and long-term effect of vascular impairments on neuronal activity and health.

Disclosures: D. Kain: None. **P. Blinder:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.02/VV21

Topic: F.07. Autonomic Regulation

Title: Effect of endogenous nitric oxide on adrenergic nerve-mediated vasoconstriction and CGRPergic nerve-mediated vasodilation in pithed rats

Authors: *H. KAWASAKI[1], S. TAKATORI[2], K. YAMAWAKI[3], Y. ZAMAMI[4];
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Abstract: ***Background***: Vascular adrenergic vasoconstrictor nerves mainly regulate tone of blood pressure (BP). Also, calcitonin gene-related peptide (CGRP)-containing (CGRPergic) vasodilator nerves participate the tone regulation. Furthermore, there are nitric oxide (NO)-containing (nitrergic) nerves, which include nitric oxide (NO) on blood vessels as vasodilator nerves. However, it remains unclear whether nitrergic nerves participate in regulation of the blood vessel tone. The present study was investigated to clarify the role of nitrergic nerves in vascular responses to spinal cord stimulation (SCS) and vasoactive agents in pithed rats..
Methods: Male Wistar rat were anesthetized and pithed. After allowing BP and heart rate to stabilize, we observed vasopressor response induced by SCS (2-8 Hz) and bolus injection of norepinephrine (NE; 250-500 ng/kg). The blood of the pithed rat before and after SCS was collected and plasma NE concentration was measured with HPLC. To evaluate vasodilator responses, the mean BP was increased by continuous infusion of methoxamine (4-15 μg/kg/min) concomitant infusion of hexamethonium (2-3 mg/kg/min) to block autonimic outflow. After the elevated BP stabilized, SCS at 4-8 Hz and bolus injections of acetylcholine (ACh), sodium nitroprusside (SNP) and rat calcitonin gene-related peptide (CGRP) were intravenously applied. Then, we evaluated the effects of NO synthase (NOS) inhibitor N-ω-nitro-L-arginine methylester hydrochloride (L-NAME; 0.5 and 3 mg/kg/h) and combined infusion of L-NAME (3 mg/kg/h) and L-arginine (300 mg/kg/h) on these vascular responses and plasma NE concentration.
Results: Pressor responses to SCS and NE in pithed rat were markedly enhanced by L-NAME infusion, while combined infusion of L-NAME and L-arginine had no effect on these responses. L-NAME infusion significantly increased release of NE evoked by SCS. When the BP was artificially increased, depressor response to ACh was suppressed and depressor to smaller dose of SNP was enhanced by L-NAME. However, L-NAME had no effect on depressor responses to SCS and CGRP, which resulted in similar to the control responses.
Conclusion: The present results suggest that endogenous NO regulates vascular tone through

endothelium function and inhibition of adrenergic neurotransmission, but not CGRPergic nerve function.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: F.07. Autonomic Regulation

Support: NINR Grant NR-013693

NCATS Grant UL1TR000124

Title: Female and male obstructive sleep apnea patients show prior diagnosis of co-morbid hypertension and mental health conditions

Authors: *E. AN[1], A. AGUILA[2], K. WATSON[3,4], M. R. IRWIN[3,5,6], R. AYSOLA[3,7,2], L. DOERING[1], R. M. HARPER[2,8], P. M. MACEY[1,2];
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Abstract: Introduction: Obstructive sleep apnea (OSA) is accompanied by regional brain injury linked to common autonomic and depressive symptoms. We examined whether such symptoms cause or a result from OSA by assessing whether OSA diagnosis precedes diagnosis of symptom cause or consequence of OSA. We hypothesize that OSA leads to brain injury, and such injury leads to autonomic, depressive and anxiety disorders; hence, OSA diagnosis should precede hypertension (HTN), depressive and anxiety disorders. **Methods**: All adult patients in the UCLA Medical System database over 10 year with an OSA diagnosis ("UCRex" database) were included. Times of diagnoses of HTN, primary unipolar depressive, and anxiety disorders were compared to the earliest OSA diagnosis using 1 sample t-tests; age factors were determined by linear regression. Relationships were assessed by sex or mixed groups separately by independent samples t-tests. **Results:** 10518 subjects had a diagnosis of OSA (4122 F, 6392 M). In 4874 subjects (2006 F, 2868 M), HTN was diagnosed substantially earlier than OSA ($P < 0.001$; mean time to diagnosis = -697 days; median = -64 days. Females showed significantly earlier HTN diagnoses than males ($P < 0.05$: female: mean time before = -786 days; median = -559 days; males: mean time before = -634 days; median = -388 days). Age was negatively related to time

from OSA to HTN diagnosis in both sexes ($P < 0.05$). In 2421 of the OSA subjects (1252 F, 1169 M), a depressive disorder was diagnosed earlier than OSA ($P < 0.001$; mean time to diagnosis = -208 days; median = -64 days. Females showed significantly earlier HTN diagnoses than males ($P < 0.05$: female: mean time before = -306 days; median = -153 days; males: mean time before = -102 days; median = -0 days). Age was unrelated to time from OSA to depressive diagnosis in any group. In 430 of the OSA subjects (213 F, 217 M), anxiety disorder diagnoses precede OSA diagnosis substantially ($P < 0.001$; mean time to diagnosis = -267 days; median = -208 days. Both sexes showed similar times between diagnoses. Age was unrelated to time from OSA to anxiety diagnosis in any group. **Conclusions:** Hypertension is diagnosed years prior to OSA diagnosis, with a longer separation in females. Depressive and anxiety disorders are diagnosed months before OSA diagnosis, although the median time from OSA to depressive diagnosis in males was 0 days. The data likely do not reflect the true onset of OSA. While the results do not support the model that OSA leads to brain injury which leads to co-morbid conditions, it is possible that OSA is being diagnosed years after it has developed. Delayed diagnosis of OSA compared to other co-morbid conditions may relate to historical under screening for OSA.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

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Program#/Poster#: 341.04/VV23

Topic: F.07. Autonomic Regulation

Support: KSCHIRT Grant 9-10A

NIH Grant 1R01HL103750

Title: Cardiorespiratory coupling in individuals with spinal cord injury

Authors: *S. C. ASLAN[1], S. HARKEMA[2], A. OVECHKIN[3];
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Abstract: Cardiovascular and autonomic dysfunctions result in hemodynamic instability after spinal cord injury (SCI). The majority of individuals with SCI experience transient episodes of low and high blood pressure (BP) in response to daily life activities. Respiration exerts significant influence on cardiovascular function through neural and mechanical mechanisms; BP

and heart rate (HR) oscillate with breathing. Patients with autonomic and respiratory dysfunctions experience abnormal breathing patterns characterized by cyclic variation of ventilation. Abnormal ventilation is accompanied by large oscillations in BP and HR, and therefore has a detrimental effect on both cardiovascular and pulmonary functions causing an increase in morbidity and mortality. Besides cardiovascular dysfunction, respiratory insufficiency is also common in SCI individuals due to paresis/ paralysis/ spasticity of the trunk muscles involved in respiration. In spite of well recognized cardiovascular and pulmonary dysfunctions after SCI, it is not known whether these individuals are prone to have abnormal breathing patterns and hemodynamic instability associated with respiration. The aim of this study was to investigate the prevalence and severity of abnormal breathing patterns and their role in BP and HR regulations in individuals with SCI. Beat-to-beat BP, HR, chest and abdomen respiratory kinematics were continuously acquired from 30 individuals with cervical and upper thoracic SCI and 10 non-injured controls during quiet breathing. Spectral power analyses of hemodynamic variables, and cross correlation between respiration with HR and BP were applied. Breathing rate was around 0.2 Hz (12 breaths per minute) in both SCI and non-injured individuals whereas the depth of the breathing had crescendo-decrescendo alterations in SCI individuals compared to no alteration in the non-injured. The frequency of these alterations was around 0.05 Hz. There was a strong correlation between respiration with HR and BP within these frequencies. Individuals with SCI have abnormal breathing patterns with cyclic variations in tidal volume causing further hemodynamic instability in these individuals. Early detection and treatment of abnormal respiration may be important to prevent further damage to cardiovascular and respiratory diseases after SCI.

Disclosures: **S.C. Aslan:** None. **S. Harkema:** None. **A. Ovechkin:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.05/VV24

Topic: F.07. Autonomic Regulation

Support: Supported by the National Institute of Nursing Research NR-013693.

Title: Sex differences in insular gyral responses to an autonomic challenge

Authors: ***N. S. RIEKEN**[1], J. A. OGREN, 91403[2], R. KUMAR[3,4], R. M. HARPER[2,4], P. M. MACEY[1,4];
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Abstract: Introduction: Sex differences in autonomic control may underlie male-female cardiovascular disease variations; the insular cortex helps coordinate such regulation. The insula consists of five main gyri, and shows a sex-based gyral distribution of neural responses to a complex sympathetic-parasympathetic challenge, the Valsalva maneuver. Here, we examined sex-related differences in insular responses to a purely sympathetic challenge, the hand grip.
Methods: We studied functional MRI (fMRI) responses to four 16-s hand grip challenges (80% maximum hand grip strength) in 22 females (age; mean±std: 50±8 yrs), and 36 males (45±9 yrs). Heart rate (HR) and fMRI signal responses were compared between and within groups (repeated measures ANOVA $P<0.05$).
Results: Females had higher resting HR than males, but showed smaller percent change HR increases to the challenge. All gyri showed similar fMRI signal patterns, with an initial rise concurrent with the HR peak, followed by a decline toward baseline. Signals in females were higher than males. Both sexes exhibited an anterior-posterior organization of insular responses, with lateralized patterns varying by gyri and sex. Females showed greater signals in the right short vs long gyri (0.2-0.3%), whereas males showed similar anterior and mid short gyri increases vs the long gyri (0.2%). Females showed higher left anterior and mid short gyri signals over the long gyri (0.2%), with the posterior short gyrus responding similarly to the anterior and posterior long gyri. Males showed greater responses in the all left short gyri vs the posterior long gyrus (0.15%), with patterns differing between the anterior and posterior long gyri (<0.1%). The mid and posterior short gyri showed no lateralization in females, but left-sided dominance in males (-0.1%). Anterior and posterior long gyri showed greater left than right activation in both sexes. The most prominent sex difference was in the anterior-most short gyrus, where females showed greater right-sided activation (0.2% through challenge), whereas males showed lower right-sided activation (0.15% from 6 sec).
Conclusions: The findings reinforce a greater role of the anterior vs. posterior insula in sympathetic activation. The anterior short gyrus showed oppositely lateralized responses in males vs females, similar to Valsalva maneuver findings. Females showed e response differences between right anterior short gyri and posterior long gyri, but such differences were not apparent in males. Insular responses to a sympathetic challenge show both amplitude and lateralization differences between females and males, possibly contributing to sex-specific autonomic patterns.

Disclosures: N.S. Rieken: None. **J.A. Ogren:** None. **R. Kumar:** None. **R.M. Harper:** None. **P.M. Macey:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

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Program#/Poster#: 341.06/VV25

Topic: F.07. Autonomic Regulation

Support: Departmental Scholarship.

Title: Central modulation of cardiac sympathetic nerve activity following acute myocardial infarction.

Authors: *R. ROY;
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Abstract: Acute myocardial infarction (MI) is a global health problem costing around 7.4 million lives every year. One of the main contributing factors to the high mortality associated with acute MI is an adverse increase in cardiac sympathetic nerve activity (SNA). Once established, the sustained increase in cardiac SNA is essentially irreversible, even with the use of sympathetic beta receptor blockers. The increased drive for SNA is most likely central in origin. However, the regions of the brain involved in the generation of increased SNA immediately after acute MI remains unknown. Therefore, we aimed to assess the activation of specific brain regions in response to acute MI. We first determined the specific regions of brain that are activated in the early stages following acute MI. Rats were transcardially perfused under anesthesia 90 min following the induction of MI. Immunohistochemistry for Fos protein was performed on brain sections as a marker of neuronal activation. MI rats had a significantly higher number of Fos-positive cells in the paraventricular nucleus than sham operated rats (P=0.0002, unpaired t-test), which included significantly greater Fos expression in MI rats. We next determined the phenotype of the activated parvocellular neurons using double label immunohistochemistry. Acute MI was associated with significantly higher number of Fos-positive oxytocin (OT) neuron compared to sham (P=0.0022, unpaired t-test). As parvocellular neurons are known to project to rostral ventrolateral medulla (RVLM), next we identified the activated parvocellular OT neuronal projection by injecting a retrograde dye into the RVLM. Significantly higher numbers of retrogradely labeled activated OT neurons were identified in the infarcted rat (P=0.001, unpaired t-test). Taken together these results suggest that the activation of parvocellular pre-autonomic OT neurons may drive increased cardiac SNA in early stages of acute MI.

Disclosures: R. Roy: None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

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JSPS KAKENHI Grant 15K12611

Title: Persistence of post-stress blood pressure elevation is induced by activated astrocytes

Authors: ***Y. HASEBE**[1,2], S. SUGAMA[3], K. TAKEDA[4,2], K. KOIZUMI[1], I. FUKUSHI[5,2], M. HOSHIAI[1], Y. KAKINUMA[3], J. HORIUCHI[5], K. SUGITA[1], Y. OKADA[2];
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Abstract: When psychological stress is loaded, the sympathetic nervous system is reflexively excited. Even after the stress is relieved, stress-induce blood pressure elevation persists. Such sustained blood pressure elevation could be a consequence of neural plasticity of the sympathetic nervous activity. Because it has been revealed that not only neurons but astrocytes play active roles in neural plasticity in various brain functions, we hypothesized that astrocytes are involved in post-stress persistent blood pressure elevation. We tested this hypothesis by analyzing the effects of arundic acid, an inhibitory modulator of astrocytic function, on responses of blood pressure and heart rate to air-jet stress in rats. Further, the effects of arundic acid on air-jet stress induced activation of neurons and astrocytes were histochemically examined. We have shown that inhibition of astrocytic activation suppressed air-jet stress induced blood pressure elevation during and after stress loading. Suppression of heart rate by arundic acid was not remarkable. Histochemically, pretreatment with arundic acid suppressed air-jet stress induced activation of neurons and astrocytes in the cardiovascular brain regions. We demonstrated that not only neurons but astrocytes are involved in stress-induced blood pressure elevation and its post-stress persistence, which could underlie the pathogenesis of hypertension in stress-loaded subjects.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.08/WW1

Topic: F.07. Autonomic Regulation

Support: British society of Neuroendocrinology project support grant

Title: Vasopressin contributes to the development of angiotensin II-dependent hypertension

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Abstract: Cyp1a1-Ren2 rats (300 - 400g) have the Cyp1a1-Ren2 transgene (comprised of the mouse Ren-2 renin cDNA fused to the cytochrome P450 promoter) inserted in the Y-chromosome of Fischer 344 (F344) rats. Ingestion of indole-3-carbinol (I3C) activates the Cyp1a1 promoter to increase Ren-2 renin gene expression and hence cause the development of angiotensin II-dependent hypertension. Administration of 0.225% I3C-containing diet increased systolic blood pressure (SBP) from 128 ± 3 mmHg at day 0 to 172 ± 2 mmHg at day 7 (n = 8). We have previously found that vasopressin magnocellular neurons exhibit increased firing rate on day 7 of I3C treatment. Vasopressin cell firing rate causes secretion of vasopressin into the bloodstream. At normal physiological levels, vasopressin causes reabsorption of water from urine through its action at vasopressin 2 receptors in the kidneys. At higher levels, vasopressin acts as a vasoconstrictor through its action at vascular vasopressin 1a receptors. To determine whether increased vasopressin secretion contributes to the development of angiotensin II-dependent hypertension, a potent vasopressin 1a receptor antagonist ((Phenylac^{1},D-Tyr(Et)^{2},Lys^{6},Arg^{8},des-Gly^{9})-vasopressin trifluoroacetate salt, 230 ng/h) was subcutaneously infused during the 7 day period of I3C treatment. In vehicle-treated rats, SBP increased from 139 ± 6 mmHg to 185 ± 4 mmHg whereas in V1-Ant-treated rats, I3C increased SBP from 137 ± 5 to 161 ± 3 mmHg (both n = 7; P = 0.003, two-way repeated measures ANOVA, Bonferroni's *post-hoc* test). SBP in non-I3C fed rats was unaffected. Hence, increased vasopressin secretion contributes to the development of angiotensin II-dependent hypertension in Cyp1a1-Ren2 rats via vasopressin 1a receptor activation.

Disclosures: A. Korpal: None. **D.O. Schwenke:** None. **C.H. Brown:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

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Program#/Poster#: 341.09/WW2

Topic: F.07. Autonomic Regulation

Support: GlaxoSmithKline funding

Title: Temporal structure of metabolic modulation of autonomic nervous system activity

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Abstract: Bioelectronics medicine is a novel therapeutic approach, which aims at developing a new class of medicines based on the neuromodulation of the activity of the autonomic nervous system (ANS). This approach could be also used to re-establish a proper metabolism. However, in order to understand where and how to apply electrical stimulations, a deeper understanding of the neural correlates of metabolic processes is needed. The carotid body and its sensory afferent, the Carotid Sinus Nerve (CSN), are involved in many crucial body functions, including the control of glucose metabolism. Recent experimental studies even suggested that blocking CSN activity has beneficial effects on type 2 diabetes. The interplay between CSN activity and metabolic processes, however, has been so far mainly investigated focusing on the average level of the elicited neural activity, so a thorough description of the temporal aspects underlying CSN activity role in metabolism is still missing. Here we recorded and analyzed the CSN responses to glucose and insulin challenges in both healthy rats (HR) and rats on high-fat high-sucrose diet (HFHSR), which has shown to be an efficient model of metabolic and insulin resistance disorders. Bursting activity in both HR and HFHSR was found to have two distinct phases following challenge onset: a short-lived decrease in burst frequency followed by a long-lasting increase. The spectral analysis of CSN signal modulation revealed that HR had a significantly lower dominant frequency (~300 Hz) than HFHSR (~400 Hz). In both challenges there was a strong increase in the power of the dominant frequency, as well as the onset of fluctuations in the 30-80 Hz range. In order to assess the effects of CSN denervation on the autonomous system sensitivity, we repeated the same analysis on a separate set of recordings from sympathetic nerve during glucose and insulin challenge. In the first set of recordings the autonomous system was intact; in the second experimental CSN denervation was performed. We were then able to

identify differences between the two conditions in terms of bursting period and overall spectral structure. These results shed light on the temporal structure of the ANS activity during metabolism, a fundamental precondition to the development of efficient neurostimulation patterns to treat metabolic disorders.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

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Topic: F.07. Autonomic Regulation

Support: National Institute of Biomedical Imaging and Bioengineering (NIBIB), NIH: K01-EB011498, Center for Functional Neuroimaging Technologies (JRP), P41-EB015896 (JRP, LLW)

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National Institute of Mental Health (NIMH), NIH: R21-MH103468 (RGG, VN)

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Title: Mapping the brainstem circuitry of transcutaneous vagus nerve stimulation (tVNS) in humans using ultrahigh-field (7T) fMRI

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Abstract: Transcutaneous vagus nerve stimulation (tVNS), which stimulates the auricular branch of the vagus nerve (ABVN), has been suggested and/or used as a neuromodulatory therapy for multiple disorders involving sympathovagal disruption, such as chronic pain, depression, and cardiovascular disorders. Animal studies have linked clinical outcomes following ABVN stimulation with medullary activation, in particular of nucleus tractus solitarius (NTS). However, this pathway is difficult to elucidate in humans, mainly due to anatomical/physiological challenges posed to brainstem functional neuroimaging. Our study applied ultrahigh-field functional MRI (7T fMRI) and cardiorespiratory physiological monitoring for enhanced spatiotemporal resolution in order to evaluate brainstem response to ABVN stimulation. Five (5) healthy subjects experienced two 7-minute block design fMRI runs, with moderately strong but not painful tVNS delivered through Ag/AgCl electrodes placed within the left cymba conchae of the ear (rectangular pulses with 450 μS pulse width, delivered at 30 Hz). Each run followed a block-design protocol with variable duration stimulation and rest periods (8 s tVNS + 14 s rest, 17 repetitions; 14 s tVNS + 20 s rest, 11 repetitions), with order randomized across subjects. Blood oxygen level-dependent (BOLD) fMRI data were collected on a Siemens 7T scanner using a Simultaneous Multi-Slice EPI acquisition with multiband factor 2 (voxel size=1.2 mm isotropic, 34 coronal slices centered on the brainstem, TR=0.89 s, TE=23 ms, 470 volumes), concurrently with electrocardiogram and respiration belt monitoring, collected at 400Hz in order to control for physiological noise. A brainstem mask defined in the ICBM152 MNI space was transformed to individual functional space and applied to preprocessed fMRI data (RETROICOR, motion correction, FWHM=2 mm spatial smoothing). General Linear Model analyses were performed (FSL Feat). A mixed-effects group map identified a cluster located in the inferior dorsal medulla and consistent with NTS. Interestingly, the cluster also encompassed dorsal motor nucleus of the vagus (DMNX) and nucleus ambiguus (NAmb). Successfully exploiting ultrahigh-field, high spatial resolution fMRI, we were able to identify medullary nuclei activated by ABVN stimulation in humans. The functional mapping of this pathway in humans is critical in developing future, brainstem nucleus targeted "electroceutical" applications incorporating ABVN stimulation, and future studies will evaluate whole brain and brainstem response, using 7T fMRI to optimize stimulation parameters of tVNS for various applications.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.11/WW4

Topic: F.07. Autonomic Regulation

Title: Ascending cholinergic neurons in the mesopontine tegmentum regulate blood pressure fluctuation during REM sleep

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Abstract: During REM sleep, large fluctuations of autonomic nervous systems occur, resulting in changes in blood pressure, heart rate or respiration which are considered to reflect the emotional changes during REM sleep. We have shown that the amygdala induces blood pressure fluctuation during REM sleep. REM sleep regulating center in the mesopontine tegmentum regulates several phasic events during REM sleep, including rapid eye movement or PGO wave. So, it is highly probable that the mesopontine tegmentum is involved also in blood pressure fluctuation during REM sleep. In unanesthetized and head-restrained rats, blood pressure fluctuation was induced by electrical stimulation of the areas including laterodorsal/pedunculopontine tegmental nuclei (LDT/PPT) or parabrachial nucleus (PBN). Single neuronal recording from the LDT revealed that about 40% of the LDT neurons showed firing correlated with blood pressure fluctuation during REM sleep. In most cases, the increase in firing occurred prior to blood pressure increase. Judging from the shape of action potentials, most of the neurons whose activity was correlated with blood pressure fluctuation were cholinergic, indicating that the cholinergic neurons in the LDT are the main population of neurons that drive blood pressure fluctuation during REM sleep. Blood pressure fluctuation induced by the mesopontine tegmental stimulation was blocked by the application of cholinergic antagonist (mecamylamine) into the amygdala. The results suggest that the ascending cholinergic system from the mesopontine tegmentum to the amygdala has a crucial role in driving blood pressure fluctuation during REM sleep.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

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Topic: F.07. Autonomic Regulation

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Kentucky Spinal Cord Injury Research Center

Title: Cardiovascular regulation in individuals with chronic motor complete and incomplete spinal cord injury

Authors: *S. WANG[1], S. ASLAN[1,4], D. LORENZ[2], A. OVECHKIN[1,4], G. HIRSCH[3], B. DITTERLINE[1], S. HARKEMA[1,4];
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Abstract: Spinal cord injury (SCI) results in abnormal cardiovascular control which chronically persists, impeding rehabilitation. It is known that level of injury is a factor in the development of orthostatic hypotension (OH), with higher prevalence in cervical SCI compared to lower level SCI. However, the relationship between injury severity and cardiovascular dysfunction is less known. We previously showed that SCI individuals had a wider variability in cardiovascular responses to orthostatic stress (OS) than non-disabled (ND) participants, and that the ASIA motor impairment scale (AIS) was not conclusive at predicting those responses. In a larger SCI population (n=86), we further sought to identify individual patterns of cardiovascular responses to OS at different time points, independent of AIS grade. In this study, continuous BP and HR were obtained from 26 ND and 86 individuals with SCI (C1-L2, all AIS grades) during supine rest and in response to OS induced by a rapid and passive sit-up (SIT). Each position lasted for 15 minutes. The first 3 min after position change is a standard time window in which neural component dominates BP control in ND. However, in our study, we observed BP fluctuations at different rates in response to OS in different SCI individuals. Therefore, we quantified the

maximum change in BP with flexible window for each individual. Using hierarchical cluster analysis, SCI participants were grouped based on patterns of systolic BP (SBP), diastolic BP (DBP) and HR responses. Following SIT, we characterized 3 primary patterns of cardiovascular responses regardless of level or severity of injury. One group had a severe decrease in SBP in their maximum windows compare to the other two groups. The second group had mild drop in SBP and the third group had normal increase in SBP. The Three groups were further divided into groups based on the time course of change, i.e., SBP was maintained or continued to decrease, as well as different DBP and HR changes. Cervical injuries were related with more severe decrease in SBP while upper thoracic injuries were related with larger increase in HR. AIS grade was not related with the cluster groups. In conclusion, BP and HR responses to orthostatic stress, including the magnitude and time course of changes, provide information regarding the degree of abnormal autonomic control of the heart and peripheral vasculature, independent of AIS grade. Classifications of patterns of cardiovascular responses will assist in stratification of autonomic impairment and decision making for cardiovascular management. The mechanisms of residual and/or compensatory cardiovascular control after SCI warrant further investigation.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.13/WW6

Topic: F.07. Autonomic Regulation

Title: Arterial stiffness predicts white matter burden in patients with mild cognitive impairment.

Authors: *B. C. TSENG[1], C.-Y. GWO[2];
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Abstract: Background: Brain white matter hyperintensities (WMH), or leukoaraiosis, is a measure of white matter damage associated with to small cerebral vessel pathologies and/or white matter fiber myelination abnormalities. The presence and extent of WMH has been linked to cognitive impairment and dementia in older adults. In addition, increases in arterial stiffness and pulse wave velocity (PWV) are the hallmark of arterial aging indicating the presence of subclinical atherosclerosis. To date, the pathogenesis of WMH and its relationship to cerebrovascular health in people with mild cognitive impairment (MCI) remains unclear. The purpose of this study was to determine the relationship between arterial stiffness as measured by

central pulse wave velocity (cPWV), WMH volume, and regional distribution of WMH subtypes in MCI patients.
Methods: Fifty-two MCI patients (male=24, female=28, age=64.8±6.7 yrs) and 30 age- and educational level-matched non-MCI older adults participated (male=15, female=15, age=65.8 ±6.6 yrs). WMH was assessed using Fluid-Attenuated Inversion Recovery (FLAIR) images on a 3T Philips MR system. Subcortical, periventricular, and deep WMH volumes were quantified and regional distributions were identified based on brain watershed territories using customized semi-automated software. Arterial stiffness was assessed using the applanation tonometry method (SphygmoCor, AtCor). cPWV was assessed between the right common carotid artery and the left femoral artery.
Results: MCI patients showed higher total, subcortical, and deep WMH volumes in anterior watershed territory than the non-MCI subjects ($P<.05$). In addition, cPWV was associated with subcortical WMH volume in anterior watershed territory (MCI $r^2=.691$, $p<.001$, non-MCI $r^2=.865$, $p<.001$). Using general linear modeling, we found a difference in regression slopes between groups ($p=0.048$, $F=4.07$).
Conclusion: Our findings revealed a relationship between central arterial stiffness and white matter integrity in brain anterior watershed territory; and this relationship appeared to. Interestingly, this relationship appears to be amplified in the MCI patients and is modulated by a threshold of pulse wave velocity. It is our speculation that arterial stiffness may add white matter burdens in brain regions that are susceptible to cerebral hypoperfusion in MCI patients. Future studies may further investigate the impact of WMH on MCI and Alzheimer's Disease.

Disclosures: B.C. Tseng: None. **C. Gwo:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

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Topic: F.07. Autonomic Regulation

Support: NIH Grant R01 NR-013625

NIH Grant R01 NR-014669

Title: Non-gaussian diffusion measures show axonal and myelin changes in patients with heart failure

Authors: *B. ROY[1], M. WOO[1], G. FONAROW[2], R. KUMAR[3,4,5,6];
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Abstract: Heart failure (HF) patients show brain axonal and myelin alterations in autonomic, respiratory, cognitive, and mood regulatory sites based on diffusion tensor imaging (DTI) Gaussian procedures. However, complex brain sites follow non-Gaussian diffusion, and thus, Gaussian diffusion-based measures may not show complete extent of axonal and myelin changes in whole-brain regions, but can be examined with non-Gaussian diffusion based diffusion kurtosis imaging (DKI). Our aim was to evaluate axonal and myelin changes in HF over control subjects using DKI-based axial and radial kurtosis procedures. We acquired two DKI series from 6 HF (age, 56.2±7.7 years; body mass index, 25.6±5.2kg/m^2, 5 male; left ventricular ejection fraction, 29.2±11.3%) and 9 control subjects (55.4±2.8 years; 26.2±2.4 kg/m^2, 6 male) using a 3.0-Tesla magnetic resonance imaging scanner. Axial and radial kurtosis maps were computed from each series, both maps realigned and averaged, normalized to a common space, smoothed, and compared voxel-by-voxel between HF and controls using analysis of covariance (SPM12; covariates; age, gender; uncorrected threshold, $p<0.005$). No significant differences in age or gender appeared between groups. However, body mass index significantly increased in HF over control subjects. Multiple brain sites showed significantly decreased axial and radial kurtosis values, indicating compromised axons and myelin in those sites, respectively, in HF over control subjects. Decreased axial kurtosis in HF emerged in the pre-frontal, frontal and parietal lobe, and lingual gyrus, and reduced radial kurtosis appeared in the insular cortices and surrounding regions, pre-frontal, frontal, parietal and inferior occipital lobe, lingual gyrus, and cerebellum. Heart failure subjects show compromised axons and myelin in areas that regulate autonomic, respiratory, cognitive, and mood functions deficient in the condition. These sites with axonal and myelin changes are comparable to areas identified by DTI-based axial and radial diffusivity measures. These findings indicate that non-Gaussian diffusion based axial and radial kurtosis measures can be used to characterize axonal and myelin changes in the condition.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

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Topic: F.07. Autonomic Regulation

Support: NIH Grant AR047410

Title: Cholinergic intrinsic cardiac ganglion neurons may glutamatergic in nature

Authors: *T. WANG[1], K. E. MILLER[2];
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Abstract: Intrinsic cardiac ganglia (ICG) are considered not only as autonomic efferent relay stations for higher neuronal innervation, but also as regional cardiac modulation centers. This may be due to sensory ICG neurons that are capable of transmitting regional cardiac environmental information to other ICG neurons. In a previous study, we identified glutamatergic ICG neurons with VGLUT1, VGLUT2 and GLS-immunoreactivity. In the current study, we further investigated the relationship of these glutamatergic neurons and cholinergic intrinsic cardiac neurons. Rat epicardium fat tissue and atrium tissue were collected and processed for immunohistochemistry, utilizing mouse anti-vesicular acetylcholine transporter (VAChT) with rabbit anti-VGLUT1, VGLUT2, or GLS to evaluate the relationship between post-ganglionic autonomic neurons and glutamatergic neurons. Sequential labeling of VGLUT1 and VGLUT2 in adjacent tissue sections was used to evaluate the co-localization of VGLUT1 and VGLUT2 in ICG neurons. Our studies yielded the following results: (1) many glutamatergic ICG neurons also were cholinergic, expressing VAChT. (2) VGLUT1 and VGLUT2 co-localization occurred in many ICG neurons with variation in their protein expression level. Investigation of both glutamatergic and cholinergic ICG neurons could help in better understanding the function of the intrinsic cardiac nervous system and its role in modulating cardiac function.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.16/WW9

Topic: F.07. Autonomic Regulation

Title: Regional brain changes in patients with cystic fibrosis

Authors: *C. TOM[1], M. WOO[2], B. ROY[3], K. AFSHAR[4], A. P. RAO[5], L. FUKUSHIMA[6], P. ESHAGHIAN[7], M. WOO[3], R. KUMAR[8];
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of USC, Los Angeles, CA; [7]Medicine, Pulmonary Dis., [8]Anesthesiology, Radiological Sciences, and Bioengineering, David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Cystic fibrosis (CF) patients show a variety of symptoms, including mood, cognitive, respiratory, and profuse sweating issues. These abnormal symptoms suggest the presence of brain injury in this condition, which can be examined with non-invasive magnetic resonance imaging (MRI) procedures. However, the tissue integrity across the whole-brain in CF patients is unclear. Our aim was to assess regional brain changes in CF over control subjects using MRI-based diffusion tensor imaging (DTI) procedures. We acquired two DTI series from 5 CF (age, 29.7±3.7 years; body mass index, 22.0±0.7 kg/m^2; 3 male) and 5 control subjects (age, 28.5±5.1 years; body mass index, 21.3±5.4 kg/m^2; 4 male) using a 3.0-Tesla MRI scanner. Using diffusion and non-diffusion images, mean diffusivity (MD) values, which indicate average motion of water molecules within the tissue and show microstructural changes, with decreased values in acute, and increased in chronic pathological condition, were calculated at each voxel from each DTI series. Both MD maps, derived from each DTI series, were realigned and averaged, normalized to a common space, smoothed, and compared voxel-by-voxel between groups using ANCOVA (covariates, age and gender; SPM12, $p<0.005$; extended threshold, 10 voxels). No significant differences in age, gender, or body mass index appeared between CF and control subjects. Various brain areas showed significantly reduced MD values in CF subjects, indicating predominant acute tissue changes, over control subjects. Sites with reduced MD values included the bilateral prefrontal and frontal, parietal, and occipital cortices, bilateral corona radiate, anterior, mid, posterior cingulate cortices; insula, cerebellar vermis, middle cerebellar peduncles, cerebellar cortices and deep nuclei, and ventral medulla. Only few areas, including the basal-forebrain and right occipital cortex, showed increased MD values in CF subjects, suggesting chronic tissue damage, over control subjects. Cystic fibrosis subjects show predominant acute tissue changes in areas that control mood, cognition, respiratory, and autonomic functions. The findings suggest that tissue changes in these regulatory sites may contribute to symptoms accompanying the condition.

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Poster

341. Autonomic Control: Cardiovascular Regulation I

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Program#/Poster#: 341.17/WW10

Topic: F.07. Autonomic Regulation

Support: NSERC

Title: Regulation of epinephrine biosynthesis by intermittent hypoxia

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Abstract: Hypertension is a cardiovascular disorder characterized by elevated blood pressure and is frequently co-morbid with obstructive sleep apnea (OSA). OSA patients experience intermittent hypoxia (IH), characterized by brief but recurring episodes of cessation in breathing during sleep. These patients produce elevated reactive oxygen species (ROS) and catecholamines as a consequence of oxidative stress, and have an increased incidence of hypertension; however the mechanisms defining this association are not clearly established. Catecholamines, such as epinephrine are involved in the regulation of blood pressure, and are increased in hypertension. Phenylethanolamine N-methyltransferase (PNMT) is the terminal enzyme in the catecholamine biosynthetic pathway involved in the synthesis of neurotransmitter/hormone epinephrine, and is found abundant in the adrenal medulla. Genetic linkage studies have associated the PNMT gene to the development of hypertension. This study examines the role of IH in the regulation of the PNMT gene and its cellular regulatory mechanisms using rat pheochromocytoma adrenal medullary (PC12) cells. Cells exposed to normoxia (21% O_2), chronic hypoxia (1 hour; 1% O_2) or IH (1 hour; alternating cycles of 1% O_2 for 30 seconds and 16% O_2 for 3 minutes) were analyzed for the transcript levels of PNMT and its regulatory transcription factors HIF1α (hypoxia inducible factor 1α), Egr1 (early growth response protein 1), Sp1 (specificity protein 1) and GR (glucocorticoid receptor) by RT-PCR. Results show that IH causes a 1.6-fold increase in levels of intron-retaining PNMT mRNA and a 1.4-fold increase in intronless PNMT mRNA compared to normoxic control; the intronless transcript generates the functional PNMT. In comparison, chronic hypoxia causes no change in intron-retaining PNMT transcript and a 1.3-fold increase in intronless PNMT transcript, suggesting that the regulation of PNMT expression is different under IH than CH, and might involve alternate splicing mechanisms. IH increases expression of HIF1α (1.3-fold), Egr1 (1.6-fold) and Sp1 (1.3-fold) mRNA. In contrast, CH increases mRNA expression of HIF1α (1.2-fold), Egr1 (1.5-fold) and Sp1 (1.2-fold). The preliminary results of this investigation help assess the cellular pathways involved in regulating PNMT expression via its transcription factors. These findings will help elucidate the mechanism by which IH regulates catecholamine synthesis, particularly as it relates to the pathogenesis of hypertension in OSA patients.

Disclosures: **R.B. Mailloux:** None. **S. Khurana:** None. **T.C. Tai:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.18/WW11

Topic: F.07. Autonomic Regulation

Title: Quantifying stress through crustacean EKG: A modified detrended fluctuation analysis(mDFA)of the nerve-heart dynamics

Authors: *T. YAZAWA;
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Abstract: The heart is governed by two nerves, the cardio-accelerator (CA) and the cardio-inhibitor (CI). Both are simultaneously active if a subject is not in a sick condition; recorded from the hermit crab heart (Yazawa, Zool. Sci. 1976). If a human approaches a spiny lobster (Panulirus japonicas), CI discharge is suppressed but CA remains active. Thus, concomitance of CA and CI is a stress-free state. In the absence of humans, CI cyclically shows a sharp rise in frequency (changing from 5 Hz to 60 Hz) with a complete cessation of CA, so, high CI with no CA. The high CI state lasts for 1-10 min and repeats every 10-20 min. As this cyclic slowdown in heart rate can be observable with an electrocardiogram (EKG), crustacean stress states are distinguishable by EKG. "CA-dominant" state is a stressful state. We deduced that this can happen in humans too. Since CI and CA directly correlate with EKG pattern, analyzing EKG patterns may be a useful tool for healthcare. We recently made a method for analyzing the healthiness of the heart: modified detrended fluctuation analysis (mDFA, Yazawa 2015 ASME). With mDFA, we looked at the cardio-vascular system as a whole. The true test of a technology is, how well it works in a real-life operational setting (Reginald Brothers, NASA, May7, 2015): We present empirical results of mDFA-tests, to quantify stress through the heartbeat (EKG) recordings, including job-related stressful EKG, caregiver's stressful EKG, as well as stress/fear EKG-response of model animals, lobsters and crabs. The method did not require sophisticated technical training or complicated mathematics. mDFA is a kind of tailored medicine, observing subjects one by one without big cohort statistics. We present a single universal result: Healthy individuals show a scaling index (SI) near 1.0 and unhealthy subjects show a decreased scaling exponent. Using mDFA, we quantified the stress of not only models but also humans. The practical mDFA worked well: (1) monitoring power-law relationships in the heartbeat interval time series by computing a scaling index (SI). (2) SI indicates that stress, anxiety, or fear with a decreased SI to ~0.7. (3) Especially in human subjects, cardiac rhythm reflected mental conditions such as chronic job stress. (4) Stress levels were confirmed through informal interviews during EKG recordings. For example, one case study of a subject in a descending aircraft exhibited a SI of ~0.7, returning to 1.0 after a safe landing. Present case studies show how wellness of subjects can be evaluated using heartbeat recordings. We conclude that mDFA

is a useful new numerical method for quantifying degrees of wellness and the transition between stress (sickness) and wellness.

Disclosures: T. Yazawa: None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.19/WW12

Topic: F.07. Autonomic Regulation

Support: IBRO/ISN Post Doc Fellowship 2016

Title: Dorsal hypothalamic DA neurons contributes to PVN RVLM circuitry and Ang II mediated sympatoexcitation

Authors: *O. M. OGUNDELE[1], C. C. LEE[2], J. FRANCIS[2];
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Abstract: Paraventricular nuclei (PVN) projections to the rostral ventrolateral medulla (RVLM)/C1 catecholaminergic neuron group forms the pre-autonomic sympathetic center involved in the neural control of systemic cardiovascular functions. However, the role of extra-hypothalamic and thalamic dopaminergic PVN inputs in the PVN-RVLM circuit remains elusive. Using retrograde neuroanatomical tracing, electrophysiology and high contrast confocal imaging, we investigated the morphology of the dopaminergic neuron groups in the dorsal hypothalamic area (DHA) and their contribution to the PVN-RVLM neural circuit. Our study showed that two distinct dopaminergic (DA) neuron sub-groups in the *Zona Incerta (Zi)* and *Reuniens thalamic nuclei (Re)* converge onto the DA neuron in the upper PVN (Convergence Zone; CZ). Retrograde label injection into the RVLM/C1 region of the brain stem produced vesicular yield in the DA neurons of dorsal hypothalamic area (Zi/Re) and PVN. Subsequent analysis showed that acute Angiotensin II treatment induced, simultaneously, excitatory post-synaptic currents (EPSC) and inflammatory response in the CZ. The EPSC was associated with repetitive evoked action potentials, increased post-synaptic density and tyrosine hydroxylase expression. Furthermore, the observed Ang-induced excitatory response was associated with elevated IGF-1R and HMGB1 in the CZ. We deduced that *Zi and Re* neurons contribute to the PVN catecholaminergic circuitry and are a target of Ang II-mediated inflammation and sympathoexcitation. Ultimately, we have shown that IGF-1R up-regulation is a possible

mechanism through which Ang II facilitates a concurrent sympathoexcitation and inflammation in DA neuron groups around the third ventricle.

Disclosures: O.M. Ogundele: None. **C.C. LEe:** None. **J. Francis:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.20/WW13

Topic: F.07. Autonomic Regulation

Support: JSPS KAKENHI Grant 24590268

Title: Reliable and noninvasive protocol for the induction of acute cardiac response to emotional stress in intact freely-moving mice

Authors: *S. SATO, T. KANBAYASHI, A. IMANISHI, K. TSUTSUI, T. SHIMIZU; Neuropsychiatry, Akita Univ. Grad. Sch. of Med., Akita, Japan

Abstract: For chronic psychological stress experiments in mice, there are standardized protocols such as restraint, forced swim, and social defeat stress tests for the studies in psychiatric disorders such as depression. On the other hand, there has been no noninvasive method to assess psychological responses to acute psychological stresses in mice. Here we show a novel noninvasive protocol to assess heart rate (HR) response as a psychological stress response in intact mice without anesthesia nor surgery i.g., transmitter implantation for telemetry system. The device for the HR recording is an electrocardiogram (ECG) plate sensor, which is placed in a conventional cage, with multiple stripes of gold-plated electrode on the surface and designed to detect ECG when at least two paws of mice on the sensor touch different electrodes separately. The multi-stripe ECG (msECG) sensor works only when the soles have emotional sweating in response to a psychological stress, because the electrode-skin contact impedance is too high with dried up soles for ECG signal detection. The emotional sweating is a biomarker of acute psychological stress, which is perceived by the emergence of the ECG signal output from the msECG sensor. Indeed, mice exhibited ECG responding to a psychological stress by hitting the mouse cage with a slight HR increase (754 ± 29 bpm; n = 7) from the HR during active state (703 ± 33 bpm). The HR response while mice were sleeping was more dramatic. Mice exhibited ECG from the beginning of sleep probably in parallel with perspiration on their soles although the mechanism is unknown. During several minutes after the sleep onset, HR gradually lowered to 340 ± 12 bpm (n = 6), below the intrinsic HR of 466 ± 19 bpm, implicating the facilitated parasympathetic nervous system activity. Interestingly, when an experimenter entered the mouse

room and gazed at the awakened mouse, the HR suddenly increased up to 608 ± 55 bpm (n = 6) from the HR of 340 bpm and thereafter, it gradually decreased. The profiles of the HR responses in 6 mice examined were basically similar and at the HR declining phase, they all exhibited bradyarrhythmia, which we consider that it might possibly be atrial fibrillation. In conclusion, we have demonstrated a novel noninvasive protocol for the assessment of acute psychological stress responses during active and sleep state from onset in intact freely-moving mice. The protocol that regards ECG detection as the alternative indicator of emotional sweating may contribute to a new progress in studies in autonomic regulation on various acute psychological stress responses in intact mice and a variety of genetically modified mouse models.

Disclosures: **S. Sato:** None. **T. Kanbayashi:** None. **A. Imanishi:** None. **K. Tsutsui:** None. **T. Shimizu:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.21/WW14

Topic: F.07. Autonomic Regulation

Support: Grant Conacyt No. 219707

Title: The role of dopamine D_2-like receptors in the inhibition of the cardioaccelerator sympathetic outflow in diabetic pithed rats

Authors: *B. VILLANUEVA-CASTILLO, E. RIVERA-MANCILLA, A. H. ALTAMIRANO-ESPINOZA, G. MANRIQUE-MALDONADO, C. M. VILLALÓN;
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Abstract: Diabetes mellitus is associated with abnormalities in the central and peripheral catecholaminergic systems. These abnormalities include alterations in both the levels of catecholamines (including dopamine) and the expression of the corresponding receptors. In this respect, our group has previously reported that the dopamine D_2-like receptors inhibiting the cardioaccelerator sympathetic outflow in normoglycemic pithed rats resemble the pharmacological profile of the dopamine D_2, but not of the D_3 or D_4, receptor subtypes; however, no study has yet reported the role of these cardiac sympatho-inhibitory receptors in diabetic rats. On this basis, the present study was designed to identify the pharmacological profile of the dopamine D_2-like receptor subtypes involved in the inhibition of the cardioaccelerator sympathetic outflow in diabetic pithed rats.
For this propose, male normotensive Wistar rats were pretreated with streptozotocin (50 mg/kg,

i.p.) in order to induce diabetes. After 4 weeks, the rats were pithed, artificially respired and pretreated with gallamine and desipramine for selective preganglionic spinal (C_7-T_1) stimulation of the cardioaccelerator sympathetic outflow. Subsequently, the effect of selective antagonists at dopamine D_2, D_3 and D_4 receptor subtypes was determined on the inhibition of the cardioaccelerator sympathetic outflow induced by 10 μg/kg.min of quinpirole (a D_2-like receptor agonist). Hence, quinpirole-induced cardiac sympatho-inhibition was: (i) unchanged after i.v. bolus injections of vehicles (1 ml/kg of saline, 0.5% DMSO or bidistilled water); and (ii) abolished after i.v. bolus injections of the subtype-selective dopamine receptor antagonists L-741,626 (D_2; 300 μg/kg), SB-277011-A (D_3; 300 μg/kg) or L-745,870 (D_4; 100 μg/kg). These results, taken together: (i) show that quinpirole-induced cardiac sympatho-inhibition in diabetic pithed rats is mediated by
activation of the dopamine D_2, D_3 and D_4 receptor subtypes; and (ii) contrast with our results previously reported in normoglycemic Wistar rats, wherein quinpirole-induced cardiac sympato-inhibition is exclusively mediated by the dopamine D_2 receptor subtype.

Disclosures: B. Villanueva-Castillo: None. **E. Rivera-Mancilla:** None. **A.H. Altamirano-Espinoza:** None. **G. Manrique-Maldonado:** None. **C.M. Villalón:** None.

Poster

341. Autonomic Control: Cardiovascular Regulation I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 341.22/WW15

Topic: F.04. Stress and the Brain

Support: CIHR

Title: The role of epigenetic regulators in the fetal programming of hypertension

Authors: *J. LAMOTHE[1], S. KHURANA[4], C. WILLIAMSON[2], C. J. BYRNE[2], S. MERCIER[3], S. THARMALINGAM[4], T. TAI[4,2,1];
[1]Biomolecular Sci., [2]Biol., [3]Sch. of Human Kinetics, Laurentian Univ., Sudbury, ON, Canada; [4]Med. Sci. Div., Northern Ontario Sch. of Med., Sudbury, ON, Canada

Abstract: The causes of hypertension are complex and involve both genetic and environmental factors. A sub-optimal environment during fetal development has been linked to the development of adult diseases including hypertension; a concept known as fetal programming. Animal studies show that timed *in-utero* exposure to high levels of glucocorticoids results in the postnatal development of hypertension in adulthood. Evidence suggests that *in utero* stress can alter patterns of gene expression, possibly a result of alterations in the topology of the genome by

epigenetic markers such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs). The objective of this study was to determine the role of epigenetic regulators in mediating the fetal programming of hypertension. Specifically, this study examined the effects of the HDAC inhibitor valproic acid (VPA) or the DNMT inhibitor 5-aza-2'-deoxycytidine (5aza2DC) on blood pressure (BP) and gene expression in programmed rats. BP measurements suggest that both VPA and 5aza2DC attenuate the development of hypertension. Programmed hypertensive animals receiving epigenetic inhibitors from weeks 12-15 displayed decreased BP comparable to unprogrammed saline control animals. Interestingly, qPCR results suggest VPA attenuates programmed increases in several catecholamine biosynthetic enzymes induced by *in utero* DEX exposure. For example, the terminal catecholamine biosynthetic enzyme phenolethanolanime N-methyltransferase (PNMT) showed a fold decrease in relative expression from 3.88 to 1.16 in males and 2.43 to 1.02 in females compared to unprogrammed saline control. Similar results were found for tyrosine hydroxylase (TH) showing a fold decrease from 3.42 to 1.23 in males and 2.35 to 1.38 in females compared to unprogrammed saline control. Interestingly, Dopamine Beta Hydroxylase expression only displayed a fold decrease from 2.29 to 1.42 and only in males relative to unprogrammed saline controls. Generally, 5aza2DC did not attenuate increased expression of all three catecholamine biosynthetic enzymes despite BP results. Further results from a custom qPCR array may provide additional insight into the role of both VPA and 5aza2DC in the attenuation of elevated BP. This study provides insight into the role of epigenetic regulation of PNMT and consequences in the fetal programming of hypertension.
Note: This research is funded by CIHR

Disclosures: J. Lamothe: None. **S. Khurana:** None. **C. Williamson:** None. **C.J. Byrne:** None. **S. Mercier:** None. **S. Tharmalingam:** None. **T. Tai:** None.

Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.01/WW16

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01MH091801

R01EY019466

NSF BCS 1539717

NCRR P41RR14075

Title: Interhemispheric asymmetry in sleep depth, arousal and behavioral response associated with the first-night effect

Authors: *M. TAMAKI, J. BANG, T. WATANABE, Y. SASAKI;
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Abstract: We experience poor sleep in a new environment; it takes longer time to fall asleep and we are woken frequently from sleep. This temporary sleep disturbance, known as the first-night effect (FNE) in human sleep research, has been regarded as a sleep disturbance due to temporary inhabitation to an unfamiliar environment. However, the FNE is so robust that even younger and healthy participants show poor sleep on the first night, suggesting that the FNE involves an ecological benefit. Interestingly, animal studies have shown that sleeping in only one brain hemisphere plays a role in monitoring the surroundings as a protective mechanism. This led us to ask whether the FNE in humans involves interhemispheric asymmetry of sleep to keep some degree of alertness in one brain hemisphere and monitor unfamiliar environments. In Exp. 1, we tested whether the FNE involves regional interhemispheric asymmetry in sleep depth or the strength of slow-wave activity (SWA, 1-4Hz). SWA during sleep was measured by magnetoencephalography (MEG) and polysomnography on the first and second sleep sessions (Day 1 and Day 2). We employed the cortically constrained minimum-norm estimate using individual anatomical magnetic resonance imaging (MRI) to source-localize SWA in cortical brain networks. The SWA was weaker in the left hemisphere than the right in the default-mode network, which is associated with mind wandering, during the deepest sleep stage, only on Day 1 when the FNE occurred. The degree of SWA asymmetry was significantly correlated with the sleep-onset latency, a sensitive parameter for the presence of the FNE. In Exp. 2, we tested whether the vigilance shows interhemispheric asymmetry during sleep with the FNE. We measured an evoked brain response to sounds using an oddball paradigm, and compared the degree of vigilance between hemispheres. The left hemisphere showed increased vigilance than the right hemisphere. This interhemispheric asymmetry in vigilance was found only on Day 1, but not on Day 2. We further asked whether the FNE involves faster behavioral response to external signals during sleep. In Exp. 3, participants were instructed to tap their fingers when they heard sounds during sleep. Sounds to the left hemisphere induced awakenings and tapping responses significantly faster than those presented to the right hemisphere. This interhemispheric asymmetry in behavioral response was found only on Day 1, not on Day 2. Our results demonstrate that the human brain involves a regional interhemispheric sleep under the FNE as a night watch and suggest that it may be advantageous for humans to keep some degree of alertness to detect unfamiliar surroundings in a new environment.

Disclosures: **M. Tamaki:** None. **J. Bang:** None. **T. Watanabe:** None. **Y. Sasaki:** None.

Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.02/WW17

Topic: F.08. Biological Rhythms and Sleep

Support: BIBS/NPNI Postdoctoral Fellowship in Translational Neuroscience

NIH NIEHS ES018915

NIMH MH079179

NINDS NS055813

S10RR023457

US Department of Veteran Affairs

Title: Genes differentially methylated in young adults with short sleep duration may regulate sleep across species

Authors: *A. C. HART[1], H. HUANG[1], Y. ZHU[2], V. KNOPIK[3,4], J. E. MCGEARY[3,4,6], M. ELIOT[5], M. CARSKADON[7,4];
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Abstract: Genes, proteins and pathways that regulate sleep are likely conserved across species. Here, we explicitly relied on this conservation and undertook a collaborative approach to identify genes that impact sleep regulation or response to sleep restriction, in both vertebrate and invertebrate species. We hypothesized that epigenetic mechanisms might regulate the expression of genes important for sleep regulation in humans. We looked for genes with differential levels of DNA methylation in college students with shorter (<6.8 h) *versus* longer (>7.8h) average total sleep duration, based on daily on-line sleep logs during the first semester of college. To identify differentially methylated human genes, we pooled blood DNA samples from 8 participants with shorter sleep and 8 with longer sleep. Then, we assessed genome-wide methylation for each group with a 450K Illumina array and identified genomic sites with significantly altered DNA methylation between groups. Based on this analysis, 52 genes were found to have differential DNA methylation. Interestingly, this list of genes included Neuropeptide Y, which has been implicated in sleep and sleep homeostasis across species. To determine if the other differentially

methylated genes play conserved roles in sleep or sleep regulation, we examined the impact of gene loss of function on sleep. For these behavioral studies, we assumed that gene function would be conserved across species and used an invertebrate model organism, looking at *C. elegans* sleep at the larval-to-adult developmental transition stage. Starting with the list of 52 differentially methylated human genes, we identified *C. elegans* orthologs for 34 genes. Then, we examined the consequences of *C. elegans* gene loss of function on sleep. Thus far, perturbation of 7 out of 51 genes alters *C. elegans* sleep. Many of these genes have not been previously implicated in sleep regulation in any species. These 7 genes may act in one or a few related pathways, based on the proteins they encode. In a different group of shorter *versus* longer sleep college students, we have been able to confirm that 2 of these 7 genes are differentially methylated, independently replicating our previous observations. We conclude that differential patterns of DNA methylation are present in young adults with different sleep duration. And, we conclude that at least some of these genes play conserved roles in sleep regulation or response to restricted sleep across vertebrate and invertebrate species.

Disclosures: **A.C. Hart:** None. **H. Huang:** None. **Y. Zhu:** None. **V. Knopik:** None. **J.E. McGeary:** None. **M. Eliot:** None. **M. Carskadon:** None.

Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.03/WW18

Topic: F.08. Biological Rhythms and Sleep

Support: JSPS KAKENHI 26380987

Title: Sleep loss induce diabetes in mice model

Authors: ***S. CHIKAHISA**[1], S. HARADA[2,1], N. SHIMIZU[1], T. SHIUCHI[1], S. NISHINO[3], H. SÉI[1];
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Abstract: Sleep-wake regulatory system and energy metabolism are strongly associated with each other. Accumulating evidence from both epidemiologic studies and laboratory studies indicates that sleep impairment may increase the risk of obesity and diabetes in humans. However, the mechanisms for the interaction of sleep loss with diabetes are still unknown. In the present study, we investigated whether sleep loss by rearing on different environment would

induce diabetes in mice, and explored its underlying mechanism. Male mice were randomly assigned to two groups to be reared on either normal sawdust (control) or wire net. Mice reared on wire net for 3 weeks showed a decreased amount of NREM sleep, an increased sleep fragmentation, and an attenuated slow-wave activity (SWA, power density of the EEG delta band and a parameter of sleep depth) compared with that of control mice. These sleep-disrupted mice showed an impaired glucose tolerance measured by glucose tolerance test (GTT). One-week treatment with sleep-inducing agent pyrilamine (histamine h1 receptor antagonist) ameliorated GTT accompanying improvement of sleep quality in mice reared on wire net. Histamine content of the cortex was also increased in mice reared on wire net. Histamine in the brain is known to be released not only from histamine neurons but also from brain mast cells. In mice reared on wire net, a number of brain mast cells were increased, although c-fos expression in histamine neurons did not differ from that of control mice. In addition, inhibition of histamine secretion from mast cells by intracerebroventricular injection of cromolyn improved sleep quality and ameliorated GTT in mice reared on wire net. Our data indicates that sleep quality is important for the glucose homeostasis, and mast cells may be involved in the sleep regulation in a mouse model for insomnia.

Disclosures: **S. Chikahisa:** None. **S. Harada:** None. **N. Shimizu:** None. **T. Shiuchi:** None. **S. Nishino:** None. **H. Séi:** None.

Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.04/WW19

Topic: F.08. Biological Rhythms and Sleep

Support: Max Plack Society

Swiss National Science Foundation

Title: First evidence of sleep in flight

Authors: ***N. C. RATTENBORG**[1], B. VOIRIN[1], S. M. CRUZ[2], R. TISDALE[1], G. DELL'OMO[3], H.-P. LIPP[4], M. WIKELSKI[2], A. L. VYSSOTSKI[5];
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Abstract: Many birds fly non-stop for days or longer, but do they sleep in flight and if so how? Birds on land can sleep with one eye open, a behavior associated with wakefulness in the opposite cerebral hemisphere and slow-wave sleep (SWS) in the other. Likewise, dolphins can swim during unihemispheric SWS. Thus, it is commonly assumed that birds alternate sleeping with the left or right hemisphere during long-distance flights, permitting them to maintain aerodynamic control and environmental awareness, while obtaining enough daily sleep to maintain attention during wakefulness. However, due to the absence of recordings of brain activity during long flights, it is unknown whether birds sleep on the wing. We tested the assumption that birds sleep in flight by recording GPS coordinates, flight altitude, head movements, and the electroencephalogram (EEG) in great frigatebirds (*Fregata minor*) flying non-stop over the ocean for up to 10 days. Surprisingly, we found that they stayed mostly awake and showed only small bouts (lasting up to several minutes) of both unihemispheric and bihemispheric SWS during flight, sleeping only 0.7 h/day (42 min), roughly 7.5% of the time sleeping on land. Although SWS was more asymmetric in flight than on land, the occurrence of bihemispheric SWS in flight demonstrates that unihemispheric SWS is not required for aerodynamic control. Instead, we found a relationship between the direction of flight and opposing interhemispheric asymmetries in EEG slow-wave (0.75-4.5 Hz) and gamma (30-80 Hz) activity, the later involved in visual attention, suggesting that frigatebirds use unihemispheric SWS to watch where they are going. Nonetheless, the low amount of sleep in flight indicates that the ecological demands for attention exceed that afforded by sleeping unihemispherically in frigatebirds, raising the possibility that other birds and marine mammals thought to rely on unihemispheric sleep also forgo sleep under demanding ecological circumstances. Determining how frigatebirds maintain adaptive performance on little sleep may provide new perspectives for understanding the adverse effects of sleep loss typically experienced by other animals.

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Poster

342. Sleep Behavior and Systems II

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Program#/Poster#: 342.05/WW20

Topic: F.08. Biological Rhythms and Sleep

Support: French General Directorate for Armament (DGA, # Contract: PDH-1-SMO-2-508 / 14ca703)

Title: No benefits of sleep extension on executive processes during an acute total sleep deprivation.

Authors: *A. RABAT[1,2], P. J. ARNAL[1,2], H. MONNARD[1], C. BOUGARD[1,2], M. ERBLANG[1], P. VAN BEERS[1,2], C. DROGOU[1,2], M. GUILLARD[1,2], D. GOMEZ-MERINO[1,2], F. SAUVET[1,2], D. LÉGER[3,2], M. CHENNAOUI[1,2];
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Abstract: Introduction: Both acute total sleep deprivation (TSD) and chronic sleep restriction (CSR) impair various aspects of cognitive processing (i.e. sustained attention, executive processes and decision-making; see Chee and Chuah, 2008; Killgore, 2010). Rupp et al. (2012) showed that a week of banking sleep before a CSR had a protective effect on sustained attention processes. In our study we have evaluated if this effect, that exists in TSD situation (Arnal et al., 2015), is also evidenced for executive processes such as inhibition and working memory. Methods: 14 healthy right-handed male subjects (31.4±3.9 years old) with a body max index of 24.0±2.0 Kg/m^2, a median chronotype and sleep need participated in a 2 experimental counter-balanced conditions (9 days crossover design): extended (EXT, 9.8±0.1 hours of TIB) and habitual sleep (HAB, 8.2±0.1 hours TIB). After 5 nights (EXT or HAB) they were tested in a 3 days laboratory design with a baseline night (either EXT or HAB), a baseline day (BASE: 07:00-00:00) followed by an extended wake period (TSD: 00:00-21:00), a recovery night (REC: 21:00-07:00) and day (REC: 07:00-18:00). Inhibition (go-nogo task) and working memory (2nback) processes were both tested at different time (BASE: 9:30, 13:00, 17:00; TSD: 21:00, 00:00, 03:00, 07:00; 9:30, 13:00, 17:00; REC: 9:30, 13:00, 17:00). Results: Over the 6 nights of EXT, total sleep time (TST), WASO, NREM 1, 2, 3 and REM sleep amounts were significantly longer compared to HAB (respectively $p<0.001$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.01$ and $p<0.001$) and with no significant differences for sleep efficiency and sleep latencies. Concerning executive processes, we both observed for HAB and EXT sessions significant increase of speed responses and errors for inhibition and a significant decrease of corrects responses for 2nback (from TSD 09:30 until the end; $p<0.001$ for both tasks). We neither observed significant differences for these two executive capacities between HAB and EXT sessions, during BASE, TSD and REC periods. Conclusions: These results pointed out that banking sleep, beneficial for sustained attention processes under TSD (see Arnal et al., 2015), is no efficient for core executive ones. We thus hypothesize a dissociation effect of lack of sleep inside the cognitive processes with a direct link on attention processes and an indirect link on executive ones as suggested by Rabat et al., in a recent study with a CSR situation (Rabat et al., 2016). Support: This work was supported by Grants the *French General Directorate for Armament* (DGA, # Contract: PDH-1-SMO-2-508 / 14ca703). Keywords: Total Sleep Deprivation, Executive processes, Banking Sleep, Inhibition, Working Memory.

Disclosures: **A. Rabat:** None. **P.J. Arnal:** None. **H. Monnard:** None. **C. Bougard:** None. **M. Erblang:** None. **P. Van Beers:** None. **C. Drogou:** None. **M. Guillard:** None. **D. Gomez-Merino:** None. **F. Sauvet:** None. **D. Léger:** None. **M. Chennaoui:** None.

Poster

342. Sleep Behavior and Systems II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: F.08. Biological Rhythms and Sleep

Support: TR-SFB 654 /Plasticity and sleep

Title: New declarative learning after sleep is enhanced by pre-sleep D-cycloserine administration

Authors: *M. ALIZADEH ASFESTANI[1], J. SCHWIDETZKY[1], E. BRAGANZA[1], S. SOEKADAR[2], J. BORN[1,3], G. FELD[1];
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Abstract: Sleep is beneficial for declarative memory consolidation and sleep can boost subsequent learning. Memory consolidation occurs based on local synaptic potentiation whereas new learning after sleep benefits form global downscaling which happens during sleep. Long term potentiation (LTP) is an important factor for synaptic consolidation. In the hippocampus LTP is mediated by N-methyl-D-aspartate-receptors (NMDA) containing the NR2A subunits and long term depression (LTD) is mediated by NR2B subunits. D-cycloserine (DCS) as NMDA-receptor co-agonist preferentially acts through NR2A containing receptors, which may favour LTP over LTD. Previously, we showed that sleep-dependent declarative memory consolidation is facilitated by DCS administration, probably due to local increases in potentiation. In the current study we investigated whether DCS during sleep impairs new learning of a similar task due to the assumed potentiating effect of DCS on the memory traces during sleep. This potentiation would reduce the capacity for new learning in the hippocampus and additionally reduce global downscaling. We designed a double blind, placebo controlled, balanced crossover study to investigate this hypothesis. Participants learned two lists of word-pairs (original lists) in the evening after their arrival and then orally received DCS or placebo before 8 hours of sleep. Sleep EEG was prepared and polysomnography was recorded during sleep. The next evening, participants were asked to learn two lists of word-pairs again. One list consisted of completely new word-pairs (new list), while the other one used the cue words form one of the original lists and matched them with new target words (interference list). Interestingly our results (n=30) indicate that learning after sleep under DCS was significantly increased independent of interference. Similar to the previous study sleep architecture was altered by the treatment. To investigate, if this effect of DCS on memory is sleep-dependent or not we have are currently performing a wake -control study.

Disclosures: **M. Alizadeh Asfestani:** None. **J. Schwidetzky:** None. **E. Braganza:** None. **S. Soekadar:** None. **J. Born:** None. **G. Feld:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: DFG TR-SFB 654 'Plasticity and Sleep'

Title: Increasing acetylcholine levels does not affect odor-induced memory reactivation during slow wave sleep

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Abstract: Sleep-associated memory consolidation is assumed to rely on the reactivation of newly acquired memory representations in the hippocampus, particularly during slow wave sleep (SWS). Mnemonic cues such as odors can be associated with a learning task and then presented again during subsequent sleep to facilitate consolidation of the learned content. Reminder cues presumably bias hippocampal memory reactivation in favor of the associated memories, allowing information to be communicated to the neocortex to finally become integrated into distributed neocortical networks for long-term storage. This information flow has been suggested to depend on the low cholinergic tone that is characteristic for SWS and leads to a disinhibition of connections between hippocampus and neocortical areas.
In the present study, we pharmacologically increased acetylcholine levels by administering the acetylcholine-esterase inhibitor physostigmine during a 40-minute sleep period. Before sleep, subjects learned card locations in a 2D object location task in the presence of an odor and this odor was again presented during SWS of the subsequent 40-minute sleep period. We expected that increased cholinergic tone would suppress performance improvements known to be triggered by odor-induced memory reactivation.
Contrary to our hypothesis, physostigmine did not suppress the memory-enhancing effect of odor-induced memory reactivation. Odor stimulation during SWS significantly improved memory retention, independently of whether subjects received placebo or physostigmine. We speculate that odor stimulation may trigger neocortical consolidation mechanisms, complementary to previously demonstrated hippocampal targets.

Disclosures: **J.G. Klinzing:** None. **B. Rasch:** None. **J. Born:** None. **S. Diekelmann:** None.

Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: DFG Grant TR-SFB 654 'Plasticity and Sleep'

Title: Sleep to abstract the gist: a long-term study on visual perceptual memories

Authors: ***N. D. LUZ**[1,2], S. DIEKELMANN[1], J. BORN[1,3], K. RAUSS[1];
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Abstract: Sleep is known to benefit the consolidation of individual episodic memories. In the long run, however, it may be more efficient to retain the abstract gist of single, related memories, which can be generalized to similar instances in the future. While episodic memory is enhanced after one sleep period, effective gist abstraction has been proposed to require multiple nights of sleep. Here we tested this hypothesis using a nonverbal version of the Deese-Roediger-McDermott paradigm. Subjects encoded 16 sets of 10 highly associated abstract shapes each at different times of day, once in the morning and once in the evening. The shapes within each set were derived from a single prototype (the 'gist' of the set), which was not shown during encoding. During retrieval, subjects were presented with three different stimulus types: previously encoded old shapes, previously unseen prototype shapes, and completely new shapes. Their task was to indicate which of the stimuli had been seen during encoding. We examined gist abstraction (i.e. the recollection of prototype shapes) and episodic memory (i.e. clear and vivid recollection of old shapes) after 20 min, after 10 hrs (including sleep or wakefulness), as well as after one year of retention. While after 10 hrs, sleep led to an enhancement of episodic memory for single items, it did not affect gist abstraction. One year later, however, we found significant gist knowledge only if subjects had slept the night after encoding, while there was no evidence of any residual memory for individual items. These findings indicate that sleep after initial encoding strengthens episodic memories in the short term and facilitates long-term gist abstraction.

Disclosures: **N.D. Luz:** None. **S. Diekelmann:** None. **J. Born:** None. **K. Rauss:** None.

Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: SB/SO/AS-19/2013

Title: Sleep deprivation negatively impacts reproductive output in *Drosophila melanogaster*

Authors: *S. POTDAR, D. DANIEL, F. A. THOMAS, S. CHIDAMBARAM, V. SHEEBA, S. LALL;
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Abstract: Sleep behaviour is ubiquitous in nature and while there are several hypotheses that explain its need in restorative functions at the synaptic, cellular and organismal levels, how sleep affects reproductive physiology is poorly understood. Several studies in the past have tried to link infertility among men and women to reduced quantity and quality of sleep. These types of studies have also been conducted in mammalian model systems, but beyond endocrinological factors, mechanistic neurobiological insights linking sleep and reproductive health have been lacking. In order to bridge this gap, we used the well-established system of fruit fly *Drosophila melanogaster*, whose genetic tool-kit, extensively studied neurobiology and physiology make it an attractive model organism. To this end, we subjected female fruit flies to chronic sleep deprivation using different methods and measured their total egg output per 24 hours for a period of 7 days. We found that, when flies are fed with caffeine for 6-7 days either during day-time or night-time their egg output is significantly reduced as compared to the control flies that did not receive caffeinated food. Furthermore, sleep loss during the night affects egg output more severely as compared to sleep loss during the day. Sleep loss does not appear to affect the quality of the eggs laid as pre-adult survivorship did not differ between control and sleep-deprived flies. Interestingly, flies carrying a loss-of-function mutation in dopamine transporter *fumin* (*fmn*), which have drastically reduced sleep as compared to their background controls, also lay significantly lower number of eggs compared to the control flies. Studies that test the effect of sleep deprivation using targeted activation of sleep and arousal circuits on egg output are underway. Thus, using several independent methods we show the negative impact of sleep deprivation on reproductive fitness of organisms, which can also explain why sleep may have evolved in the animal kingdom.

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Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: CRC-15-04-KIST

MH100820

Title: Repetitive sleep deprivation dysregulates the cortico-cortical connectivity of gamma and theta oscillation in REM sleep

Authors: *B. KIM[1,2], B. KOCSIS[3], E. HWANG[1], J. CHOI[1,2];
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Abstract: REM sleep reorganizes temporal connectivity in frontal region, decreasing gamma frequency coupling between frontal and other cortical areas. We recently observed that 5 days of repetitive partial sleep deprivation (SD1 - 5, 18 hours of SD per day) induced monotonic increase of gamma (30 - 40 Hz) oscillation in frontal cortex and a persistent increase of fast theta (8 - 10 Hz) oscillations in all the cortical regions in mice. Here, in order to address the functional role of these increased gamma and fast theta oscillations, we investigated the cortico-cortical connectivity of 38 channel EEG using phase synchronization index and applied network analysis to characterize the influence of sleep deprivation. We found that both theta and gamma network generators behaved differently in acute sleep deprivation (SD1) and in chronic sleep deprivation (SD3, SD5) conditions. For example, the strength of inter-cortical connectivity increased in both gamma and theta networks in SD1, but decreased as sleep deprivation continued. The breakdown of fronto-temporal network connectivity of both theta and gamma oscillations was conspicuous in SD5. On the other hand, the strength of intra-cortical connectivity of the gamma network within frontal cortex increased throughout the sleep deprivation days and this strengthened frontal gamma coupling was sustained even after cession of sleep deprivation. This biphasic behavior of cortical network in SD days may represent compensatory adjustments of neuronal connectivity in an acute sleep deprivation condition, which was not sustained in chronic sleep deprivation condition. Besides, the weakened coupling of gamma and theta oscillation between frontal and parietal/temporal/central cortex may suggest that the increased theta and gamma oscillation in chronic sleep deprivation do not contribute the global network synchronization rather are confined to local circuitry.

Disclosures: **B. Kim:** None. **B. Kocsis:** None. **E. Hwang:** None. **J. Choi:** None.

Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS087550

Title: Differential effect of sleep deprivation and behavioral sate on excitatory vs inhibitory neurons in CA1

Authors: *J. E. HEISS, A. M. THOMAS, T. S. KILDUFF;
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Abstract: The hippocampal formation has been proposed to be a specialized neural structure dedicated to the acquisition of memories during wakefulness that are later transferred to the cerebral cortex for long-term storage and consolidation during sleep. Therefore, hippocampal neuronal activity should be strongly modulated by behavioral state. However, when recorded outside their place field, most rodent hippocampal cells show <20% changes in firing rate across behavioral states. In contrast, different types of hippocampal interneurons exhibit heterogeneous behavioral state-dependent activity. Sleep deprivation (SD) impairs hippocampal-dependent memory but whether this involves an increase or a decrease in neuronal activity is not clear. One line of evidence suggests that during prolonged wake, synaptic strength and expression of AMPA receptors increases, which should increase overall neuronal activity. On the other hand, SD disrupts cAMP signaling, reduces NMDA-mediated currents and increases the levels of adenosine, which should reduce neuronal activity. In order to clarify how hippocampal neurons change their pattern of activity at different circadian times and in response to SD, we performed microendoscopic Ca^{2+} imaging of CA1 neurons in freely-behaving mice in combination with telemetric EEG/EMG recordings which enabled us to determine the activity of hundreds of cells simultaneously in conjunction with assessment of sleep/wake states in each mouse. Overall, we imaged thousands of excitatory neurons using AAV-Syn-GCaMP6f in WT mice and hundreds of inhibitory neurons using AAV-FLEX-GCaMP6f injected in Gad2-IRES-Cre mice. We found that the CA1 neuronal population is heterogeneous and different neuronal types can have opposite behaviors, rendering the common practice of averaging activity from all cells misleading. A majority of excitatory neurons showed reduced activity after 6h SD, while most inhibitory neurons showed the opposite, suggesting that SD can disrupt the excitation/inhibition balance in hippocampal networks. During a 1 h imaging period, clusters of neurons with strong behavioral state dependency were identified but the composition of these clusters changed during the next imaging period several hours later, although the number of neurons per cluster remained roughly constant. These results suggest a dynamic interaction between CA1 neurons, their

afferent inputs, and the neuromodulators affecting the activity of these cells across behavioral states, as well as differential effects of SD on excitatory vs inhibitory neurons.

Disclosures: **J.E. Heiss:** None. **A.M. Thomas:** None. **T.S. Kilduff:** None.

Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH MH101147

DA035805

DA029565

DA023206

DA034856

DA030379

the Pennsylvania Department of Health

Title: Prefrontal cortex to accumbens projections regulate reward seeking after sleep deprivation.

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Abstract: Sleep profoundly affects the emotional and motivational state. In humans and animals, loss of sleep often results in enhanced motivation for reward, which has direct implications for health risks as well as potential benefits. Current study aims at understanding the mechanisms underlying sleep deprivation (SD)-induced enhancement of reward seeking. Young adult mice (8 - 12 weeks old) were trained to self-administer sucrose pellet until a stable baseline was achieved. They then underwent acute SD for 6 hr during the first half of the light phase, during which they had full access to food and water. When tested immediately after SD, mice exhibited selective increase in sucrose self-administration but not food intake, suggesting enhanced motivation for reward. In the nucleus accumbens (NAc), a key brain region regulating emotional and motivational responses, we observed a decrease in the ratio of the overall excitatory over inhibitory synaptic inputs onto NAc principle neurons after SD. The shift was partly mediated by

reduced glutamatergic transmission of presynaptic origin. Further analysis revealed that there was selective reduction of the glutamate release probability at the medial prefrontal cortex (mPFC)-to-NAc synapses, but not those from the hippocampus, thalamus, or the basal lateral amygdala. To reverse this SD-induced synaptic alteration, we expressed the stabilized step function opsin (SSFO) in the mPFC; optogenetic stimulation of SSFO at mPFC-to-NAc projection terminals persistently enhanced the action potential-dependent glutamate release. Finally, intra-NAc optogenetic stimulation of SSFO selectively at mPFC-to-NAc terminals restored normal sucrose seeking in mice with SD, without affecting food intake. Our results highlight the mPFC-to-NAc projection as a key circuit-based target for sleep to regulate reward-motivated behaviors.
Key words: sleep, reward, mPFC, accumbens, self-administration, SSFO.

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Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Support: CIHR (MOP-130502)

Title: Gaba and muscarinic receptors on motor trigeminal neurons are homeostatically regulated with sleep deprivation

Authors: *H. TOOSSI, E. DEL CID-PELLITERO, B. JONES;
McGill Univ., Montreal, QC, Canada

Abstract: Muscle tone is regulated across sleep-wake states, being maximal in waking, reduced in slow-wave sleep (SWS) and absent in paradoxical or REM sleep (PS or REM). Such changes in tone are evident in the masseter muscles and shown to correspond to changes in activity and polarization of the motor trigeminal (Mo5) neurons, which are disfacilitated during SWS and hyperpolarized and inhibited during REM sleep (Nakamura et al., 1978). This inhibition during REM sleep depends upon GABAergic and cholinergic input upon both $GABA_A$ and $GABA_B$ and also acetylcholine muscarinic 2 (AChM2) receptors (Rs) (Brooks and Peever 2012; Grace et al., 2013). In the present study, we examined by immunohistochemistry in mice whether $GABA_A$, $GABA_B$ and AChM2 Rs are present on Mo5 neurons and might undergo homeostatic changes as a function of the neurons' activity during sleep deprivation (SD) during the day when mice

normally sleep the majority of the time. Applying quantitative stereological image analysis of dual immunofluorescent stained sections, we determined that the proportion of Mo5 neurons positively immunostained for $GABA_A$Rs was significantly higher following SD (~65 %) as compared to sleep control (SC, ~32 %) and sleep recovery (SR, ~47 %). The luminance of the $GABA_A$Rs was also significantly higher in SD as compared to SC and SR. Although, $GABA_B$Rs and AChM2Rs were observed in all Mo5 neurons (100%), the intensity of these receptors was also significantly higher following SD as compared to SC and SR. We conclude that the density of $GABA_A$, $GABA_B$ and AChM2 receptors significantly increases on Mo5 neurons as a function of and homeostatic adjustment to their prolonged activity during SD.

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Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Title: The role of nuclear peroxisome proliferator activated receptor alpha (PPAR) in sleep recovery after sleep deprivation in rats

Authors: *A. SARRO-RAMIREZ[1], G. ARANKOWSKY-SANDOVAL[2], E. MURILLO-RODRÍGUEZ[3], K. GUZMÁN[4];
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Abstract: The peroxisome proliferator-activated receptor alpha PPAR is a member of the nuclear receptor superfamily that has been suggested as a modulator of several physiological functions. The PPARα recognizes as an endogenous ligand the anorexic lipid mediator oleoylethanolamide which displays wake-inducing properties. Despite that recent evidence indicates that activation of PPARα by synthetic agonists such as Wy14643 enhances waking as well as the extracellular contents of wake-related neurotransmitters. The aim of this study was to characterize if PPARα regulates sleep rebound after total sleep deprivation. We report that after 6h of TSD activation of PPAR by pharmacological systemic administration of OEA, promoted alertness by blocking the sleep rebound after TSD. Besides, wake-linked compounds such as dopamine, norepinephrine, serotonin, or adenosine collected from nucleus accumbens were

enhanced after TSD in OEA-treated animals. These sleep and neurochemical results were mimicked after injection of PPARα agonist Wy14643. Opposite findings were observed if PPARα antagonist MK-886 was administered to rats. Our results strengthened the hypothesis that PPARα might modulate sleep and neurochemical homeostasis after sleep deprivation.

Disclosures: **A. Sarro-Ramirez:** None. **G. Arankowsky-Sandoval:** None. **E. Murillo-Rodríguez:** None. **K. Guzmán:** None.

Poster

342. Sleep Behavior and Systems II

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Program#/Poster#: 342.15/XX8

Topic: F.08. Biological Rhythms and Sleep

Title: Spike timing rigidity is maintained in bursting neurons under pentobarbital-induced anesthetic conditions

Authors: R. KATO[1], M. YAMANAKA[2], *M. KOBAYASHI[1];
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Abstract: Pentobarbital potentiates γ-aminobutyric acid (GABA)-mediated inhibitory synaptic transmission by prolonging the open time of $GABA_A$ receptors. In contrast, it is unknown how pentobarbital modulates the activities of cortical local circuits *in vivo*. We performed extracellular unit recording from rat insular cortical neurons under both awake and anesthetic conditions. Spike firing was analyzed by random matrix theory (RMT), which enables us to distinguish whether events involve regularity. RMT has been applied not only in nuclear physics but also in statistical and multivariate analyses in many research fields, including biophysics. An experimentally recorded spike train is converted to the spike train of the average interval one at any local time by the unfolding transformation. This procedure removes the system-specific average spike density. Unfolding transformation defines an average spike interval locally in time and enables the comparison of different activity states on a universal scale. Neurons with high spontaneous firing frequency (> 5 Hz) and bursting were classified as HFB neurons (n = 10), and those with low spontaneous firing frequency (< 10 Hz) and without bursting were classified as non-HFB neurons (n = 48). Most of HFB neurons have shorter spike width than non-HFB neurons. Systemic injection of pentobarbital (30 mg/kg) reduced firing frequency in all HFB neurons and in 78% of non-HFB neurons. RMT analysis demonstrated that both HFB and non-HFB neurons showed an increase in the number of neurons with repulsion by pentobarbital. Under awake conditions, in 50% of HFB and 40% of non-HFB neurons, the decay phases of

normalized histograms of spontaneous firing were fitted to an exponential function, which indicated that the first spike had no correlation with subsequent spikes. In contrast, under pentobarbital-induced anesthesia conditions, the number of non-HFB neurons that were fitted to an exponential function increased to 85%, but almost no change in HFB neurons was observed. These results suggest that under both awake and pentobarbital-induced anesthetized conditions, spike firing in HFB neurons is more robustly regulated by preceding spikes than by non-HFB neurons, which may reflect the $GABA_A$ receptor-mediated regulation of cortical activities.

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Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

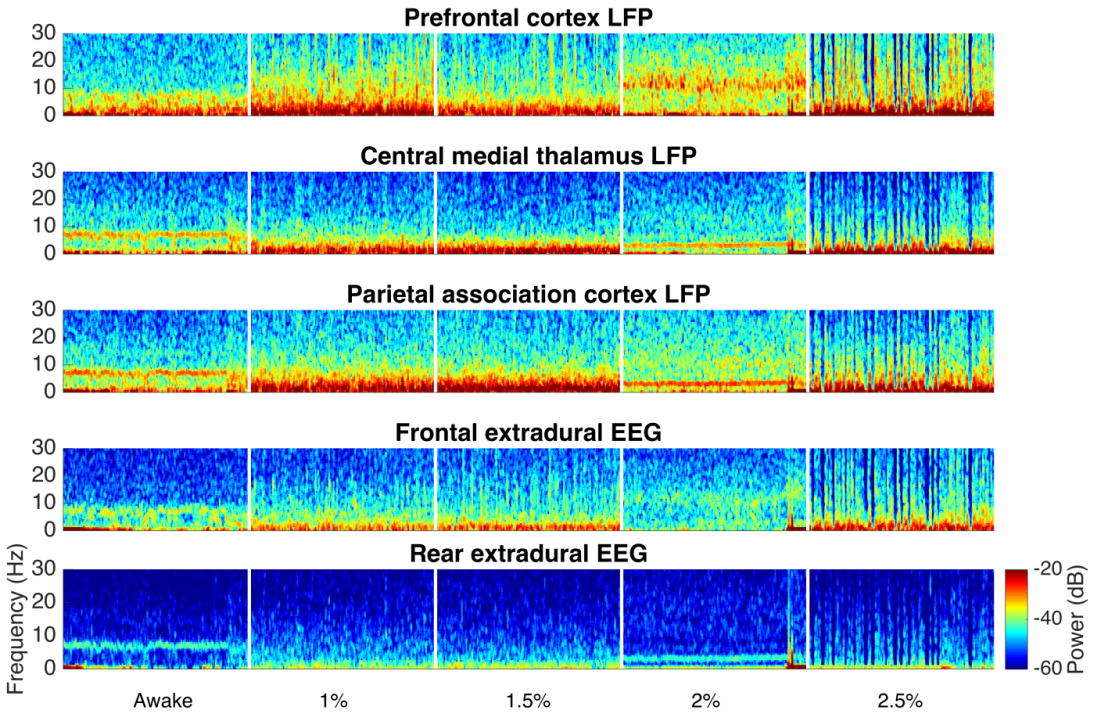
Support: NIH TR01-GM104948

Title: Multi-site intracranial recordings in rats under propofol and sevoflurane anesthesia

Authors: ***J. A. GUIDERA**[1], N. E. TAYLOR[2], J. T. LEE[3], K. Y. VLASOV[3], J. PEI[3], E. N. BROWN[2], K. SOLT[2];
[1]Anesthesia, Massachusetts Gen. Hosp., Cambridge, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]MIT, Cambridge, MA

Abstract: INTRODUCTION: Sevoflurane and propofol are general anesthetics that induce characteristic frontal EEG oscillations in humans, including alpha (8 - 12 Hz) oscillations. In rodents, however, alpha oscillations are typically absent in extradural EEG recordings. This may be due to fundamental differences in the brains of rodents and humans, or the large differences in size and location of the frontal cortex in the two species. In this study we conducted multi-site intracranial LFP recordings and extradural EEG recordings in rats under general anesthesia, to test the hypothesis that sevoflurane and propofol induce similar brain oscillations in rodents and humans. **METHODS:** Three anesthetized male Sprague-Dawley rats were implanted with extradural skull screws and intracranial electrodes stereotaxically targeted to the prefrontal cortex, parietal association cortex, and central medial thalamus. In a separate surgery, central venous catheters were placed in the femoral vein. After full recovery from surgery, the rats were placed in an anesthetizing chamber and EEG/LFPs were recorded as they inhaled progressively higher doses of sevoflurane (0%, 1%, 1.5%, 2% and 2.5%), or received an anesthetic dose of propofol (>= 5 $mg \cdot kg^{-1} min^{-1}$ iv). Spectrograms were computed from the recordings. **RESULTS:**

At 2.0% sevoflurane, delta oscillations were prominent in all recordings. Although alpha oscillations were present in the frontal extradural EEG, they were far more robust in the LFP recordings from prefrontal cortex. Similar results were obtained with propofol anesthesia. **CONCLUSIONS:** At anesthetic doses of sevoflurane and propofol, the frontal alpha oscillations observed in human frontal EEG recordings are present in LFP recordings from rat prefrontal cortex. These results suggest that rodents provide an adequate model to study human brain oscillations under sevoflurane and propofol anesthesia. Simultaneous neurophysiological recordings from multiple brain sites in rodents may allow us to better understand how and where anesthetic-induced brain oscillations are generated in humans.



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Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.17/XX10

Topic: F.08. Biological Rhythms and Sleep

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Swartz Foundation

Howard Hughes Medical Institute

Title: Nonlinear spatiotemporal analysis of sleep spindles

Authors: *A. L. SAMPSON[1,2], C. LAINSCSEK[1,2], S. S. CASH[3,4], E. HALGREN[2], T. J. SEJNOWSKI[1,2];
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Abstract: Sleep spindles are discrete pulses of 11-17 Hz oscillations recorded in the electroencephalogram (EEG) during stage 2 non-REM sleep. Since they are believed to arise from the activity of thalamocortical circuitry, spindles have become a subject of study for their potential roles in memory consolidation and other cognitive functions. Disrupted or abnormal spindle activity has also been associated with certain psychiatric and neurological disorders. Delay differential analysis (DDA) has proven effective as a tool for detecting sleep spindles. DDA is a time domain classification framework based on embedding theory in nonlinear dynamics. An embedding reveals the nonlinear invariant properties of a dynamical system from a single time series. In DDA, we combine a delay embedding with a differential embedding by relating delayed versions of the original time series to its derivative in a polynomial function. The coefficients of the terms of this model, as well as the least-squares error provide a low-dimensional nonlinear functional basis onto which the data can be mapped. By building this basis on the dynamical structure of the data we eliminate the need for preprocessing of the data, and the low number of features greatly reduces the risk of overfitting. A DDA model that was trained on a single channel from one subject was applied to a wide range of data from different subjects, channels, and recording systems.
Here we apply DDA analyses to stereoelectroencephalogram (SEEG) and electrocorticogram (ECoG) recordings from patients with intractable epilepsy. For a three-term model, we selected one set of delays for each sampling rate and applied the model to 15 test recordings from eight subjects. The mean area under the receiver operating characteristic (ROC) curve across these recordings was 0.84. Using the same model, we examine the timecourses of the features across the spatial grid of channels during the detected spindles. This allows insight into the degree of synchrony between brain regions during spindles. Of particular interest are the ECoG recordings, which provide broad coverage of the cortical surface, allowing for the study of the relative strength and timing of spindle activity across a large area. We find that peak spindle intensity occurs at different times in the frontal, parietal, and temporal cortices, showing oscillation among brain regions.

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Poster

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NSF Graduate Research Fellowship

Title: Theta oscillations, not slow spindles, precede down state troughs

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Abstract: Down states are periods of neuronal quiescence during non-REM sleep N2-3, and are known to group faster oscillations such as sleep spindles. This phenomenon was first demonstrated in vivo in anesthetized animals and was later shown non-invasively in humans using EEG. Previous work has suggested slow and fast spindles occur on different phases of the slow oscillation, with slow spindles occurring just prior to the trough of the down state. In this study, we further characterize the timing of spindles relative to cortical down states using bipolar stereoencephalography (sEEG) recorded from patients with intractable epilepsy.
Event-related histograms for each channel indicated that both slow (> 10 and ≤ 12 Hz) and fast (> 12 and < 16 Hz) spindles occur after the down state trough. However, time-frequency plots averaged across all down states revealed an increase in power in the 5-12 Hz range just prior to the trough. To determine whether this power was due to oscillations or the steep drop in the local field potential (LFP), we averaged the raw LFP in the top 25% of down states with increased theta power (5-8Hz) just prior to down state trough. These raw recordings were locked to the first peak preceding the down state trough and revealed discernable theta oscillations lasting about 500 ms. Interestingly, the magnitude of these oscillations was more pronounced for N2 than for N3.

These findings demonstrate both slow and fast spindles occur after the trough of the down state, but that some down states exhibit a short oscillation in the theta range just prior to their trough. Whether these oscillations have the same generators as slow or fast spindles, or whether they are simply very slow spindles, remains to be seen. Functionally, non-REM sleep is believed to be important for memory consolidation, however the mechanisms by which this occurs are unknown. In addition to improving our understanding of sleep dynamics, the relative timing of these rhythms also provides important constraints for mechanisms of sleep-dependent memory consolidation.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant MH60670

Department of Anesthesiology, University of Michigan Medical School

Title: Hidden subcortical sleep

Authors: *B. A. GROSS[1], J. J. EMRICK[2], B. T. RILEY[3], G. R. POE[1];
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Abstract: Researchers determine sleep states by measuring brain activity from a single site, assuming that sleep states occur at the same time throughout the brain. The assumption that the whole brain exhibits sleep simultaneously is fundamental to experiments, e.g. depriving animals of sleep. We sought to determine if sleep state scores can differ between two separate structures: the neocortex and the hippocampus.
We measured electrical signals (electroencephalograms and electromyograms) during sleep from the neocortex and hippocampus of five freely-behaving adult male rats. We assigned sleep-waking states in 10-second epochs to a 4h recording from each structure based on standard scoring criteria, then compared and analyzed simultaneous states and signals at each site.
We found that the total amount of each state was similar between the hippocampus and neocortex, but that sleep states at simultaneous epochs, assessed separately from the

hippocampal and neocortical signals, were scored as different as often as the same ($p = 0.82$). Furthermore, we found that the progression of states often flows through asynchronous state pairs. For example, the hippocampus progressed from transition-to-REM to REM before the neocortex 39% of the time whereas they progressed in synchrony 16% of the time.
We demonstrate that neocortical and hippocampal sleep-waking states can differ in the same epoch. Electrode location thus impacts estimations of sleep architecture, state transition timing, and in sleep manipulation experiments, percent time in sleep states. Therefore, models assuming brain state homogeneity should not be applied to the sleeping or waking brain under normal conditions.

Disclosures: **B.A. Gross:** None. **J.J. Emrick:** None. **B.T. Riley:** None. **G.R. Poe:** None.

Poster

342. Sleep Behavior and Systems II

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Program#/Poster#: 342.20/XX13

Topic: F.08. Biological Rhythms and Sleep

Title: Chronic immobilization stress modify sleep pattern and several metabolic parameters in both stress and recovery period in rats

Authors: *A. JIMENEZ-ANGUIANO[1], A. L. GUZMAN-GUZMAN[1], A. I. GOMEZ-MORALES[1], A. K. LEON-OLGUIN[1], G. BLANCAS-FLORES[2], J. VELAZQUEZ-MOCTEZUMA[1];
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Abstract: Stress produces several physiological changes in the organisms. It has been shown that acute stress induced an important increase in REM sleep and modify the energy balance. However, the effect of chronic stress in sleep and metabolic parameters (MP) during both stress and recovery period (RP) has not been completely studied. To determine the effect of chronic stress on sleep pattern and several MP, we used 18 male Wistar rats (250-300 g). Electrodes were implanted for conventional sleep recordings and we quantified the levels of cholesterol, triglycerides, uric acid, glucose, and several transaminases. Animals were grouped under the following conditions: 1) Control, without stress and with water and food *ad libitum* (n=6), 2) Immobilization Stress (IS) for 10 days during the first 2 hrs of darkness period (n=6). 3) Control of IS with no stress, water and food during the time of the SI (n=6). At the beginning, in the first day and every third day inmmediately after the IS we realized polysomnographic recordings during eigth hours and measured body weight (BW) and MP. After the period of IS we continued

the measurements of sleep and MP each week for a month. IS during the first and third days increased Slow Wave Sleep II and REM sleep and reduced the latency of sleep in relation to control groups. During the RP of the IS, the duration of sleep cycles were reduced. BW decreased in animals with IS and increased during the RP. In the MP, the IS tend to decrease glucose levels and increased the levels of many transaminases. From the obtained results we suggest there is an important effect of stress on sleep and metabolic regulation during both, stress and RP.

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Poster

342. Sleep Behavior and Systems II

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the Department of Veterans Affairs

Title: Sleep and sleep-related motor activity in female rats

Authors: Y.-H. CHENG[1], K.-C. HSIEH[2], L. RAMANATHAM[1], J. M. SIEGEL[3], *Y.-Y. LAI[4]; [1]Psychiatry, UCLA, Los Angeles, CA; [2]VAGLAHS Sepulveda, Los Angeles, CA; [3]UCLA/VAGLAHS Sepulveda, Los Angeles, CA; [4]Psychiatry, UCLA/VAGLAHS Sepulveda, North Hills, CA

Abstract: Restless legs syndrome (RLS) and periodic leg movement (PLM) disorders occur across all ages and affect 5 -10% of the population. RLS has been reported to be a major cause of insomnia and is one of the most common movement disorders. Despite the fact that women constitute 70% of RLS patients, studies using female animals have not been reported. In this study, we used 4 male and 3 female young adult Sprague-Dawley rats, weighing 260-300 g, to address the question whether gender plays a role in the generation of RLS in rats. Rats were surgically implanted with electrodes for EEG and EMG (neck and hindlimb) recordings. Baseline sleep and motor activity recordings were performed for 3 and 12 days for male and female rats, respectively. Thus, 3-day baseline recordings were obtained each phase (proestrus,

estrus, metestrus, and diestrus) of the estrus cycle in female rats. We found that the hematocrit level in female rats (43.6 ±1.2) is significantly lower than male rats (47.1 ± 0.1). Sleep patterns during the light phase, dark phase or over 24 hours did not differ between male and female rats ($p=0.78$, ANOVA), with the exception of a decrease in rapid eye movement (REM) sleep during the dark phase of the proestrus stage in female rats ($p<0.01$, post-hoc). Periodic leg movements in quiet wake were not observed in male rats, but were seen in female rats. Periodic leg movements in slow wave sleep (SWS) were significantly higher in female rats (26.8 ± 7.6) than in male rats (5.3 ± 3.8), over 24 hours recording ($p<0.001$, df=5, ANOVA). Statistical differences in PLM in sleep were not found across the estrus phases ($p=0.67$, ANOVA) in female rats. Isolated leg movements in sleep were not different between male and female rats ($p=0.78$). Systemic injection of pramipexole (PR, 0.01 or 0.02 mg/kg) had no effect on sleep in female rats (ANOVA, $p>0.3$), but decreased PLM in sleep in 2 female rats, whereas, the third animal did not show a response to PR injection. We conclude that lower hematocrit levels in female rats may contribute to the generation of RLS.

Disclosures: Y. Cheng: None. **K. Hsieh:** None. **L. Ramanatham:** None. **J.M. Siegel:** None. **Y. Lai:** None.

Poster

342. Sleep Behavior and Systems II

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Grant-in-Aid for Scientific Research (C) (26380987) to S.C

Title: Short time running exercise enhances sleep pressure in mice

Authors: *N. SHIMIZU[1], Y. YOSHIOKA[2], S. CHIKAHISA[1], Y. KITO[1], T. SHIUCHI[1], H. SEI[1];
[1]Dept. of Integrative Physiology, Inst. of Biomed. Sci., Tokushima Univ. Grad. Sch., Tokushima, Japan; [2]Student lab, Tokushima Univ. faculty of medicine, Tokushima, Japan

Abstract: Many studies suggest the sleep regulation would be affected by metabolic change. However, those mechanisms are still unclear. In previous study, we have reported a possibility that ketone body metabolism contribute to the regulation of sleep homeostasis (Chikahisa S et al., *Neuropharmacology,* 2014). On the basis of this research, we hypothesized that short time

running exercise may induce an enhancement of ketone body metabolism and then influence the sleep regulation.
Male ICR mice (aged 10 weeks) were subjected to running exercise for 0 (control), 15, 30, or 60 minutes by using treadmill. A start time of exercise was selected in beginning at light phase. Running speed was set at 100 rpm/min, which does not enhance a lactate release in each running time. To eliminate an effect of sleep loss, all mice were deprived sleep during non-running phase. Total forced awaking time (running + sleep deprivation) was unified in 75 min. After the running, we performed the sleep recording.
Mice exercised for 30 and 60 minutes showed an enhancement of slow-wave activity (SWA) during non-rapid eye movement (NREM) sleep which is used as the index of sleep pressure compared with control mice. Furthermore, mice exercised for 30 and 60 minutes were also observed not only an increase in the amount of NREM sleep but also shortening of sleep latency. Ketone bodies of plasma were increased in mice exercised for 30 and 60 minutes. mRNA expression of 3-hydroxy-3-methylglutaryl-CoA synthase 2, a limiting enzyme of ketone bodies production, in liver and cortex were enhanced. These findings indicate that short time exercise make sleep deeper depending on running time, which may be related to enhancement of ketone body metabolism.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: Department of Veterans Affairs

Title: Chronic inflammation of the preoptic-hypothalamic sleep-regulatory systems contributes to changes in sleep-wake organization in aging.

Authors: A. KOSTIN, A. ALAM, J. GERA, R. SZYMUSIAK, D. MCGINTY, *N. ALAM; VA Greater Los Angeles Healthcare Syst., North Hills, CA

Abstract: INTRODUCTION: Both animal and human studies indicate that the brains of older subjects are in a heightened inflammatory state, even in the absence of overt disease. Evidence also suggests that chronic inflammatory processes play a role in cellular aging including neuronal aging. Chronic sleep disturbance, including frequent awakenings during sleep,

decreases in nonREM and REM sleep amounts and nonREM slow wave activity, is another hallmark of aging. We examined the hypothesis that chronic inflammation of sleep-regulatory preoptic-hypothalamic median preoptic nucleus (MnPN) and ventrolateral preoptic area (VLPO) contributes to changes in sleep-wake organization in aging. METHOD: Experiment-1: Tissue samples were collected from MnPN and VLPO of 3 months (n=2) and 24 months old (n=2) male Fisher-344 rats and analyzed for IL-6 and TNFα mRNA using qRT-PCR and proteins using Western blot. Experiment-2: The sleep-wake profiles of rats were recorded during baseline and during chronic infusion of artificial cerebrospinal fluid (aCSF) or lipopolysaccharide (LPS; 0.11µg/h) into the MnPN (n=2) or VLPO (n=2) using an Alzet mini-osmotic pump for 2 weeks. RESULTS: Compared to young rats, both mRNA and protein levels of TNFα and IL-6 were elevated >3-fold in both the MnPN and VLPO of old rats. Five days of LPS infusion into the MnPN of young rats decreased both nonREM (54 ± 3% vs. 40 ± 4%) and REM sleep (11 ± 2% vs. 5 ± 1%) amounts and increased sleep fragmentation as marked by increase in number of awakenings and more frequent and shorter sleep bouts during lights-on phase. On the other hand, during the dark-phase, waking was decreased (77 ± 1% vs. 62 ± 8%) and sleep intrusions were increased. These effects persisted during the remainder of LPS treatment. Chronic LPS infusion into the VLPO produced similar changes in sleep-wake organization. CONCLUSION: The sleep-regulatory MnPN and VLPO in old rats exhibit signs of chronic inflammation as evident by elevated basal levels of inflammatory molecules. LPS-induced chronic inflammation of the MnPN and VLPO in young rats produced sleep-wake features that are similar to those observed in aging. Taken together, these preliminary findings suggest that chronic inflammation of sleep-regulatory MnPN and VLPO potentially contributes to sleep disturbance that accompany aging.

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Poster

342. Sleep Behavior and Systems II

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Topic: F.08. Biological Rhythms and Sleep

Title: Characterizing sleep, circadian rhythms, and eye closure in Acomys cahirinus (Cairo spiny mouse) using EEG, EMG, piezoelectric sensors, and video

Authors: *L. E. GUERRIERO[1], C. WANG[1], T. C. BROOKS[1], A. A. AJWAD[2], S. SUNDERAM[2], A. W. SEIFERT[1], B. F. O'HARA[1];
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Abstract: To better understand the functions and origins of sleep, sleep should be studied across many different species. Although it is well conserved throughout mammals, more than 99% of publications focused on sleep in mammals are done in only three species: humans, the house mouse (*Mus musculus*), and the Norway rat (*Rattus norvegicus*). We aim to characterize sleep and wake in *Acomys cahirinus,* the Cairo spiny mouse. These spiny mice, native to Northern Africa, are larger and live longer than *Mus musculus*. Previous work, by Wang *et al. (see companion poster),* have shown that *A. cahirinus* and *M. musculus* have relatively similar sleep and wake profiles, but with a few interesting differences. In order to truly understand these differences in sleep architecture of *A. cahirinus*, electroencephalogram (EEG) recordings were done. These recordings have been extensively gathered from *M. musculus* and are important for discerning nonREM from REM sleep, and a variety of other measures such as spectral power in different frequency bins, especially EEG delta power during nonREM sleep. For each recording, animals were placed in single cages and set up with a tethered EEG and EMG headmount. During EEG recordings, the mice were also monitored using a well validated, non-invasive, piezoelectric system, that picks up breathing rhythms to determine sleep or wake. By combining EEG, EMG, Piezo data, and IR (infrared) camera recordings, sleep status can be determined with greater accuracy and with additional variables such as breathing. Current data show that *A. cahirinus* have a few major differences in sleep from *M. musculus*. *A. cahirinus* have a significantly longer daily sleep and have a higher amount of REM sleep. Other differences from *M. musculus* sleep structure are seen during the night, the active time of both of these nocturnal rodents. *A. cahirinus* are awake at dark onset, but appear to sleep more than *M. musculus* after the middle of the night. Most strikingly, it was noticed that *A. cahirinus* do not close their eyes during sleep, during both the light and dark periods. This raises further questions about *A. cahirinus* sleep architecture, adaptations, and evolution, and why it differs from *M. musculus.*

Disclosures: **L.E. Guerriero:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions. **C. Wang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions. **T.C. Brooks:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions. **A.A. Ajwad:** None. **S. Sunderam:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions. **A.W. Seifert:** None. **B.F. O'Hara:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions.

Poster

342. Sleep Behavior and Systems II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 342.25/XX18

Topic: F.08. Biological Rhythms and Sleep

Title: A comparative study of sleep and circadian rhythms between the house mouse (Mus musculus) and African spiny mouse (Acomys cahirinus)

Authors: *C. WANG[1], T. C. BROOKS[1], L. E. GUERRIERO[1], A. A. AJWAD[2], S. SUNDERAM[2], A. W. SEIFERT[1], B. F. O'HARA[1];
[1]Dept. of Biol., [2]Dept. of Biomed. Engin., Univ. of Kentucky, Lexington, KY

Abstract: The study of sleep and circadian behavior in different organisms can provide valuable insight for understanding behavioral, physiological and environmental influences on these processes. Interestingly, two species of African spiny mice, *Acomys russatus* (Golden spiny mouse) and *Acomys cahirinus* (Cairo spiny mouse) have been reported to exhibit different circadian rhythm patterns in locations where the two species overlap. Both species are primarily nocturnal in different habitats, but in areas of overlap *A. cahirinus* exhibit nocturnal behavior, while *A. russatus* becomes more diurnal. However, very few studies on the circadian activity of these species are available and nothing is known of their sleep behavior, which can be the dominant force in driving other diurnal variables. Therefore, we have begun to study one of these species (*A. cahirinus*) in greater detail alongside the well-studied house mouse (*Mus musculus)* using a well validated, non-invasive, piezoelectric system, that picks up all movements during wake, and the breathing rhythms during sleep. In these studies, we found *A. cahirinus* and *M. musculus* to be primarily nocturnal, but with clearly distinct behavioral patterns. Specifically, the activity of *A. cahirinus* sharply increases right at dark onset, which is common in nocturnal species, but surprisingly, decreases sharply just one hour later. These differences may be related to foraging differences between these species, or may be related to the socialized behavior of *A. cahirinus* and its poorer adaptation to isolation as compared to *M. musculus*. In order to confirm the exact sleep patterns of *A. cahirinus* in the original cage and experimental cage, we set up four IR cameras surrounding the cage to record activity and electroencephalogram (EEG) recording. With IR camera recording in the single and group cage conditions, we found that *A. cahirinus* is more active before the middle of the night period than after middle of the night period in both single and group cages, and this decreased activity in the latter half of the night is much greater than *M. musculus*. With an EEG recording approach, we confirmed that after middle of the night time period, wake percentage is less than before the middle of the night time period. During IR camera recording observations, we also found that *A. cahirinus* do not close their eyes during sleep periods of the day or night. Eye closure and sleep has not been systematically studied across mammals, but is clearly a rare behavior, that we will investigate further.

Disclosures: **C. Wang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **T.C. Brooks:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **L.E. Guerriero:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **A.A. Ajwad:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **S. Sunderam:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **A.W. Seifert:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc. **B.F. O'Hara:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solution Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solution Inc..

Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.01/XX19

Topic: F.08. Biological Rhythms and Sleep

Title: mGluR1-PLCβ4 signal is critical for sleep architecture

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

Abstract: Sleep is composed of multiple states including non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep which have characteristic electroencephalogram (EEG), respectively. REM sleep displays low amplitude EEG pattern in theta frequency range (4-9Hz) and NREM sleep displays high amplitude and multiple EEG patterns composed of slow wave (<0.5 Hz), delta wave (0.5-4 Hz) and sleep spindle (10-15 Hz) by the frequency. Especially, the brain rhythms at transition from wake to NREM sleep show the dramatic change from irregular and low amplitude pattern to relatively regular oscillatory and large amplitude pattern reflecting the synchronized thalamocortical oscillatory activity. Thalamocortical oscillations have been described to be initiated from intra-thalamic circuit composed of GABAergic thalamic reticular nucleus (TRN) neurons and glutamatergic thalamic cortical (TC) neurons and then to be transmitted to cortical neurons. Remarkably, mGluR1 found on TC relay cells, postsynaptic to axon terminals from cortical layer 6 could regulate the cortico-thalamic input. Here we investigated the role of thalamic mGluR1- phospholipase C β4 (PLCβ4) pathway

in controlling sleep architecture using PLCβ4-deficient mice. PLCβ4$^{-/-}$ mice displayed the increased NREM and total sleep amount with altered brain rhythm during sleep. Especially, delta power density during NREM sleep was increased, parallel with enhanced intra-thalamic oscillations. These results indicate a crucial role of TC neurons in the generation of delta waves and stabilization of NREM sleep state.

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Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant RO1HL116845

Title: Microinjections of carbachol into dorsomedial pons elicit REM sleep in naturally sleeping rats

Authors: ***V. B. FENIK**[1,2], N. J. CARBALLO[1,3], I. RUKHADZE[1,3];
[1]VA Greater Los Angeles Healthcare Syst., Los Angeles, CA; [2]WebSciences Intl., Los Angeles, CA; [3]Med., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Important data for understanding neurochemical mechanisms of REM sleep-related atonia of pharyngeal muscles have been obtained using pontine microinjections of carbachol, a cholinergic agonist, to elicit REM sleep-like state in anesthetized rats (Fenik et al., 2005). However, it was reported that carbachol applied iontophoretically into sublaterodorsal (SLD) nucleus in naturally sleeping rats triggers wakefulness rather than REM sleep (Boissard et al., 2002). We sought to determine effects of carbachol microinjected in naturally sleeping rats using the same approach, volume and concentration used in anesthetized rat studies.
Sprague-Dawley rats were implanted for chronic recording of EEG and neck EMG. Experiments were conducted in head-restrained rats after they were fully adapted and displayed normal sleep-wake patterns. In each rat, single microinjections of carbachol (10 mM, 10 nl) were placed into pontine reticular formation using glass pipettes. Pontamine Skye Blue (2%) was added to the carbachol solution to mark injection sites. The carbachol solution was pressure injected at 2-4 PM while the injection volume was monitored using a pocket microscope.
Carbachol microinjections that were placed into the dorsomedial pons at AP-8.72 (n=2) elicited REM sleep. The duration of these carbachol-induced REM sleep bouts (2.4 and 2.8 min) and the

EEG frequencies, which were verified by the spectral power analysis, were identical to naturally occurring REM sleep state. The latencies of the responses (66 and 31 s) corresponded to the distances between carbachol injection sites and the SLD nucleus (0.8 and 0.64 mm, respectively). These distances tended to be larger in our naturally sleeping rats than in anesthetized rats, which might be due to expected impaired sensitivity of the latter. Carbachol that was injected more ventral and caudal (1.2 mm from SLD nucleus at AP-8.84, n=1) triggered wakefulness with a latency of 1.5 s and duration of 18 min. This carbachol response was similar to carbachol activating effects at ventral pontine sites in anesthetized rats.
Our findings suggest that microinjections of carbachol into pontine reticular formation produce similar effects in both anesthetized and naturally sleeping rats. These results contradict the absence of the responses to carbachol that was applied iontophoretically into the SLD nucleus in head-restrained rats (Boissard et al., 2002), probably because carbachol may cover a larger area of reticular formation during microinjections as compared to the iontophoretic ejection.

Disclosures: **V.B. Fenik:** None. **N.J. Carballo:** None. **I. Rukhadze:** None.

Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.03/XX21

Topic: F.08. Biological Rhythms and Sleep

Title: Effects of breath-holding on subjective drowsiness

Authors: T. KIKUCHI[1], M. TAKAYOSE[1], *R. KOSHIZAWA[2], K. ARAI[1], K. FUJIMOTO[3], Y. SANO[3], K. SHIROISHI[4], N. GYODA[5];
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Abstract: [Background]Drowsiness is often induced by monotonous work. About 10% of ship accidents occur as a result of operator dozing, causing serious accidents, including death. Therefore, the development of drowsiness mitigation methods that can be easily carried out during work is significant. In this study, as a first step to investigate whether breath-holding can reduce or prevent drowsiness, we examined the effects of breath-holding on the degree of drowsiness reported by university students. [Method]Students attending classes on "Physiology" at the university were recruited to investigate the degree of subjective drowsiness at the time of its occurrence and after breath-holding. The degree of subjective drowsiness was measured using a Visual Analog Scale (below, VAS). Breath-holding was performed up to 90 percent of the

subjects' subjective limits. Participation in the survey was optional, and valid responses were obtained from 327 students (19.62 ± 1.52 years). Subjects were classified into three groups, according to the size of the VAS value at the time of drowsiness: Low (0.0-3.3); Middle (3.4-6.6); and High (6.7-10.0). [Result]VAS values were significantly reduced after breath-holding (High: 8.14 → 5.27, $p<0.001$; Middle: 5.46 → 3.51, $p<0.001$; Low: 2.11 → 1.57, $p<0.05$). In all three groups, the correlations between duration of breath-holding and the VAS value were not significant. In addition, no statistically significant differences were noted among the three groups in the duration of the breath-hold. The relative decreases in the VAS values in High (35.3%) and Middle (35.7%) were significantly greater than Low (25.6%), ($p<0.01$). No significant difference was observed between High and Middle. [Conclusion]Breath-holding was shown to reduce subjective drowsiness. Results also suggest that the magnitude of reduction is greater when VAS values are higher (greater degree of drowsiness). In addition, the drowsiness reduction was observed even when the degree of drowsiness was small, suggesting that breath-holding may be effective in drowsiness prevention. In the future, we plan to investigate the relationship of breath-holding and physiological indicators such as brain waves and pulse waves.

Disclosures: **T. Kikuchi:** None. **M. Takayose:** None. **R. Koshizawa:** None. **K. Arai:** None. **K. Fujimoto:** None. **Y. Sano:** None. **K. Shiroishi:** None. **N. Gyoda:** None.

Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: MEXT 15H05935

JSPS FIRST Program

Uehara Memorial Foundation research grant

Takeda Science Foundation Research Grant

Title: Forward genetics approach in identification of novel sleep/wakefulness related gene(s).

Authors: ***S. J. KIM**[1,2], T. FUJIYAMA[2], C. MIYOSHI[2], N. HOTTA[2], S. KANNO[2], A. IKKYU[2], M. KAKIZAKI[2], T. MATSUOKA[2], S. MIZUNO[3], I. MIURA[4], T. SUZUKI[4], K. VIVEK[5], J. S. TAKAHASHI[5], S. TAKAHASHI[3], S. WAKANA[4], H. FUNATO[2,7], M. YANAGISAWA[2,6];
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Abstract: This study employs a forward genetic approach attempting to identify novel genes regulating sleep-wakefulness behavior and to elucidate the molecular pathway governing sleep regulation. Through a high-throughput monitoring of ENU-mutagenized mice for measuring comprehensive sleep parameters, several pedigrees showing heritable sleep abnormalities have been established including *Sleepy2* mutant pedigree which is characterized by long NREM sleep time. Whole-exome deep sequencing, combined with a linkage analysis identified a single nucleotide change of A to T in the splicing acceptor site of a gene, here termed *Sleepy2*. *Sleepy2* has been reported to be involved in a variety of biological functions and diseases but had not previously been implicated in sleep physiology.

To prove the causal relationship between the identified gene mutation and sleep phenotype, the same mutation was reproduced using the CRISPR/Cas9 genome-editing technology. The ENU-induced single nucleotide change (herein, referred to as *SA* mutation) in the splicing acceptor site results in two variant forms of the mRNAs, which induces truncation of the encoded gene. Based on reverse transcription polymerase chain reaction (RT-PCR) data, this mutation may cause severe instability of the mRNA that leads to degradation by nonsense-mediated decay. The *SA* mutation in *Sleepy2* results in loss of functional *Sleepy2* protein. Indeed, the homozygous mutant mice exhibit null-like phenotypes when compared with previously reported data. Moreover, the *SA* mutation is similar to single nucleotide polymorphism (SNP) mutation of *Sleep2* related to a known human disease.

The polysomnography analysis based on EEG/EMG recording in *Sleepy2*^{CRISPR} mutant mice line were conducted to prove the causal relationship between the ENU-identified mutation and perturbed sleep phenotype. The preliminary screening of ENU-mutant mice showed hypersomnolence. Indeed, the same sleep disturbance was observed in the CRISPR-driven mutant mice line.

Since *Sleepy2* is reported to regulate epigenetic change of genomic DNA, it is essential to investigate the mechanistic function of the *Sleepy2* and the effect of this particular mutant variant form in various cellular processes and signaling pathways governing sleep and other vital physiological functions. Further investigation and scrutiny will reveal novel modulators of the system and help us gain fundamental understanding of sleep. Equally important, proteins encoded by the *Sleepy2* genes are highly prominent as drug targets, opening up the possibility of future drug development exploiting the new signaling pathway.

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Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

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Program#/Poster#: 343.05/YY1

Topic: C.01. Brain Wellness and Aging

Support: P01AG017628

HL07953

Title: The unfolded protein response regulates behavioral state

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Abstract: The Unfolded Protein Response (UPR) is a cellular process that regulates protein homeostasis in response to endoplasmic reticulum (ER) stress. UPR activation occurs when misfolded proteins accumulate in the ER and leads to the downregulation of protein synthesis, upregulation of molecular chaperones, and increased protein degradation. Previous work from our lab has demonstrated that the UPR chaperone BiP promotes sleep in the fly. However, the involvement of other UPR molecules in regulating sleep has not been explored. In the following study, we examined the role of the UPR sensors protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and inositol-requiring enzyme 1 (IRE1) in regulating sleep and wake behavior in *Drosophila melanogaster*. Using both pharmacological and genetic approaches, we examined the sleep effects of inhibiting PERK and IRE1α activity. For the pharmacological experiments, wildtype flies were administered food containing either vehicle or the PERK inhibitor GSK2606414 and the IRE1α inhibitor STF080310. Following food administration of GSK2606414, wildtype flies display significantly reduced nighttime sleep during the night in wildtype flies. In contrast, STF080310 significantly reduced total sleep during the day. Molecular analyses of flies treated with the PERK and IRE1α inhibitors demonstrate that levels of BiP expression in individual flies are positively correlated with sleep time. RU486-inducible expression of IRE1α RNAi in neurons mimicked the effect observed upon IRE1α inhibitor administration. The effects of PERK RNAi expression in neurons will also be assessed. The results from this study suggest that the UPR proteins PERK and IRE1α promote sleep in *Drosophila*. It is possible that during wake, upregulated protein synthesis in the brain leads to a gradual increase in UPR activation that may represent a cellular sleep-promoting signal. Both dysregulated sleep and sustained UPR activation have been implicated in the pathophysiology of numerous neurodegenerative diseases. Thus, there is strong translational incentive to understand the mechanisms underlying UPR-mediated sleep regulation.

Disclosures: S. Ly: None. **A.I. Pack:** None. **N. Naidoo:** None.

Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.06/YY2

Topic: F.08. Biological Rhythms and Sleep

Support: MIddlebury College Startup Funds

Title: Reductions in local and global sleep need following pharmacological depotentiation in rat cerebral cortex

Authors: C. CARROLL[1], H. HSIANG[1], S. SNYDER[1], J. FORSBERG[1], *M. B. DASH[2]; [1]Neurosci., [2]Psychology, Middlebury Col., Middlebury, VT

Abstract: Sleep is a ubiquitous phenomenon whose fundamental function remains to be definitively characterized. The synaptic homeostasis hypothesis (Tononi and Cirelli, 2003) proposes that the function of sleep is to maintain appropriate levels of synaptic activity by globally reducing synaptic strength during sleep to counteract waking-induced synaptic plasticity. Consistent with this hypothesis, molecular and electrophysiological indices of synaptic potentiation are enhanced after waking and decrease following sleep (Vyazovskiy et al., 2008) while learning increases NREM slow-wave activity (SWA; Huber et al., 2004), an electrophysiological measure of enhanced sleep need (Achermann and Borbely, 2003). These results however, do not directly test whether synaptic strength is responsible for generating sleep need. Recent research has established that pharmacological inhibition of protein kinase Mzeta following injection of zeta-inhibitory peptide (ZIP) reduces synaptic strength (Migues et al., 2010; Sacktor, 2012). One hour before light onset, we administered local injections of ZIP (2.5 µL of 5 nmol/µL ZIP) into the cortex of freely-behaving rats to assess whether pharmacological depotentiation directly affects sleep need. Compared to the effects of a saline control delivered 24hrs prior, ZIP injections delivered to a single cortical area (motor cortex; N=8) selectively reduced SWA during the first four hours following injection (-31.64 ± 13.52%). This reduction was specific to electrodes near the injection site. Despite affecting local SWA levels, the singular ZIP injection did not significantly alter sleep architecture (percent of light period: ZIP: NREM = 39.27 ± 2.07%, REM = 7.64 ± 1.21%, Saline: NREM = 42.66 ± 2.84%, REM = 7.84 ± 0.71%). By contrast, our preliminary results (N=2) indicate that increasing the spatial extent of synaptic depotentiation by simultaneously injecting ZIP throughout much of the cerebral cortex (including both motor cortices and left/right parietal cortices) is associated with alterations in sleep architecture including a ~10-15% reduction in NREM sleep during the light period. Together, these results provide direct experimental support for the synaptic homeostasis hypothesis and demonstrate that alterations in cortical synaptic strength directly regulate both global and local sleep need.

Disclosures: **C. Carroll:** None. **H. Hsiang:** None. **S. Snyder:** None. **J. Forsberg:** None. **M.B. Dash:** None.

Poster

343. Sleep Regulators and Pharmacology

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Program#/Poster#: 343.07/YY3

Topic: F.08. Biological Rhythms and Sleep

Title: Hypnotic, anxiolytic and anticonvulsant effect of the methanolic extract of Ricinus communis (Euphorbiaceae) leaves

Authors: *O. O. SUNDAY[1], L. D. IOR[2], S. ADEDOYIN[2];
[1]Univ. of Jos, Jos, Plateau State, Nigeria; [2]Dept. of Pharmacol., Univ. of Jos, Jos, Nigeria

Abstract: The Anticonvulsant, Hypnotic and Anxiolytic properties of Ricinus communis (Euphorbiaceae) were investigated. Anticonvulsant activity was investigated using invitro anti convulsant model (using Pentyleneterazole), while hypnotic effect was evaluated using phenobarbitone induced sleep in rats and the Anxiolytic effect was evaluated using mice marble burring test. Results obtained showed that Ricinus communis (Euphorbiaceae) significantly reduced the number of convulsive episodes from 5 episodes to 2 episodes at the dose of 200 mg/Kg, while 400 mg/Kg of the extract increased the number of convulsion episodes from 5 to 12 episodes. This result showed that R. communis attenuated convulsion at low doses, but aggravated convulsion at higher doses. At the doses tested, the extract significantly prolonged the onset of convulsion. These results were comparable to that of phenobabitone (30 mg/Kg) at 200 mg/Kg of the Extract of R. communis, with phenobarbitone showing higher potency. Results obtained from the hypnotic test showed that the extract of R. communis (400 mg/Kg and 800 mg/Kg) significantly reduced the onset of sleep, the extract of R. communis (200 mg/Kg, 400 mg/Kg and 800 mg/Kg) prolonged the duration of sleep in Phenobarbitone induced sleep model. The results obtained were comparable with the results obtained with diazepam, but diazepam was more potent. Results from the anxiolytic studies revealed that the extract of R communis has anxiolytic activity. The extract of R communis (200 mg/Kg, 400 mg/Kg and 800 mg/Kg) significantly reduced the number of marbles buried by mice from 8.4 to 2.40, 0.60 and 0.00 respectively. The observed results showed that the R. communis possesses Central Nervous activity. The activity tends to be depressive and may explain its use in folkloric medicine for the management of central Nervous system disorder.

Disclosures: **O.O. Sunday:** A. Employment/Salary (full or part-time): Full. **L.D. Ior:** A. Employment/Salary (full or part-time): Full. **S. Adedoyin:** None.

Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH/NINDS K08NS069667

Tross Research Fund University of Iowa

Koostra Fellowship Maastricht University

Van Leersum Beurs (KNAW)

Title: Serotonergic dorsal raphe neurons in hypercapnia-induced arousal from sleep

Authors: *N. LEIBOLD[1,3], H. R. SMITH[4], C. M. GINAPP[1,2], D. A. RAPPAPORT[4], G. F. BUCHANAN[1,4];
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Abstract: All mammals must sleep. Prolonged sleep deprivation results in reduced cognition, reduced motor performance, and in extreme cases, death. The ability to rapidly arouse from sleep in response to life-threatening stimuli is a critical reflex conserved across mammals. One such arousal stimulus is hypercapnia, which would generally signify an airway obstruction; however, the precise locus of action of carbon dioxide (CO_2) remains controversial. Serotonin (5-HT) neurons in both the midbrain and medulla, the former thought to be involved in sleep-wake regulation and the latter in breathing regulation, are sensitive to CO_2 and pH, and thus are an attractive candidate for mediating CO_2 arousal. However, whether the rostral or caudal sites are more important is unclear. Here we tested the hypothesis that 5-HT neurons in the dorsal raphe nucleus (DRN) and not those in the medullary raphe are critical for arousal to CO_2. Adult male wildtype (WT; *Lmx1b*$^{f/f}$ or C57BL/6J) or 5-HT neuron deficient (*Lmx1b*$^{f/f/p}$) mice were implanted with EEG/EMG headmounts and microdialysis cannulae directed toward the DRN or medullay raphe. After recovery from surgery and acclimation to the testing apparatus, normal (5% CO_2, pH 7.4) or acidified (25% CO_2, pH 6.8) artificial cerebrospinal fluid was microdialyzed into the DRN or medullary raphe during wakefulness and sleep and effects on sleep architecture and breathing were assessed. Acidification of DRN, but not medullary raphe during sleep caused arousal in WT animals. However, acidosis-induced arousal was lost in *Lmx1b*$^{f/f/p}$ mice. Stimulation of the medullary raphe in WT mice with acidosis during wake or sleep increased

ventilation. This effect was also lost in *Lmx1b*$^{f/f/p}$ mice. Taken togeher these data indicate that DRN 5-HT neurons are important in mediating CO_2 arousal, while medullary 5-HT neurons affect breathing only. Deficits in CO_2-induced arousal responses likely contribute to diseases such as the sudden infant death syndrome (SIDS), sudden unexpected death in epilepsy (SUDEP), and obstructive sleep apnea (OSA). Therefore, understanding this protective mechanism may lead to interventions to reduce morbidity and mortality from these diseases.

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Poster

343. Sleep Regulators and Pharmacology

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Program#/Poster#: 343.09/YY5

Topic: F.08. Biological Rhythms and Sleep

Support: Merck & Company

NIH T32 HL007909

Title: Identifying genes and gene networks underlying sleep and psychiatric behaviors in an F2 mouse population

Authors: *V. GAO[1,2], P. JIANG[2], J. SCARPA[3], M. VITATERNA[2], A. KASARSKIS[3], F. TUREK[2];
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Abstract: Sleep disturbances are known to be both a cause and symptom of psychiatric disorders, but the exact nature of this relationship is unclear. We wished to investigate how sleep and psychiatric behavior are related at the levels of phenotype, genotype, and gene expression. A large population of (C57BL/6 x 129) F2 mice were tested in a battery of behavioral tests (elevated plus maze, open field arena, fear conditioning, forced swim test, and tail suspension test), and also had their sleep measured under baseline conditions and in response to sleep deprivation and restraint stress. The mice were also genotyped and had gene expression levels measured using microarrays from cortex, hippocampus, hypothalamus, and thalamus. Principal components analysis was used to cluster phenotypes and discover underlying relationships between them. Weighted gene coexpression network analysis and clustering were used to identify modules of coexpressed genes in each tissue. Quantitative trait loci analysis and a causal

inference test were used to identify genes whose expression levels are causal to phenotype variation. These analyses reveal relationships between phenotypes, as well as relationships between phenotypes and gene expression. We identify some coexpression modules which are correlated with both sleep and psychiatric behavior. These modules are investigated further, identifying key regulator genes which occupy key nodes in the network. We find that one particular set of genes are highly associated with sleep measurements in all four brain tissues, and Gene Ontology enrichment analysis suggests that this gene set is involved in neural membrane and lipid regulation. This multiscale analysis generates hypotheses about sleep and psychiatric regulation and identifies putative causal genes which can then be tested experimentally with drug targeting or genetic manipulation.

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Poster

343. Sleep Regulators and Pharmacology

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.10/YY6

Topic: F.08. Biological Rhythms and Sleep

Support: Department of Veterans Affairs

Title: Effects of subarachnoid infusion of CRF receptor-1 antagonist on sleep and preoptic neuronal activity in rats

Authors: *S. KUMAR[1], I. GVILIA[2,3], K.-C. HSIEH[2,3], S. RAI[2,6], K.-T. CHEW[2], D. MCGINTY[2,7], R. SZYMUSIAK[2,4,5];
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Abstract: Introduction: Antagonism of corticotropin releasing factor (CRF), by intracerebroventricular (ICV) administration of CRF receptor-1 (CRF-R1) antagonist reduces spontaneous waking and elevates NREM sleep time during the dark phase in rats. ICV administration of CRF-R1 agonist decrease sleep, negatively impact homeostatic response to sleep loss and suppresses activity of GABAergic neurons in the ventrolateral preoptic area (VLPO) and median preoptic nucleus (MnPO). In this study, we infused CRF-R1 antagonist (antalarmin; ANT) directly into the subarachnoid space (SA) rostral to the preoptic hypothalamus late in the light phase and quantified effects on spontaneous sleep-waking and c-

Fos expression in preoptic neurons. Methods: Groups of rats were subjected to continuous SA infusion of ANT for 3 hrs, initiated 8 hrs after lights-on (ZT8). Rats received continuous SA infusion (0.2µl/min) of 1% dimethyl sulfoxide (DMSO) in artificial cerebrospinal fluid as vehicle (n=4) or ANT (2 µg, n=3 or 6 µg, n=5) during ZT 8 to ZT 11. Rats were left undisturbed with continuous EEG and EMG recordings. At the end of 3 hours of infusion, rats were immediately euthanized and brain tissue harvested and processed for immunostaining of c-Fos-protein and glutamic acid decarboxylase (GAD). Results: ANT-treated rats exhibited significant increases in time spent in nonREM sleep (Vehicle, 37.9±2.1%; 2 µg, 41.6±2.8%; 6 µg, 54.4±1.3%) and REM sleep (Vehicle, 7.6±1.2%; 2 µg, 9.9±2.7%; 6 µg, 13.1±1.03%), and decreases in time spent awake (Vehicle, 54.7±2.9%; 2 µg, 48.4±1.4%; 6 µg, 32.4±1.7%) during 3 hrs of infusion. The percent of GAD+ neurons expressing Fos was increased in treated rats in the rostral MnPO (Vehicle, 11.8±1.6%; 2 µg, 13.9±2.6%; 6 µg, 20.0±2.0%), caudal MnPO (Vehicle, 10.5±2.0%; 2 µg, 12.3±2.1%; 6 µg, 17.3±1.1%), extended VLPO (Vehicle, 12.4±2.2%; 2 µg, 16.0±3.4%; 6 µg, 23.4±2.6%) and VLPO core (Vehicle, Vehicle, 11.4±1.3%; 2 µg, 13.9±2.2%.5; 6 µg, 19.4±1.6%). Conclusion: These results support the hypothesis that CRF-R1 mediated reductions in preoptic GABAergic neuronal activity suppress sleep and promote wakefulness at circadian times when endogenous CRF signaling is elevated.

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Poster

343. Sleep Regulators and Pharmacology

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Program#/Poster#: 343.11/YY7

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR MOP-136969

CIHR MOP-136967

NSERC 298475

Title: The role of CL thalamic nucleus in the modulation of cortical slow oscillation in mice

Authors: *A. OZUR[1], S. CHAUVETTE[2], I. TIMOFEEV[3];
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Abstract: It was recently demonstrated that the thalamus plays an essential role in the generation of fully expressed cortical slow oscillation. However, the possible distinct role, played by the specific vs. non-specific thalamic nuclei, in the generation and modulation of the cortical slow oscillation was not investigated.
Last year we presented data showing that the inactivation of first-order (specific) thalamic nuclei with muscimol (GABAA-agonist) decreased the slow/delta power in the corresponding primary cortical area, while the inactivation of higher-order (non-specific) nuclei with muscimol significantly reduced the slow/delta power in all investigated cortical regions. Thus, the non-specific thalamic nuclei play a major role in the regulation of the global cortical slow-wave activity.
We hypothesize that the global reduction of slow/delta power in neocortex in areas affected by the thalamic inactivation is based on two non-exclusive possibilities: (a) the activities of neurons responsible for the reduction in slow/delta power are reduced (amplitude or duration of states); (b) the synchrony of neural activities between local subpopulations of neurons is reduced. We performed intracellular recordings from cortical neurons combined with local field potential (LFP) recordings in anesthetized mice and we inactivated thalamic CL nucleus to test the first hypothesis. After the inactivation of CL nucleus the LFP recordings showed a decrease of slow-wave activities in all investigated cortical areas. Intracellular recordings from frontal and somatosensory cortical areas revealed that the duration of active states of the slow oscillation was significantly decreased.
We conclude that non-specific thalamic nuclei contribute to the maintenance of cortical active states during slow oscillation.

Disclosures: **A. Ozur:** Other; CIHR MOP-136969, CIHR MOP-136967, NSERC 298475. **S. Chauvette:** None. **I. Timofeev:** None.

Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS027881

Title: Effects of the noisy orexin current on firing and input-output gain of cholinergic neurons in the laterodorsal tegmental and pedunculopontine tegmental nuclei of mice.

Authors: ***M. ISHIBASHI**, N. E. MOLINA, I. GUMENCHUK, C. S. LEONARD;
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Abstract: Orexin (hypocretin) neuropeptides act through two G-protein coupled receptors (OX1 and OX2) and are essential for normal arousal and sleep. These peptides produce a slow, post-synaptic depolarization which is mediated by an unusually noisy cation current in some neurons, including serotonergic (5-HT) dorsal raphe (DR) neurons and cholinergic laterodorsal (LDT) and peduculopontine (PPT) tegmental neurons. We recently found that this noisy current provides significant high-frequency input to both these serotonergic and cholinergic neurons. Moreover, by using a dynamic clamp to add a virtual noisy orexin conductance (vGorx), we found that this noisy current engages an intrinsic Ca^{2+}-dependent resonance that peaked in the theta and alpha frequency range (4 - 14 Hz) and extended up to 100 Hz. While noise often degrades system performance, it can also be useful, particularly in non-linear systems, like neurons, to enhance the effectiveness of weak inputs. In experiments described here, we investigated how this noisy current interacts with other inputs to induce spiking by using whole-cell recordings from visualized cholinergic LDT and PPT neurons in brains slices from mice expressing dTomato fluorescence in cholinergic neurons. Based on earlier work showing that balanced synaptic noise can produce a divisive modulation of input-output gain in neocortical pyramidal neurons (Chance et al. Neuron, 2002 doi:10.1016/S0896-6273(02)00820-6), we compared the effect of a virtual orexin conductance (vGorx with noise) to that of a corresponding vGorx (without noise) on firing and input-output gain of cholinergic neurons. Results from these experiments revealed: 1) The vGorx with noise triggered more spikes and sustained firing for a longer duration than the vGorx without noise over a wide range of conductances and 2) In contrast to balanced synaptic noise in pyramidal neurons, the noisy vGorx produces an additive rather than divisive effect on input-output gain of LDT and PPT cholinergic neurons. Since we recently found orexin also enhances the late afterhyperpolarization of these neurons, future experiments are needed to examine the combined impact of noise and the enhanced AHP, since it is possible AHP enhancement contributes to gain modulation. Nevertheless, the collective evidence so far indicates that the postsynaptic actions of orexin transcend simple excitation to regulate the signal encoding ability of the target neurons.

Disclosures: **M. Ishibashi:** None. **N.E. Molina:** None. **I. Gumenchuk:** None. **C.S. Leonard:** None.

Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.13/YY9

Topic: F.08. Biological Rhythms and Sleep

Support: K12 HD43489-14

P50 MH103222

Title: Sexually dimorphic increase in kynurenic acid and impaired contextual memory after acute sleep deprivation in rats

Authors: *A. BARATTA, A. D. BUCHLA, A. POCIVAVSEK;
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Abstract: Poor sleep quality afflicts millions of Americans and contributes to cognitive impairments and various psychiatric disorders. While restful periods at night have been associated with memory consolidation, prolonged periods of poor sleep quality result in neurocognitive dysfunction. Our current experiments were designed to test the hypothesis that kynurenic acid (KYNA), an astrocyte-derived metabolite of the kynurenine pathway (KP) of tryptophan degradation, is elevated in the brain after sleep disturbances. KYNA is an antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors, and elevations in KYNA negatively impact learning and memory. As sleep patterns are sexually dimorphic and fluctuations in hormones impact sleep, we investigated the effect of sleep deprivation (SD) on KP metabolism in both male and female adult Wistar rats. Animals were sleep deprived by gentle handling for 6 h from Zeitgeber time (ZT) 0 to ZT 6, where ZT 0 is the start of the light-phase. KP metabolites were analyzed in the brain and plasma immediately after SD. In the hippocampus, KYNA levels were 2.4-fold elevated in male rats after SD (control: 103 ± 14 fmoles/mg protein; SD: 243 ± 55 fmoles/mg protein; $P < 0.05$). In contrast, KYNA levels in female animals were not significantly elevated after SD (control: 47 ± 8 fmoles/mg protein; SD: 75 ± 13 fmoles/mg protein; $P = 0.1$). Irrespective of sex, brain levels of the KP metabolite 3-hydroxykynurenine remained unchanged after SD. In the serum, no significant changes in KYNA or its bioprecursor kynurenine were observed in male or female rats. In separate adult animals, we tested contextual memory using the passive avoidance paradigm (PAP). Animals undergoing behavioral testing were sleep deprived from ZT 0 to ZT 6. PAP training occurred at ZT 3 and following training, animals underwent three additional hours of SD. Twenty-four hours after training, animals were tested in the retention trial. In male rats, SD induced significant PAP deficits, evidenced as decreased avoidance latency during the retention trial (control: 155 ± 34 s; SD: 31 ± 6 s; $P < 0.01$). Conversely, in female animals, the avoidance latency was not significantly reduced after SD (control: 82 ± 25 s; SD: 41 ± 14 s; $P = 0.3$). Collectively, our results demonstrate a striking sexual dimorphism in the elevation of hippocampal KYNA and contextual memory retention after an acute period of SD. Ongoing experiments are designed to assess the possible role of estrogen and other hormones in protecting the female rodent from a deleterious increase in brain KYNA after SD and preventing KYNA elevations during SD by inhibiting the enzyme kynurenine aminotransferase (KAT) II.

Disclosures: **A. Baratta:** None. **A.D. Buchla:** None. **A. Pocivavsek:** None.

Poster

343. Sleep Regulators and Pharmacology

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Support: Human Frontier Science Program (RGY0076/2012)

Canadian Fund for Innovation (CFI)

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NeuroCure (SPP1665)

Title: Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness

Authors: *C. G. HERRERA[1], M. BANDARABADI[1], M. CARUS CADAVIECO[2], K. SCHINDLER[1], A. PONOMARENKO[2], T. KOROTKOVA[2], A. ADAMANTIDIS[1,3]; [1]Dept of Neurol., Inselspital Univ. of Bern, Bern, Switzerland; [2]NeuroCure Cluster of Excellence, Leibniz Inst. for Mol. Pharmacol. (FMP), Berlin, Germany; [3]Dept. Klinische Forschung DKF, Univ. of Bern, Bern, Switzerland

Abstract: In mammals, during non-rapid eye movement (NREM) sleep, synchronous synaptic activity in the thalamocortical network generates these low-frequency oscillations that include predominant slow wave (< 1 Hz) associated with delta oscillations (1-4 Hz) and spindles (11-15 Hz). Here, we investigated whether TRN cells - the main inhibitory input to thalamo-cortical networks - integrate sleep-wake signals from subcortical circuits using optogenetics coupled to high-density electrophysiology in mice. Various opsins (ChETA, ArchT) were genetically to targeted to the lateral-hypothalamic GABA (LH_{GABA}) neurons in VGAT::Cre mice. Transduced animals were chronically implanted with optic fibers, multiple tetrodes (LH/TRN/VB/Cortex) and EEG/EMG. Optical stimulations were delivered time-locked to specific brain states before OFF-line analysis of neuronal activity and brain state transitions. Here we identified a monosynaptic GABAergic connectivity between $LH_{\mathbf{GABA}}$-$TRN_{\mathbf{GABA}}$. Optogenetic activation of this circuit mimic naturalistic activity of $LH_{\mathbf{GABA}}$-$TRN_{\mathbf{GABA}}$ circuit across brain states, and induced rapid arousal during NREM, but not REM, sleep, through direct inhibition of TRN cells and subsequent dis-inhibition of thalamo-cortical networks. In contrast, optogenetic silencing of $LH_{\mathbf{GABA}}$-$TRN_{\mathbf{GABA}}$ transmission increased the duration of NREM sleep and amplitude of delta

oscillations. During deep anesthesia, activation of this circuit induced sustained cortical arousal. Collectively, these results demonstrate that TRN cells integrate subcortical arousal inputs selectively during NREM sleep, and participate in sleep homeostasis while possibly modulating sensitivity to sensory inputs during sleep and unconscious states.

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Poster

343. Sleep Regulators and Pharmacology

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Title: T-type calcium channel inhibition in thalamic reticular nucleus reduces sleep spindles in mice

Authors: *C. SHUKLA[1], S. THANKACHAN[1], J. M. MCNALLY[1], J. T. MCKENNA[1], C. YANG[1], R. E. BROWN[1], R. W. MCCARLEY[2], R. BASHEER[1];
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Abstract: Cav3.3 T-type calcium channels are expressed in parvalbumin (PV)-containing GABA thalamic reticular neurons (TRN) and are thought to generate burst firing necessary for sleep spindle generation. Cav3.3 is also a risk gene for schizophrenia, where spindle abnormalities are present. The role of Cav3.3 T-type channels in the control of sleep and/or spindles has been examined using Cav3.3 knockout mice but not with acute, local

pharmacological blockade. Therefore, here we examined the inhibitory effect of a selective T-type inhibitor, TTA-P2, in TRN.
In vitro, whole-cell patch-clamp recordings of identified PV neurons were made in slices from young (13-22d) PV-tdTomato mice. *In vivo*, mice were implanted with both bilateral microdialysis cannulae targeting TRN (AP -0.7, ML $\pm$1.3, DV -4.0) and EEG/EMG electrodes. Experiments were as follows: Day1, aCSF from ZT 2-ZT 6 (baseline, BL). Day 2, TTA-P2 (1μM) microdialysis infusions from ZT 2-6 when mice mostly sleep. EEG was recorded both days and the effect of the drug on sleep-wake state was scored using Sirenia-Pro. A custom Matlab script was then utilized to automatically detect individual spindles (10 - 15Hz) in the EEG, allowing analysis of drug effects on spindle density during NREM sleep (spindles/min NREM sleep). Data was compared with the BL values and further related to the histological location of the probe.
In vitro, TRN-PV neurons exhibited low-threshold spikes/inward currents after removal of hyperpolarizing currents/voltage steps respectively, which were blocked by TTA-P2 (3 μM, n=4, 4), confirming the expression of low threshold T-type Ca channels in TRN PV neurons. *In vivo*, in three mice the probe was localized in TRN on both sides (bilateral hits) and in 4 mice the probe was localized in TRN on one side and outside TRN on the other (unilateral hits). We observed that in both groups (bilateral and unilateral hits), perfusion of 1 μM TTA-P2 resulted in a decrease in NREM spindle density (Unilateral: -17. 68%, Bilateral: -39.68%). Collectively, uni- and bi-lateral hits (N=7 mice) showed a statistically significant decrease (-27.11±8.5%, p=0.03, t-test) without any significant effect on NREM sleep.
In summary, TRN PV neurons express T-type calcium channels whose activation is blocked by TTA-P2. In contrast to a constitutive, global knockout of Cav3.3, which reduces spindles and fragments NREM sleep, our results demonstrate that localized pharmacological blockade of T-type channels in TRN inhibits spindle density *without affecting* NREM.

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Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 343.16/YY12

Topic: F.08. Biological Rhythms and Sleep

Title: Importance of histamine clearance for brain functions

Authors: *T. YOSHIKAWA[1], F. NAGANUMA[1], T. NAKAMURA[1], T. IIDA[1], A. KARPATI[1], T. MOCHIZUKI[2], K. YANAI[1];
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Abstract: Brain histamine functions as a neurotransmitter to regulate several processes including stress response, sleep-wake cycle and appetite. Recently, brain histamine deficit was observed in neuropsychiatric disorders such as Alzheimer's disease and narcolepsy, suggesting the dysfunction of histaminergic nervous system might exacerbate brain func. Although histamine in the brain is mainly inactivated by histamine N-methyltransferase (HNMT), roles of HNMT in histamine concentration and brain function are still unclear. In this study, we analyzed HNMT knockout (KO) mice to elucidate the importance of HNMT in the mouse brain. First, we confirmed that HNMT KO mouse brains did not have HNMT enzymatic activity. Brain histamine concentration of KO mice was 6-fold higher than that of wild type mice, supporting the essential role of HNMT in the inactivation of brain histamine. Locomotor activity during dark (active) period was decreased in KO mice, although their motor functions were not impaired. Detailed analysis of locomotor activity showed that immobility time during active period was increased in KO mice, suggesting that HNMT deficiency induced abnormal sleep-wake cycle. Electroencephalogram recording revealed that wake duration of KO mice in light (resting) period was extended with compensatory extension of sleep duration in active period. A histamine H1 receptor (H1R) antagonist pyrilamine cancelled the prolonged wakefulness during resting period. Thus, abnormally elevated histamine by HNMT deficiency activated H1R and disrupted normal sleep-wake cycle. Most KO mice had skin injuries by fighting, indicating high aggressive behaviors in KO mice. Resident-intruder tests showed that aggressive behaviors were elevated in KO mice. Aggressive biting behaviors were increased in KO mice using a semi-automated apparatus. Zolantidine, an H2R antagonist, abolished the aggression of KO mice. These results indicated the importance of HNMT and H2R in aggressive behaviors. In the present study, we demonstrate that HNMT plays an important role in the regulation of brain histamine concentration. Histamine inactivation by HNMT contributes to normal sleep-wake cycle and suppression of aggressive behaviors.

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Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS082876

Title: Deletion of trace amine-associated receptor 1 attenuates behavioral response to caffeine

Authors: *M. D. SCHWARTZ[1], J. B. PALMERSTON[1], D. L. LEE[1], M. C. HOENER[2], T. S. KILDUFF[1];
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Abstract: Trace amines (TAs) are endogenous amino acid metabolites that are structurally similar to the biogenic amines. TAs are endogenous ligands for trace amine-associated receptor 1 (TAAR1), a GPCR that modulates dopaminergic, serotonergic, and glutamatergic activity. Selective TAAR1 full and partial agonists exhibit similar pro-cognitive, antidepressant- and antipsychotic-like properties in rodents and non-human primates, suggesting TAAR1 as a novel target for the treatment of neurological and psychiatric disorders. We previously reported that TAAR1 partial agonists are wake-promoting in rats and mice, and that TAAR1 knockout (KO) and overexpressing mice exhibit altered sleep-wake and EEG spectral composition. Here, we report that locomotor responses to the psychostimulants modafinil and caffeine are attenuated in TAAR1 KO mice. TAAR1 KO mice and WT littermates were instrumented for EEG and EMG recording and implanted with telemetry transmitters for monitoring locomotor activity (LMA) and core body temperature (Tb). Following recovery, mice were administered modafinil (25, 50, 100 mg/kg), caffeine (2.5, 10, 20 mg/kg) or vehicle p.o. at ZT6 in balanced order. In WT mice, both modafinil and caffeine dose-dependently increased hourly LMA for up to 6h following dosing, whereas only the highest dose of each drug increased LMA in KO mice, and did so for less time after dosing. In WT mice, total LMA summed over 6h following dosing significantly increased following modafinil (50 & 100 mg/kg) and caffeine (10 & 20 mg/kg) whereas, in KO mice, only modafinil (100 mg/kg) significantly increased total LMA. Furthermore, total LMA response was significantly attenuated in KO mice compared to WT at all doses of caffeine. Tb was increased in parallel with LMA in both genotypes. In contrast to the known hypersensitivity of TAAR1 KO mice to amphetamines, our results show that TAAR1 is a critical component of the arousing response to two widely-used psychostimulants with very different mechanisms of action. Together with our previous findings, these data suggest that TAAR1 is a previously-unrecognized component of an endogenous wake-modulating system.

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Poster

343. Sleep Regulators and Pharmacology

Location: Halls B-H

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Topic: F.08. Biological Rhythms and Sleep

Title: Pharmacological characterization of primidone in the sleep-wake cycle of rats

Authors: *M. SALAS-CRISOSTOMO[1], K. GUZMÁN[2], F. SARLAT-ACUNA[3], N. ELLIS-INFANTE[3], G. ARANKOWSKY-SANDOVAL[4], E. MURILLO-RODRÍGUEZ[3];
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Abstract: Primidone is a drug that has been widely used for the management of several health conditions, such as epilepsy and Parkinson's disease. Despite its efficiency, the mechanism of action of primidone remains unknown. Here, we described the pharmacological effects of primidone on sleep in rats. Male Wistar rats (n=24) were implanted with electrodes to record EEG and EMG. Next, animals were divided in four groups and they received either of the following treatments: Vehicle, primidone (10, 25 and 50mg/Kg, i.p; each group). All experimental challenges were carried out at the beginning of the lights-on period (08:00h). The sleep-wake cycle was recorded during the following 4h post-injections and total sleep time was analyzed. We found that compared to control rats, primidone promoted dose-dependent increases in wake (W) duration whereas the drug reduced significantly slow wave sleep (SWS) and rapid eye movement sleep (REMS; $P< 0.05$). Sleep parameters such as frequency, mean duration and latency for W, SWS and REMS, showed statistical changes in animals treated with primidone ($P< 0.05$). In this regard, primidone (50mg/kg) increased frequency and mean duration of W ($P< 0.05$). Opposite result were observed for SWS and REMS ($P< 0.05$). In this very first study, we described the wake-promoting properties of primidone in rats.
Further studies will be needed to address the mechanism of action of this compound.
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Poster

343. Sleep Regulators and Pharmacology

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Topic: F.08. Biological Rhythms and Sleep

Support: University of Missouri

Title: Trichostatin A attenuates insomnia associated with alcohol withdrawal and normalizes sleep-wakefulness

Authors: *R. SHARMA, A. SHARMA, P. SAHOTA, M. THAKKAR;
Neurol., Harry S Truman Mem. Veterans' Hosp., Columbia, MO

Abstract: Background: Insomnia is amongst the most common, severe and protracted symptom in patients in recovery from alcoholism. In fact, untreated insomnia in recovering alcoholics can precipitate relapse. However, the underlying cause of insomnia observed in alcoholics is not well understood. Previously, we have shown that insomnia observed during alcohol withdrawal was associated with downregulation of genes controlling sleep and changes in histone acetylation. We hypothesized that systemic administration of histone deacetylase inhibitor Trichostatin A (TSA) will attenuate insomnia observed during alcohol withdrawal.
Methods: Two experiments were performed in adult male C57BL/6J mice surgically instrumented for chronic monitoring of sleep-wakefulness.
Experiment 1: Mice were divided in two groups: Mice in the ***Alcohol*** group were exposed to liquid diet containing 6.8% (v/v) alcohol for 21 days followed
by exposure to normal rodent chow diet. ***Controls*** were exposed to isocaloric control liquid diet for the same duration. Sleep-wakefulness was continuously recorded for 21 days during alcohol exposure and during 7 days post-alcohol (withdrawal period).
Experiment 2: Mice were exposed to liquid diet containing 6.8% (v/v) alcohol for 21 days. Two week into alcohol consumption, mice were divided into two groups: TSA and Control. Mice in the TSA group were systemically administered TSA (at light onset; 2 mg/kg, ip) beginning every other day for 5 days with the last dose on 2nd day of withdrawal. Controls were administered vehicle (10% DMSO) instead of TSA. Sleep-wakefulness was continuously recorded until the end of the experiment.
Results:
Experiment 1: As compared to controls, mice exposed to alcohol displayed significant increase in wakefulness (insomnia) with a concomitant reduction in sleep during first five days of withdrawal.
Experiment 2: As compared to control group, mice in the TSA groups showed a reduction in wakefulness, especially during the first five days of the light period.

Conclusions: Based on our result we suggest that inhibition of histone deacetylase may be critical in attenuating insomnia associated with alcohol withdrawal. This may also have important implications in developing a therapeutic strategy for the treatment of alcoholism.

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Poster

343. Sleep Regulators and Pharmacology

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Topic: H.01. Animal Cognition and Behavior

Support: VA Career Development Award BX001677

JSMF Collaborative Award 220020346

Title: Flumazenil, a GABA antagonist, delays return of righting reflex in mice after isoflurane anesthesia but does not significantly alter dendritic spine density

Authors: J. A. FIDLER, Y. A. JAMAL, L. A. SHAPIRO, *P. S. GARCIA;
Anesthesiol., Atlanta VA Med. Ctr. / Emory Univ., Decatur, GA

Abstract: The γ-aminobutyric acid (GABA) antagonist flumazenil has been shown to improve the quality of recovery after surgery with general anesthesia (Drobish et al., A A Case Rep. 4:148-50) and improve vigilance in patients with hypersomnic disorders. We have previously reported that flumazenil can hasten cortical activation after isoflurane anesthesia and mitigate post-anesthesia sleep disruption in a rodent model. Here, a single intraperitoneal dose of 3.0 mg/kg flumazenil administered to young, healthy C57BL/6 mice significantly delayed emergence, as measured by return of righting reflex, from a single, 60-minute exposure to 1.5% isoflurane (saline: 131.0 ± 51.1 sec; flumazenil: 262.4 ± 127.9 sec; $p = 0.0016$, t-test). A post-anesthetic trend toward increased exploratory behavior was observed in mice administered flumazenil versus saline when placed into a photo-beam recording chamber, indicating a possible improvement of post-anesthesia outcome in mice treated with flumazenil, regardless of the effect on time of emergence. While Golgi staining and neuronal analysis in the hippocampus of brains, harvested 72 hours post-anesthesia, did not reveal significant effects of treatment, there were trends toward increased dendritic arborization in CA1 pyramidal neurons, and possible increased soma size in granule cells, in the brains of mice administered flumazenil versus saline. The increase in time to emergence in mice administered a single IP dose of flumazenil at cessation of isoflurane anesthesia seen here may be an *in vivo* manifestation of weak or partial agonism by

flumazenil at the GABAA receptor, which has previously been observed *in vitro* using heterologous expression systems. This effect has also been anecdotally observed in some patients with idiopathic hypersomnia who report an initial period of worsening drowsiness preceding a more wakeful state after administration of intravenous flumazenil. The *in vivo* receptor-level interaction of flumazenil was further explored in a GABAAR α4 subunit knockout mouse model. A sticky-dot removal test performed immediately following return of righting reflex from isoflurane anesthesia potentially implicates the extra-synaptic/intra-synaptic balance of GABBAAR subunits in successful recovery from anesthesia without delirium.

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Poster

344. Neurocircuitry of Human Emotion

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 344.01/ZZ3

Topic: G.03. Emotion

Support: NIH RO1MH098348

Title: Stress-induced changes in resting state functional connectivity vary with history of violence exposure

Authors: *H. E. DARK[1], N. G. HARNETT[1], A. GOODMAN[1], M. WHEELOCK[1], S. MRUG[1], M. A. SCHUSTER[2], M. N. ELLIOTT[3], S. TORTOLERO[4], D. C. KNIGHT[1];
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Abstract: *Introduction*: Resting state functional connectivity (rsFC) is a well-established measure for assessing brain connectivity patterns in emotion processing systems. For example, after an acute stressor, individuals demonstrate increased amygdala rsFC with the posterior cingulate cortex, ventromedial prefrontal cortex (vmPFC), and hippocampus (Vaisvaser et al. 2013; Veer et al. 2011). Similarly, childhood maltreatment is associated with increased amygdala rsFC with the hippocampus, parahippocampal gyrus, inferior temporal gyrus, and brainstem (Dean et al. 2014). Further, individuals with PTSD compared to non-PTSD controls demonstrate diminished medial PFC rsFC with the amygdala, hippocampus, and parahippocampal gyrus (Jin et al. 2014). Hence, those with a significant history of prior life stress may have impaired emotion regulation abilities, leaving them more susceptible to the effects of acute stress. The present study sought to examine the effects of cumulative violence exposure (CVE) on

laboratory stress-induced changes in rsFC.
Methods: Anatomically defined regions of interest (ROIs) were selected using the existing emotion processing and regulation literature. Participants completed two 6-minute resting state-functional MRI (fMRI) scans prior to (pre-stress) and after (post-stress) completing an adaptation of the Montreal Imaging Stress Task (MIST; Dedovic et al. 2005). Functional MRI data were obtained using a 3T Siemens Allegra MRI scanner. Standard high-resolution T1 weighted structural images (MPRAGE) were collected to serve as anatomical reference for the functional data.
Statistical Analyses: An analysis of covariance was conducted to determine whether post-stress rsFC varied with CVE using pre-stress rsFC as a covariate.
Results: Results revealed that CVE predicted post-stress rsFC (controlling for pre-stress rsFC) among several areas (e.g., amygdala rsFC with the hippocampus, vmPFC, dorsolateral PFC, and dorsomedial PFC; and hippocampal rsFC with the dlPFC and vmPFC) such that individuals with low, moderate, and high levels of CVE demonstrated differences in rsFC post-stress.
Discussion: FC of the amygdala, hippocampus, and PFC are important for emotion expression and regulation processes. Altered FC in these areas may increase vulnerability to stress-related psychiatric illness (Thomason et al. 2015). The present study identified changes in FC as a function of CVE, suggesting that individuals exposed to violence may have altered neural circuity in emotion regulation networks post stress, potentially increasing susceptibility to stress-related psychiatric illnesses.

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Poster

344. Neurocircuitry of Human Emotion

Location: Halls B-H

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Program#/Poster#: 344.02/ZZ4

Topic: G.03. Emotion

Support: This work was funded by the Dutch Ministry of Defence

Title: Resting-state functional connectivity in combat veterans suffering from anger and aggression

Authors: T. VARKEVISSER[1], T. GLADWIN[1], L. HEESINK[1], J. VAN HONK[2], *E. GEUZE[3,4];

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Abstract: Aims: Anger and aggression problems are common amongst military personnel in the aftermath of deployment, and are thought to arise, at least in part, due to diminished frontal-limbic connectivity. Here, we sought to further explore this hypothesis by conducting resting-state functional connectivity analyses in veterans suffering from anger and aggression. **Methods**: Male combat veterans with (n = 28) and without (n = 30) anger and aggression problems underwent resting-state fMRI. Voxel-wise regression analyses were conducted with the following seed-regions as predictors: the basolateral amygdala (BLA), centromedial amygdala (CMA), anterior cingulate cortex (ACC), and anterior insula (AI). For each analysis, white-matter, cerebrospinal fluid, global mean signal, and motion parameters were included as nuisance variables. **Results**: We observed a decrease in negative functional connectivity between the (*left*/*right*) BLA and (*left*) dorsolateral prefrontal cortex (DLPFC) in veterans suffering from anger and aggression, relative to combat controls. Also, combat veterans with- versus those without anger and aggression problems revealed a decrease in anti-correlations between the (*left*) ACC and (*left/right*) precuneus. **Conclusions**: In this study, anger and aggression problems in combat veterans were found to be associated with decreased negative functional connectivity between the BLA and DLPFC. A loss of inhibition in this circuit may signal an inability to regulate one's emotions in the face of social-environmental demands. Decreased negative functional connectivity was also found between the ACC and precuneus. A loss of inhibition in this circuit may signal a propensity to attribute the mental and emotional states of oneself and of others (i.e., Theory of Mind) as being hostile.

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Poster

344. Neurocircuitry of Human Emotion

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 344.03/ZZ5

Topic: G.03. Emotion

Support: This study was financially supported by the Dutch Ministry of Defense

Title: Amygdala response to emotional pictures in veterans with anger and aggression

Authors: *L. HEESINK[1,2,3], R. KLEBER[2], M. VINK[1], E. GEUZE[1,3];
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Abstract: INTRODUCTION: Anger and aggression are common mental health problems after military deployment (Heesink, Rademaker, Vermetten, Geuze, & Kleber, 2015). Anger and aggression are related to differences in social emotional processing. During the viewing of angry faces, heightened amygdala activity has been found in patients with impulsive aggression (McCloskey et al., 2016). However, in patients with anger and aggression problems, it is unknown whether negative (non-facial) pictures will also elicit a stronger amygdala response.
METHODS: 29 military veterans with anger and aggression problems and 30 veterans without these problems (all males) participated in this study. During an fMRI scan 32 negative, 32 positive and 32 neutral pictures from the IAPS were presented for two seconds. After that, participants rated the pictures by pressing a button.
RESULTS: Data collection is completed. Preliminary analyses revealed that patients with anger and aggression rated more pictures incongruently. The task reliably activates the amygdala, middle temporal gyrus, medial superior frontal gyrus and the hippocampus. ROI analyses will be used to assess differences in amygdala reactivity to negative pictures between the two groups.
CONCLUSIONS: This study will reveal whether patients with anger and aggression problems process emotional situations differently compared to controls. This might provide insights into potential treatments for individuals suffering from anger and aggression.
Heesink, L., Rademaker, A., Vermetten, E., Geuze, E., & Kleber, R. (2015). Longitudinal measures of hostility in deployed military personnel. *Psychiatry Research*, *229*(1-2), 479-84.
McCloskey, M. S., Phan, K. L., Angstadt, M., Fettich, K. C., Keedy, S., & Coccaro, E. F. (2016). Amygdala hyperactivation to angry faces in intermittent explosive disorder. *Journal of Psychiatric Research*, *79*, 34-41.

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Poster

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Program#/Poster#: 344.04/ZZ6

Topic: G.03. Emotion

Support: CNPq

Title: Effects of anger induction on human behavior - an agonistic behavior pattern

Authors: *R. M. DE ALMEIDA, J. C. CABRAL;
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Abstract: Anger is an emotion of frequent occurrence in human beings and is considered to have a deleterious potential. However, there are few studies on the role of anger on human behavior, especially with experimental protocols. Moreover, studies that have sought to empirically assess the causal mechanisms between agonistic behavior constructs are still relatively scarce. To evaluate the effects of anger induction in humans, we conducted an experiment with 75 male graduate students residing in Porto Alegre, Brazil, who were randomly selected and individually contacted. The subjects were randomly assigned in control (n = 37) and anger (n = 38) groups. We used an anger induction procedure to test the hypothesis that this emotion increases the pursuit of hierarchical status and agonistic behaviors. We measured the electromyographic activity of the corrugator muscle, testosterone and cortisol levels, as well as aggression and dominance, through behavioral tasks. As a result was found that experimental group showed higher levels of dominance behavior and aggressiveness. These results were not dependent on steroid hormone concentrations, nor the ratio of testosterone and cortisol, but it was dependent on maintenance of electromyographic activity during the collection of the dependent measures. Thus, when sufficiently intense, anger induction can provoke an increase in the occurrence of dominance and aggressive behavior, which can indicate action tendencies for the establishment and maintenance of agonistic behavior in humans.

Disclosures: R.M. De Almeida: None. **J.C. Cabral:** None.

Poster

344. Neurocircuitry of Human Emotion

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Program#/Poster#: 344.05/ZZ7

Topic: G.03. Emotion

Support: R01 MH080116

Title: Developmental perturbation of dopamine signaling increases adult aggression

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Abstract: Adult pathological aggression has been associated with hyperactivity of the dopamine (DA) neurotransmitter system. To that end, our lab has identified an adolescent (postnatal days

32 to 41) sensitive time window during which increased DA signaling affects the development of circuitries underlying adult aggression in mice. We established that DA transporter blockade during P32-41 increases adult aggressive behavior. Furthermore, heightened sensitivity to amphetamine in adulthood suggests DAergic hyperfunction in these aggressive mice. This hypothesis is confirmed by changes seen in underlying DA physiology following periadolescent DA perturbations. We demonstrate that the behavioral changes are associated with hyperactivity of the Ventral Tegmental Area (VTA) DAergic neurons, both *in vivo* and in slices. We are now dissecting the role of the DA-associated circuitry in adult aggression using *in vivo* optogenetics. We find that ChR2-based activation of VTA, and not Substantia Nigra Pars Compacta DAergic neurons, increases aggressive behavior. This supports the hypothesis that increased VTA DAergic activity underlies enhanced aggression. We next optogenetically probe VTA-target regions, and have identified that direct activation of DAergic terminals in the Lateral Septum mimics the enhanced aggression observed with VTA DAergic stimulation. We thus conclude that DA appears to be acting through the Lateral Septum to induce aggression. Together, our data provide insight into a DA period that may determine the developmental trajectory of key DA aggression neurocircuitry. This might ultimately aid prevention and treatment approaches for neuropsychiatric disorders characterized by unprovoked violence, an issue that carries serious consequences in society today.

Disclosures: D. Mahadevia: None. **C.M. Teixeira:** None. **Q. Yu:** None. **D. Suri:** None. **M. Ansorge:** None.

Poster

344. Neurocircuitry of Human Emotion

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 344.06/ZZ8

Topic: G.03. Emotion

Title: The relevance of coordinated brain and heart interactions to human personality and emotions

Authors: *E. SHOKRI-KOJORI[1], D. TOMASI[1], N. VOLKOW[2];
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Abstract: The resting state of the human brain has received overwhelming attention since the inception of fMRI. Yet the relevance of coordinated brain activity at rest for predicting human behavior has been largely unknown. Although recent evidence suggests that resting state networks may emerge from a composite of lag threads, it is unclear whether there are meaningful associations between the timing of resting state networks and body physiology including cardiac

and respiratory fluctuations. Motivated by the evidence that cardiovascular health is related to personality and emotions, we tested the hypothesis that the relative timing of resting state networks and cardiac fluctuations, particularly in low fMRI frequency components (< 0.1 Hz), contributes to aspects of human personality and emotions. For this purpose, in a cohort of 203 participants (127 females) from the Human Connectome Project dataset, we characterized the amplitude and phase of coherence between 7 cortical resting state networks (RSNs) and cardiac and respiratory recordings in 4 consecutive fMRI runs (TR = 0.72 s). For both cardiac and respiratory fluctuations, we found that the low frequencies in sensory related RSNs share reproducible amplitude of coherence ($p < 0.05$, Bonferroni), an effect that was robust to an ICA-based denoising procedure. For the phase of associations between RSN and physiology, we found that the slow-rate cardiac fluctuations precede those in RSNs, and slow respiratory fluctuations lag behind the RSNs. However, only the phase between the cardiac and sensory-related fluctuations (< 0.1 Hz) was significantly reproducible ($p < 0.05$, Bonferroni). Finally, for each individual we characterized the consistency in the timing between RSNs and physiology using a phase dispersion (PD) index for cardiac (PD-card) and respiratory (PD-resp) fluctuations. Although there were no significant associations between PD-resp and behavioral measures, the PD-card significantly predicted personality ($p < 0.0001$) and emotion factors ($p = 0.002$). In summary, we showed that slow rhythms in heart and respiration serve as leading and trailing edges of a temporal "envelope" within which brain networks are distributed. We showed that the consistency of the brain networks in this temporal envelope, in particular relative to the heart, predicts positive personality traits and emotional inclinations. These findings highlight the relevance of coordinated physiological and cortical activity to shaping a main dimension of complex human behavior.

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Poster

344. Neurocircuitry of Human Emotion

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Topic: G.03. Emotion

Support: NRF-2015R1A2A2A04006136

Title: Intersubject differences in dynamic functional connectivity associated with successful use of cognitive reappraisal and expressive suppression

Authors: *S. JUN, S. HAN;
Psychology, Yonsei Univ., Seoul, Korea, Republic of

Abstract: We regulate emotions every day in a wide variety of ways. Two most commonly adopted emotion regulation strategies are cognitive reappraisal which changes the way one feels and interprets about emotion-eliciting events into positive manner and expressive suppression which changes the way one responds to the events. The present study aims to examine the association between the inter-subject differences in dynamic functional connectivity while regulating negative emotions and the degree of neutralization which denotes successful emotion regulation. In our study, we used functional magnetic resonance imaging. Eighteen subjects viewed and rated the emotionality of negative and neutral images before they come to the experiment in order to measure the degree of emotional neutralization during the experiment which had four conditions: reappraise-negative, suppress-negative, look-negative, and look-neutral. During the study, an instructive cue designating which emotion regulation strategy should be used were given for every trial and subjects used the given strategy while watching a negative or neutral image for 12 seconds followed by evaluation phase reporting their at-the-moment feeling. Behavioral results show that overall degree of neutralization was not significantly different between reappraisal and suppression. However, subjects were able to regulate their negative emotions better when they explicitly adopted either emotion regulation strategies relative to when they used natural strategy. Using the neuroimaging data, we found that the lingual gyrus and bilateral inferior orbitofrontal regions were used for instructed emotion regulation, and the supra marginal area, thalamus and middle cingulate gyrus were used for natural emotion regulation. Using dynamic functional connectivity analysis that takes into account temporal variability of local functional connectivity over short periods of time, we not only expect to find which regions with high inter-subject functional connectivity are associated with either of emotion regulation strategies but also whether the regulatory phase with higher inter-subject functional connectivity are related to higher level of neutralization. Taken together, our study will provide the strategy-specific regions commonly used across subjects and their role in emotion regulation performance.

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Poster

344. Neurocircuitry of Human Emotion

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Topic: G.03. Emotion

Support: NIH Grant R01MH107513-01

Title: Verbal and nonverbal communications convey distinct emotional qualities through shared neural circuitry

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Abstract: Little is known about the underlying brain organizations that mediate verbal and nonverbal communication of emotion, in part due to the inability of current data acquisition systems to emulate natural conditions. Such direct communication and its relationship to emotional salience can be investigated with simultaneous acquisitions of BOLD signals from two interacting subjects using functional near-infrared spectroscopy (fNIRS), offering significant ecological validity. In this study, we test the hypothesis that verbal and nonverbal modes of communication differ in their capacity to send and receive emotional qualities such as valence and arousal. BOLD signals reflecting concentrations of deoxy-hemoglobin (comparable to fMRI signals) were acquired using a whole-head fNIRS system consisting of 84 channels divided evenly between two interacting subjects with a Shimadzu LABNIRS system. Dyads of 36 subjects participated two conditions of communication: verbal (dialogue) and non-verbal (drumming), each using alternating 15 sec epochs of "sending" (drumming or talking to partner) and "receiving" (listening to partner). In each 15 sec epoch, both partners were presented with the topic of communication: a photo from a pseudorandomized subset of the International Affective Picture System (IAPS). Emotional qualities of the topics were subdivided into valence (happy versus unhappy) and arousal (excited versus calm), which have been quantified for each photo by IAPS. When collapsing across verbal (talking) and nonverbal (drumming) communication conditions, general linear model (GLM) contrast comparisons of valence and arousal both revealed peak neural activity in the Right Superior Temporal Gyrus (rSTG) – a canonical social- and emotion-sensitive area of the brain ($p<0.05$). Contrast comparisons further revealed that during the talking condition, a cluster in the rSTG (68, -40, 22) shows increased sensitivity to positive valence over negative valence ($p<0.05$). In contrast, during the drumming condition, the same region of rSTG shows increased sensitivity to high arousal over low arousal ($p<0.05$). This natural dual-brain communication paradigm reveals a neural specificity for processing varied emotional qualities regardless of verbal or nonverbal form. Further, these findings suggest that modes of communication vary with respect to conveyance of emotional qualities; talking is optimized for valence, while drumming is optimized for arousal. Given the rising popularity of non-traditional modes of clinical therapy (e.g. drum circles for PTSD), this novel finding offers a neural basis for future research to optimize clinical care in mental health.

Disclosures: **R. Rojiani:** None. **X. Zhang:** None. **A. Noah:** None. **J. Hirsch:** None.

Poster

344. Neurocircuitry of Human Emotion

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Topic: G.03. Emotion

Title: The ‘rasa’ in the ‘raga’ - brain networks of emotion responses to North Indian Classical ragas

Authors: *A. MATHUR, N. C. SINGH;
Natl. Brain Res. Ctr., Haryana, India

Abstract: The central notion in North Indian Classical Music is that of a *raga*. The word ‘*raga*’ originates in Sanskrit and is defined as 'the act of colouring or dyeing' (the mind and mood/emotions in this context) and therefore refers metaphorically to 'any feeling of love, affection, sympathy, desire, interest, motivation, joy, or delight’. It is believed that an artist uses particular note combinations, to create a mood (*rasa*) that is unique to the *raga*. We conducted a behavioural study in which 122 participants from India rated their experienced emotion for 12 *ragas* on Likert scale of 0-4 (with 0 being ‘not at all felt’ to 4 being ‘felt the most’) for each of the following emotions - happy, romantic, devotional, calm, angry, longing, tensed, and sad. The results of the behavioural study conducted online revealed that *ragas* were indeed capable of eliciting distinct emotions. Eight ragas that were rated highest on ‘happy’ and ‘tensed’ emotion, four in each category were chosen for a subsequent functional neuroimaging experiment to investigate the neural circuits underlying these emotional responses. A separate group of 29 participants rated *raga* sequences selected from the behavioural study for ‘happy’ and ‘tensed’ emotion. The *raga* condition contrasted with rest revealed a network of areas implicated in emotion processing namely, bilateral mOFC, ACC, caudate, nucleus accumbens, insula, precuneus, auditory association areas and right lOFC, amygdala, hippocampus - para hippocampus gyrus complex and hypothalamus. Further flexible factorial analysis revealed a main effect of emotion (happy/tensed) in the bilateral mOFC. Our results provide evidence for the first time that *ragas* elicit basic emotion circuitry. They also reveal that valence information may be coded in the mOFC. Together, we demonstrate the ability of North Indian Classical *ragas* as tone sequences capable of eliciting distinct emotional responses.

Disclosures: **A. Mathur:** None. **N.C. Singh:** None.

Poster

344. Neurocircuitry of Human Emotion

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Topic: G.03. Emotion

Support: NSERC Grant

Title: Differentiating neural activity associated with implied motion and emotion in the cervical spinal cord using spinal fMRI

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Abstract: Recent fMRI studies of the brain and spinal cord (i.e., spinal fMRI) have found that emotion modifies neural activity, enhancing the individual's ability to initiate motoric responses. Unfortunately, much of this neuroimaging emotion research has used stimuli that confound emotion and implied movement (i.e., images depicting overt motion or situations in which movement is imminent). The current study addresses this issue by measuring neural activity in the cervical spinal cord (which innervates the upper limbs) during the passive perception of four types of stimuli: (1) negative images that imply movement, (2) negative images that do not imply movement, (3) neutral images that imply movement, and (4) neutral images that do not imply movement. An analysis of the data from seventeen participants demonstrated that images depicting negative stimuli that imply movement elicited greater activity in the cervical spinal cord than images depicting negative stimuli that do not imply movement. This activity was greater in the right-lateralized ventral (motoric) lower cervical and dorsal (sensory) upper cervical spinal cord segments. Similarly, images depicting neutral stimuli that imply movement elicited greater activity than neutral stimuli that do not imply movement. The activity was greater in bilateral ventral and dorsal lower, and bilateral ventral upper cervical spinal cord activity. Results indicate that implied movement elicits activity above and beyond that evoked by the perception of emotion in the spinal cord, suggesting that the motion implied by visual stimuli should be controlled for in future affective neuroscience studies.

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Poster

344. Neurocircuitry of Human Emotion

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Topic: H.02. Human Cognition and Behavior

Support: "Brain machine Interface Development" under the Strategic Research Program for Brain Sciences by AMED of Japan

Postdoctoral fellowship of the Fond de recherche du Québec - Santé (FRQS)

Title: Aligning brains to extinguish naturally occurring fears with multivoxel neurofeedback

Authors: *V. TASCHEREAU-DUMOUCHEL[1,3], A. KOIZUMI[3,4], A. CORTESE[3,4,5], M. KAWATO[3,5], H. LAU[1,3,2];
[1]Psychology, [2]Brain Res. Inst., Univ. of California - Los Angeles, Los Angeles, CA; [3]Dept. of Decoded NeuroFeedback, Advance Telecommunication Res. (ATR) Inst. Intl., Kyoto, Japan; [4]Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communication Technol. (NICT), Osaka, Japan; [5]Fac. of Information Sci., Nara Inst. of Sci. and Technol., Nara, Japan

Abstract: Decoded Neurofeedback (DecNef) is a powerful new technique in functional magnetic resonance imaging (fMRI) that allows for participants to be trained to activate multivoxel patterns associated with the representation of specific visual stimuli (Shibata et al., 2011, Amano et al., 2016). Using this technique, participants could potentially learn to activate the multivoxel representations of feared objects, which could act as a form of unconscious exposure therapy. Exposure therapies are the most effective psychological treatments for naturally occurring fears (Wolitzky-Taylor et al., 2008), but are limited by high levels of attrition. Indeed, consciously facing feared objects is a highly unpleasant experience that drives many patients to drop out of treatment. We previously demonstrated that DecNef can be used to unconsciously extinguish fear responses associated with conditioned stimuli (i.e. skin conductance response and amygdala activity) (Koizumi et al., submitted). If DecNef can also be used to extinguish naturally occurring fears, this might represent a potentially important improvement of exposure therapies, eradicating the unpleasantness of the procedure by achieving exposure unconsciously. To make sure that patients would never have to be exposed to feared objects during this procedure, we utilized hyperalignment (Haxby et al., 2011), a novel technique through which the multivoxel patterns of a specific object can be learned on a set of individuals and translated to a new target participant. We developed a 1-hour fMRI experiment that can be used to construct the decoders of 30 different animals. We used the functional images recorded during this procedure to determine hyperalignment parameters that allow transforming functional data of a group of participants to the native space of a target participant. We show in a group of

20 participants that a decoder constructed on 19 of them can accurately discriminate a given animal in a new participant that was not included in the decoder construction (up to ~ 90% accuracy). Since hyperalignment can be constructed on any visual presentation, our results suggest that hyperalignment parameters could be determined without any conscious exposure to the feared object and that the data of other participants could be used to infer the patterns used in DecNef. Taken together, these results are the first to suggest that a completely unconscious exposure therapy could be achieved using DecNef, which would potentially eliminate the aversive experience that leads many patients to drop out of exposure therapy.

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Poster

344. Neurocircuitry of Human Emotion

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Topic: H.02. Human Cognition and Behavior

Support: This research was financially supported by the European Commission's Health Cooperation Work Programme of the 7th Framework Programme, under the Grant Agreement n° 602186 (BRAINTRAIN).

Title: Real-time fMRI self-regulation of functional network connectivity during a visual motion task

Authors: *J. ECK[1,2], Q. NOIRHOMME[1,2,3], M. ROSENKE[4,2], S. BRUNHEIM[5,1], F. KRAUSE[1,2], C. BENJAMINS[1], M. LUEHRS[1,2], R. GOEBEL[1,2,6];
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Abstract: In recent years real-time fMRI (rtfMRI) neurofeedback (NF) has been increasingly used as a tool to accomplish self-regulation of brain activation in healthy controls as well as in patient populations. In the most common protocols, participants are instructed to try to up- or downregulate a feedback signal that is derived from a single brain region. Changes in local brain activity, however, are often accompanied by more complex changes in network activity patterns. As many clinical conditions can be linked to pathological changes in cortical network

connectivity, it is of growing interest to investigate the feasibility of using functional network connections as a direct feedback signal in rtfMRI-NF studies. To date, these attempts have been limited. Here we present a proof-of-concept study investigating whether functional connectivity between two cortical network nodes can be successfully utilized in rtfMRI as a feedback signal. Eight participants were asked to modulate the size of an optic flow stimulus (i.e. a radially moving dot cloud). A target circle of three different sizes was overlaid on top of the dot cloud. Participants were told that modulation of attention would be a good initial strategy to modulate stimulus size, but they were encouraged to alter and optimize their strategy depending on the feedback. NF trials lasting twenty seconds were interleaved with twenty seconds of rest. The feedback signal was derived from two nodes of an occipital-parietal attention network involved in visual motion perception: a region in the posterior parietal cortex (PPC) and human middle temporal area MT/V5. Both were defined for each participant individually in a functional localizer task prior to four rtfMRI-NF runs. The feedback signal was based on the functional connectivity between MT/V5 and PPC, as calculated with partial correlation controlling for the effect of noise by using the white matter signal.
Most participants successfully differentiated two levels of functional connectivity. Fine-grained regulation to all three different target states was not possible, likely due to the limited training and limited number of points used for the computation of the partial correlation.
Our preliminary results indicate that functional connectivity of brain networks, as estimated by partial correlation, can be successfully altered by participants using rtfMRI neurofeedback. This proof-of-concept demonstration opens up new possibilities for clinical interventions targeting the change of abnormal functional network states. Future research should further investigate the option of fine-grained connectivity regulation in a full multi-session NF protocol.

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Poster

344. Neurocircuitry of Human Emotion

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Topic: H.02. Human Cognition and Behavior

Support: 3120 DFG-Graduiertenschul

Title: Effects of a virtual reality (VR)-based functional near-infrared spectroscopy (fNIRS) neurofeedback (NF) intervention on highly-impulsive college students.

Authors: *J. HUDAK[1,2], F. BLUME[1,3], T. DRESLER[1,2], C. GAWRILOW[3,1], A.-C. EHLIS[1,2]; [1]LEAD Grad. Sch. and Res. Network, Tuebingen, Germany; [2]Psychophysiology and Optical Imaging, Clin. for Psychiatry and Psychotherapy, Tuebingen, Germany; [3]Sch. Psychology, Eberhard Karls Univ. Tuebingen, Tuebingen, Germany

Abstract: We conducted an electromyography (EMG) - controlled randomized functional near infrared spectroscopy (fNIRS) neurofeedback (NF) experiment set inside of a virtual classroom using the Oculus Rift head mounted display. The experiment was novel in its use of a virtual classroom, its intensive and truncated design, and its use of a sub-clinical population of highly-impulsive college students. Designed as a pilot study preceding a much larger study with schoolchildren with attention deficit hyperactivity disorder (ADHD), we wanted to test the effects of the intervention on a population of healthy adults displaying parts of the ADHD phenotype (high impulsivity) and many of the neuropsychological characteristics underlying the disorder. We designed a virtual classroom in which the overhead lighting was controlled by means of the subjects' dorsolateral prefrontal cortical (dlPFC) oxygenated hemoglobin (O2Hb) concentration relative to a baseline conducted before each trial. When subjects increased O2Hb concentration, i.e. activated their prefrontal cortex, then the light brightened; it darkened with deactivation. N = 20 subjects underwent eight training sessions across two weeks: n = 10 subjects were included in the treatment group while n = 10 subjects were in the control group and used their *m. supraspinatus* muscles to control the lighting. These training sessions were bookended by a pre and post test in which we measured fNIRS O2Hb average amplitudes in fronto-temporal areas on two executive functioning tasks: a go/no-go and an N-back task. Additionally, the subjects performed a stop-signal task for behavioral data analysis. Emphasis during the first week of training was on learning the neurofeedback paradigm. In the second week, activation/deactivation ratio increased from 50 to 80%, in an attempt to train the implicit activation of the dlPFC in classroom situations. Results indicated a significant reduction in commission errors (impulsive false alarms) on the go/no-go task with a simultaneous increase in prefrontal O2Hb concentration, for the treatment group but not for the control group. In addition, treatment subjects showed a reduction in reaction time variability on the stop-signal task. These results indicate a clear effect of the NF intervention in reducing impulsive symptoms. This is especially encouraging because the design contained comparatively few sessions and significant results were obtained for a small pilot sample. We expect even better results with a clinical, and child population.

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Poster

344. Neurocircuitry of Human Emotion

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Title: Embodied learning: How interoceptive signals from the heart interact with anxiety in fear conditioning and extinction

Authors: *S. N. GARFINKEL[1,2], C. D. GOULD VAN PRAAG[2], M. ENGELS[4], D. WATSON[2], T. DUKA[3], H. CRITCHLEY[2];
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Abstract: The physiological state of bodily arousal influences emotional experience and can guide learning and memory. We sought to define how interoceptive signals from the cardiovascular system influence the learning and extinction of fear. Forty healthy individuals participated in a novel fear conditioning and extinction paradigm in which stimuli were time-locked to different phases of the cardiac cycle; presented either at cardiac systole, when arterial baroreceptors signal the timing and strength of individual heartbeat to the brain, or at diastole, in-between heartbeats when these baroreceptors are quiescent. During the acquisition of fear conditioning in a partial reinforcement paradigm, two stimuli (CS+systole and CS+diastole) were paired with the delivery of a shock and compared to stimuli that were never paired with shock (CS-systole, CS- diastole). During extinction learning, the contingencies between these stimuli in relation to cardiac phase (systole or diastole) remained constant (N=19), or were switched (N=21). Fear processing was quantified from electrodermal activity (skin conductance responses; SCRs) and subjective reports. There were three major findings: First, stimuli presented at systole evoked greater fear responses, with enhanced SCRs to CS-systole. Systole therefore disrupted the differential learning of conditioning fear and safety. This effect extended to the second day, where fear memory for stimuli originally presented at systole remained elevated. Second, the systolic heightening of fear responses was driven by individuals with high trait anxiety. Third, memory for fear (CS+ and CS-) was sensitive to the short-term cardiovascular context (systole and diastole) and was disrupted when these contingencies switched. Together our findings highlight how emotional learning and memory access is guided by interoceptive signals; including the congruency of cardiac phase during encoding and retrieval. The impact of heart signals on fear responses further reflects individual differences in

trait anxiety. Body and mind are dynamically coupled and these findings illustrate how feedback from states of autonomic arousal can help shape cognition and emotion.

Disclosures: S.N. Garfinkel: None. **C.D. Gould van Praag:** None. **M. Engels:** None. **D. Watson:** None. **T. Duka:** None. **H. Critchley:** None.

Poster

345. Depression: Human Postmortem Studies

Location: Halls B-H

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Program#/Poster#: 345.01/AAA3

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Campbell Family Foundation Operating Grant

R01 MH077159-09

Title: Proteomic analysis of postmortem anterior cingulate cortex reveals persistent disease effects across MDD states

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Abstract: *Background:* Major depressive disorder (MDD) is a severe mental illness and according to the WHO, the leading cause of lost years of productivity. Moreover, patients with mood disorders (including MDD) account for ~60% of completed suicides. MDD subjects are characterized by emotion dysregulation, low mood and feelings of anxiety. Most MDD patients suffer from a chronic illness with recurring episodes of increasing symptom severity, longer duration, shorter or partial remission periods and increasing resistance to antidepressants, which is suggestive of neuro-progressive pathology where outcomes and treatment efficacy are inversely correlated with earlier disease onset and increased numbers / length of depressive episodes.
Predictions: Proteomic analysis of the sgACC samples from MDD patients will show a persistent disease effect across episodes and remission states, in comparison to controls.
Methods and Results: Postmortem sgACC samples obtained from four MDD cohorts at various stages of disease or remission and one control cohort (n=90), were analyzed by LC-MS/MS then subjected to label free quantitation using MaxQuant software. Differentially-expressed label free

quantified proteins were identified by the random intercept model (RIM) with parameter selection using the smallest Bayesian Information Criterion to account for potential covariates. MDD-specific covariates (e.g., psychosis, alcohol dependence, antidepressant drug use and death by suicide) were tested in MDD cases only using post-hoc analysis of variance. This analysis yielded 192 differentially expressed proteins at $p \leq 0.1$ and RIM coefficient effect $\geq \pm 0.26$ (-log2(MDD/Control)) thresholds. The most robust findings suggest the presence of a persistent disease pathology across MDD episodes and remission that involves dysregulation of cytoskeletal organization by Rho GTPase signaling, integrin signaling, axonal guidance and GABA or glutamate receptor signaling related proteins. Additionally, changes in axonal guidance related proteins were specific to MDD subjects in current episodes, whereas alterations in other proteins groups were also observed in the remitted subjects.
Summary: Findings from this work demonstrate the existence of persistent disease effects in all MDD patients, regardless of MDD episode or remission phase. The nature of protein changes results confirm previous MDD studies showing changes in presynaptic neurotransmission, synaptic function, cytoskeletal re-arrangements and energy metabolism. Additionally, we show enrichment in phosphatidylglycerol biosynthesis I and CDP-diacylglycerol biosynthesis related proteins.

Disclosures: **E. Scifo:** None. **M. Pabba:** None. **F. Kapadia:** None. **C. Ma:** None. **D.A. Lewis:** 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; investigator-initiated research support from Pfizer, and in 2012-2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-Myer. **G.C. Tseng:** None. **E. Sibille:** None.

Poster

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Sex specific transcriptional signatures in human depression

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Abstract: Introduction Besides being 2-3 times more susceptible to major depressive disorder (MDD) than males, females also exhibit different symptomatic profiles, antidepressants responses, and biological adaptations to stress. These sex-specific differences are believed to be accompanied by different transcriptional signatures across brain regions. This study aims at defining the sex-specific transcriptional signatures and gene expression networks in the brain associated with MDD and characterizing their mechanistic implications.
Methods RNAseq was performed on brain regions from postmortem brains of humans with MDD and controls. Transcriptional profiles of NAc and PFC from male and female mice after chronic variable stress (CVS) were also analyzed. For both humans and mice, differential analysis was performed with voom Limma and gene expression networks were constructed and analyzed through weighted gene co-expression network analysis (WGCNA). Viral mediated gene transfer in mice was used to assess the functional relevance of our findings.
Results Males and females with MDD exhibit drastically distinct transcriptional signatures. Several hundreds of genes were differentially expressed across every brain region in depressed males and depressed females but with a strikingly small overlap, results that were also found in mice after CVS. We identified both common and divergent gene networks between depressed males and depressed females associated with a gain or loss of connectivity in MDD and enriched for differentially expressed genes in males or females. By virally manipulating genes within these networks, we induced stress susceptibility in a sex-specific fashion in mice. Viral knockdown of Dusp6 (downregulated in female MDD and stressed mice) in PFC increased stress susceptibility in females only. Consistent with Dusp6's action as an ERK phosphatase, we found higher levels of phospho-ERK, but not total ERK, in PFC of both females with MDD and female mice after CVS. IHC confirmed these findings in mice and showed that these effects are specific to a cell subpopulation in layer 5 of PFC. Dusp6 knockdown also increased the frequency but not amplitude of sEPCS in PFC of female but not male mice. RNAseq performed on PFC tissue after Dusp6 knockdown highlighted its impact on the gene network's structure and identified several potential gene targets interacting with Dusp6 to mediate its molecular, physiological, and behavioral effects.
Conclusions Our findings suggest that males and females with MDD show largely distinct transcriptional signatures in brain, which control the activity of molecular cascades and consequent behaviors in a sex-specific fashion.

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Poster

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH

HDRF

Title: Exploring the role of long non-coding RNAs in depression

Authors: *O. ISSLER, B. LABONTÉ, I. PURUSHOTHAMAN, B. J. HARTLEY, D. M. WALKER, C. J. PEÑA, Z. LORSCH, K. J. BRENNAND, L. SHEN, E. J. NESTLER;
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Abstract: Depression is a common, chronic, and debilitating disorder. The molecular mechanisms underlying depression are only partially understood and there is a great need for new antidepressant drugs, particularly for the large proportion of treatment-resistant patients. Long non-coding RNAs (lncRNAs) are a large class of regulatory transcripts, which represent a substantial portion of the human genome. To date there is virtually no characterization of the role of lncRNAs in depression. To address this issue, we utilized unique and comprehensive genome-wide profiles of RNAs, in several brain regions from both male and female post-mortem depressed human subjects. Overall, lncRNAs, mostly from the biotypes of long intragenic RNAs (lincRNAs) and antisense, represent about a third of the differentially regulated genes in depressed subjects compared to controls. We identified a complex pattern of differentially regulated lncRNAs that are region-and sex-specific. To bioinformatically identify target genes of regulated lncRNAs, we performed correlation analysis between the expression levels of lncRNAs and those of protein-coding genes in males compared to females. Next, RNA-seq results of loss- and gain-of-functions of lncRNAs are overlaid with the findings from DNA sequences of Chromatin Isolation by RNA Purification (ChIRP), in human neuron progenitor cells (NPCs). In parallel, to establish a causal role for specific lncRNAs and their target genes as part of the molecular mechanisms affecting depression, the expression levels of lncRNAs and their targets genes are manipulated in the relevant brain sites in male and female mice followed by phenotyping at the behavioral and molecular levels. Preliminary data indicate that depression- and anxiety-related behaviors were altered by overexpression of human-specific depression-related lncRNAs in female mice. These studies promise a fundamentally new view of brain molecular adaptations that contribute to depression risk and will identify novel targets for improved treatments or biomarkers.

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Poster

345. Depression: Human Postmortem Studies

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Support: Pritzker Neuropsychiatric Disorders Research Consortium

NIH R01MH104261

ONR N00014-12-1-0366

Hope for Depression Research Foundation

Title: Fibroblast growth factors 2 and 9 may act as molecular organizers in anterior cingulate cortex and hippocampus to mediate circuit function in MDD

Authors: *E. L. AURBACH[1], M. H. HAGENAUER[1], K. E. PRATER[1], W. E. BUNNEY[2], R. M. MYERS[3], J. D. BARCHAS[4], A. SCHATZBERG[5], J. Z. LI[1], F. MENG[1], S. J. WATSON[1], H. AKIL[1];
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Abstract: The neurotrophic hypothesis posits that changes in the expression and function of growth factors in the brain underlie the pathophysiology of Major Depressive Disorder (MDD). Previous work implicated two members of the fibroblast growth factor (FGF) system in MDD and affective dysregulation in animal models. We have previously shown that FGF2 as an endogenous anxiolytic and antidepressant molecule whose expression is downregulated in the depressed brain, while FGF9 has anxiogenic and pro-depressant properties and is upregulated in the depressed brain. However, it is unknown if FGF2 and/or FGF9 act in isolation to mediate these effects or if they are part of a larger ensemble of genes which become selectively dysregulated in MDD. Because we hypothesized that relative levels of FGF2 and FGF9 might be important to MDD pathophysiology, we examined diagnosis-specific relationships in expression between FGF2, FGF9, and FGF receptors, and we found regional patterns of alteration with MDD. In the anterior cingulate cortex, correlations between FGF family members were lost in

MDD, while in the hippocampus, new relationships emerged. These changes were related to alterations in correlated gene expression of transcripts related to fundamental biology and circuit function, supporting the hypothesis that FGF2 and FGF9 may influence affect by acting as molecular organizers whose effects become dysregulated during MDD. Future studies will validate these findings in other datasets and on other platforms, and explore the possibility that coordinate dysregulation of FGF2 and FGF9 with an ensemble of other genes can serve as a biological signature of MDD.

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Poster

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Pritzker Neuropsychiatric Research Consortium

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Hope for Depression Research

Title: Altered levels of polyamine metabolic genes in various sub-nuclei of the human amygdala in major depressive disorder

Authors: *V. SHARMA[1], M. HAGENAUER[1], S. CHAUDHURY[1], R. C. THOMPSON[1], R. M. MYERS[2], A. F. SCHATZBERG[3], J. D. BARCHAS[4], W. E. BUNNEY[5], H. AKIL[1], S. J. WATSON[1];
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Abstract: The dynamic homeostasis of the level of polyamines plays a definitive role in regulating structure and functions of the cell. Due to the polycationic nature of these molecules, they play an important role in the cell growth, proliferation, transcription, translation, repair of extracellular matrix and cell signaling processes. Increased levels of polyamines cause

detrimental effects to the cell by induction of apoptosis and cell transformation. The altered expression of genes responsible for synthesis and/ degradation of polyamines have been linked to the neurodegeneration of cells in different regions in the brain resulting in pathophysiology of various psychiatric conditions like schizophrenia, bipolar disorder and major depressive disorder. Major depressive disorder (MDD) patients present with diverse yet debilitating symptoms implicating dysfunction of multiple brain regions of which the amygdala is an important regulatory center. The amygdala plays an important role in emotion, motivation and other higher cognitive functions that contribute to the subjective value to the stimulus. However, still there is a scant knowledge describing the effects of altered levels of expression of polyamine metabolic genes in the human amygdala particularly in MDD. Additionally, the human amygdala is a heterogeneous structure comprising of numerous sub-nuclei that vary in size and shape throughout the anterior-posterior axis. We used laser capture microdissection (LCM) to dissect the nuclei from the human amygdala precisely into ten divisions (Lateral (L), Basal (B), Accessory Basal (AB), Central (CE), Medial (M), Cortical (CO), Periamygdaloid Cortex (PAC), Amygdalohippocampal Area (AHA), Anterior Amygdaloid Area (AAA) and Paralaminar (PL) nuclei). To assess the changes in the levels of polyamine metabolic genes in various sub-nuclei of human amygdala, postmortem human brain of normal healthy and MDD subjects were used for LCM based microarray gene expression analysis and qRT-PCR. Preliminary results from microarray data show differentially the altered level of polyamine metabolic genes in various sub-nuclei of human amygdala. The results are discussed in light of the function of various sub-nuclei of human amygdala, which will help us in understanding the role of polyamines and their metabolic enzymes in the functioning of human amygdalar sub-nuclei in major depressive disorder.

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

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Colciencias (Health Sciences Program, # 823-2015)

Title: A comprehensive regional analysis of genome-wide expression profiles for major depressive disorder

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Abstract: Objective: Major depressive disorder (MDD) is a global health challenge. In recent years, a large number of genome-wide expression studies (GWES) have been carried out to identify the transcriptomic profiles for MDD. The objective of this work was to carry out a comprehensive meta-analysis of available GWES for MDD. Method: GWES for MDD with available raw data were searched in NCBI GEO, Array Express and Stanley databases. Raw GWES data were preprocessed and normalized and meta-analytical procedures were carried out with the Network Analyst program. 743 samples from 24 primary studies were included in our meta-analyses for blood (Blo), amygdala (Amy), cerebellum (Cer), anterior cingulate cortex (ACC) and prefrontal cortex (PFC) regions. Afunctional enrichment analysis was carried out. Results: We identified 35, 793, 231, 668 and 252 differentially expressed (DE) genes for Blo, Amy, Cer, ACC and PFC regions. A region-dependent significant enrichment for several functional categories, such as gene ontologies, signaling pathways and topographic parameters, was identified. There was convergence with other available genome-wide studies, such as GWAS, DNA methylation analyses and miRNA expression studies. Conclusions: This is the largest meta-analysis for GWES in MDD. The examination of convergence of genome-wide evidence and of the functional enrichment analysis provides a global overview of potential neural signaling mechanisms dysregulated in MDD. Our comprehensive analysis of several brain regions identified lists of DE genes for MDD that are interesting candidates for further studies.

Disclosures: Y. González: None. **G.P. Guio:** None. **D.A. Forero:** None.

Poster

345. Depression: Human Postmortem Studies

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Program#/Poster#: 345.07/AAA9

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CIHR

FRQS

Title: The Effect of Early Life Adversity on the Oxytocinergic System: from childhood maltreatment and suicide to natural variation in rat maternal care

Authors: *D. ALMEIDA, L. FIORI, G. TURECKI;
McGill Group For Suicide Studies, Verdun, QC, Canada

Abstract: Oxytocin is a mammalian neurohypophysial hormone which acts primarily as a neuromodulator in the CNS. The early development of secure attachments, relationship quality, and the ability to regulate and manage emotions are all instances of psychological resources influenced by this system. Previous studies have shown that early life adversity might act to perturb the oxytocinergic system during critical developmental periods. A body of literature also supports alterations in the oxytocinergic system as a predisposing factor for suicidal behaviour. As such, our research looks into the expression of genes regulating the oxytocinergic system in the prefrontal cortex of male suicide completers with versus without a history of childhood maltreatment, along with healthy controls. Our expression data indicates an effect of abuse on genes involved in oxytocin metabolism and function. As a second aim of our studies, we have investigated *oxtr* expression in adult male rats raised with low versus high early maternal care. Rats of the low maternal care group displayed a similar *oxtr* expression pattern in the cingulate cortex as with what was found in suicide completers with a history of abuse. These data thus point to a similar effect of the early life environment on oxytocinergic function in both humans and rodents.

Disclosures: D. Almeida: None. **L. Fiori:** None. **G. Turecki:** None.

Poster

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: FRSQ Grant

Title: Analysis of myelin-associated genes and proteins in postmortem samples of uncinate fasciculus from suicides having experienced early life adversity

Authors: *M. J. SHAW[1], A. TANTI[2], M. DAVOLI[3], N. MECHAWAR[3], G. TURECKI[3];
[1]Integrated Program in Neurosci., McGill Univ., Verdun, QC, Canada; [2]McGill Univ., Montreal, QC, Canada; [3]Mcgill Univ., Montreal, QC, Canada

Abstract: Objective The main hypothesis driving this project is that cerebral white matter alterations may be evidenced in postmortem samples from depressed suicides, and such alterations result from changes in the population of oligodendrocytes and/or their precursors as

well as in myelin produced by mature oligodendrocytes. To test this hypothesis, are analyzing postmortem human uncinate fasciculus (UF) samples, comparing data between depressed suicides exposed or not to early life adversity (ELA), and matched sudden-death controls. Our aims are to (i) determine the densities of myelinating oligodendrocytes and their precursors using immunohistochemistry and stereology, and to (ii) assess myelin integrity through histochemistry and molecular approaches.
Methods Matched frozen UF samples are being analyzed from 17 depressed suicides having suffered from ELA, 15 depressed suicides without ELA, and 17 healthy controls having died suddenly. The expression of major myelin protein constituents are determined by immunoblotting and qPCR. *Histology and immunohistochemistry (IHC)* Fixed tissue samples will be cut serially using a cryostat. Subsets of sections will be processed for Luxol Fast Blue staining of myelin. Stained slides will be scanned with high resolution, and quantitative image analysis of myelin density performed with ImageJ. Using established protocols routinely used by our group, adjacent sections will be processed for free-floating IHC using primary antibodies directed against Olig2 to visualize oligodendrocytes and their precursors. Immunostaining will be visualized with peroxidase-DAB, and cell numbers determined with stereology using an optical fractionator (OF) probe (StereoInvestigator; mbf Bioscience).
Results Preliminary results suggest that the the expression of myelin-related proteins is altered in the UF of depressed suicides having suffered from ELA. In particular, myelin-related proteins myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) show significantly elevated expression in depressed suicides having suffered from ELA as compared to depressed suicides who have not experienced ELA.
Conclusions This project constitutes the first comprehensive postmortem study of UF myelin and myelinating cells in depression and ELA, and should help gain a better understand of the neurobiological substrates of mood disorders. Cellular and molecular evidence of altered myelination in this white matter tract may reflect altered transmission between the temporal and frontal lobes following ELA.

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Poster

345. Depression: Human Postmortem Studies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH RO1 MH 56528

RO1 MH98554

Title: Abnormal gene expression of proinflammatory cytokines in the postmortem brain of depressed suicide victims

Authors: *H. ZHANG, H. RIZAVI, X. REN, G. PANDEY;
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Abstract: There is growing evidence of the abnormalities of the immune systems in the pathogenesis of depression. Several studies suggest that inflammatory mediators play a critical role in the pathophysiology of both major depression and suicidal behavior. Whereas the levels of cytokines have been studied in great details in depression, the role of cytokines in suicide and suicidal behavior is less clear. In an earlier study, we reported abnormal gene expression of proinflammatory cytokines in the lymphocytes of depressed patients. To further examine if major depressive disorder (MDD) and suicidal behavior is also associated with abnormal gene expression of cytokines, we examined protein and mRNA expression of proinflammatory cytokines in postmortem brain of depressed suicide victims. In this study, we determined the protein and mRNA expression of proinflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α, and TNF-β, IL-10, IL-13 in prefrontal cortex (PFC) of depressed suicide victims and normal control subjects. The postmortem brain tissues were obtained from the Maryland Brain Collection. Protein levels of cytokines were determined by ELISA, and mRNA levels of cytokines were determined by the qPCR method. We found that the protein and mRNA levels of proinflammatory cytokines TNF-α, TNF-β, IL-1β, and IL-6 were significantly increased and protein and mRNA levels of the anti-inflammatory cytokine IL-10 were significantly decreased in the PFC of depressed suicide (DS) victims compared to controls. No significant differences were observed in the protein and mRNA levels of IL-8 and IL-13 in the PFC of DS subjects and normal controls. These observations suggested an up-regulation of the proinflammatory cytokines in suicide and down-regulation of one of the anti-inflammatory cytokines in suicide and possibly depression.

Disclosures: **H. Zhang:** None. **H. Rizavi:** None. **X. Ren:** None. **G. Pandey:** None.

Poster

345. Depression: Human Postmortem Studies

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American Foundation for Suicide Prevention (SRG-XXXX-001778-1209)

Title: Molecular insights of dysregulated microrna network in locus coeruleus of suicide brain.

Authors: *B. ROY[1], M. PALKOVITS[2], G. FALUDI[2], Y. DWIVEDI[2];
[1]Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL;
[2]Psychiatry and Behavioral Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Norepinephrine (NE), one of three catecholamine neurotransmitters in the brain, is produced primarily by neurons in the locus coeruleus (LC). Retrograde and ultrastructural examinations reveal that the core of the LC and its surrounding regions receive afferent projections from several brain areas which provide multiple neurochemical inputs to the LC with changes in LC neuronal firing being a highly coordinated event. On the other hand, NE containing fibers from the LC innervate nearly the entire brain. Although NE, NE receptors, and mediated signaling system have been studied in relation suicide as well as psychiatric disorders that significantly increase the risk of suicide; the molecular network changes within LC have not been studied. Molecular and cellular events like epigenetic modifications influencing gene expression are the major focus of research in stress-related disorders including major depression and suicide. MicroRNA (miRNA) is one of the candidate epigenetic modifiers from small non coding RNA family which has the innate ability to induce disease phenotype by regulating expression of a large number of genes in a cohesive and coordinated fashion. In this study, we examined miRNA networks by analyzing 768 miRNAs in LC of 9 depressed-suicide subjects and 11 matched healthy controls. Low density qPCR based expression macroarray (TLDA) revealed differential regulation of 13 miRNAs in LC of suicide brain. Interaction between altered miRNAs and target genes showed dense interconnected molecular network, in which multiple genes were predicated to be targeted by the same miRNAs. Functional clustering of predicated target genes yielded stress induced disorders such as anxiety, hyperactive behavior, post traumatic disorders that collectively showed the complex nature of suicidal behavior. Altogether, our study for the first time reveal the involvement of LC based dysregulated miRNA network in disrupting cellular pathways associated with suicidal behavior.

Disclosures: **B. Roy:** None. **M. Palkovits:** None. **G. Faludi:** None. **Y. Dwivedi:** None.

Poster

345. Depression: Human Postmortem Studies

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The American Foundation for Suicide Prevention

The Diane Goldberg Foundation

The Brain and Behavior Research Foundation

Title: Poly ADP-ribose polymerase are more in subjects with major depressive disorder and disintegrate neural progenitors in dentate gyrus of human hippocampal formation

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Abstract: Adult neurogenesis in human hippocampus was first showed by Altman's and Das and confirmed in the adult mammalian and human brain. Angiogenesis and neurogenesis are co-regulated in human and mice, and are altered in major depression (MDD) and in response to antidepressant treatment. Proliferation of neural progenitors cells (NPCs) and their differentiation into mature granule neurons (GNs) are altered in animal models of depression and by stress, as well as by antidepressants. We have found that treatment with selective serotonin reuptake inhibitors (SSRI) is associated with more neural progenitor cells (NPCs), mitotic cells and GNs selectively in anterior dentate gyrus (DG) in MDD. In untreated MDD we found fewer GNs than in controls and SSRI-treated MDD. We do not know if depression and stress-mediated stimuli, or SSRI, elicit changes in gene expression by activating proteins involved in maturation, differentiation and cell viability. Poly [ADP-ribose] polymerase (PARP-1) is a DNA-binding protein that protects survival via enzymatic DNA repair, activated by DNA strand breaks. It is unknown if PARP-1 is expressed in NPCs, neuroblast and GNs in the human DG and if it is

involved in the regulation of neurogenesis in MDD and in response to SSRI treatment.
To investigate signaling mechanisms involved in the regulation of neurogenesis in MDD and with SSRI treatment in human, we assessed the cellular localization of PARP in mature GNs, NPC and immature neuroblasts in human DG using triple immunofluorescence labelling (1:1K PARP, Abcam; 1:50K NeuN; 1:3K Nestin and 1:2K PSA-NCAM) and confocal microscopy (Olympus America Inc., Melville, New York, NY). Clinical data were obtained using psychological autopsy; toxicology and neuropathology exams were performed on all samples. Triple-labeling fluorescence confocal microscopy showed PARP-1 expression in NPCs soma and in the lumen of vasculature, on endothelial cells stained with CD31. Nestin antibody stained the entire blood vessels and the cell perikaryon as well as dendrites of NPC. We found that expression of PARP in NPCs co-labeled with Nestin leads to disintegration of cell soma of NPCs and pruning of dendrite processes and neurites. PSA-NCAM-immunoreactive neuroblasts also showed co-localization with PARP-1 staining. We found PARP-1 co-localized with NeuN in DG neurons in the DG and in the hilus.
Findings suggest that PARP is expressed in human NPCs, neuroblasts and GNs and could regulate their survival in MDD and with SSRI treatment, possibly explaining our findings of fewer GNs in MDD. Quantification of NPCs and neuroblasts expressing PARP-1 is warranted to assess if PARP-1 expression affect maturing cell numbers.

Disclosures: **M.K. Jaiswal:** None. **A. Dwork:** None. **V. Arango:** None. **G. Rosokija:** None. **J. Mann:** None. **R. Hen:** None. **M. Boldrini:** None.

Poster

345. Depression: Human Postmortem Studies

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Title: Epigenetic and transcript aberrations of the galanin system in major depressive disorder

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Abstract: Major depressive disorder (MDD) is a common, serious and heterogeneous illness with a varying rate of progression, an often inconsistent response to treatment and mostly without established mechanism(s). However, exposure to stress is an important factor and can confer lasting behavioral and biological effects that in addition may be modified by epigenetic factors like DNA methylation. Current pharmacological treatments of MDD often target the monoamine transporters, but the results are far from satisfactory, also because the drugs may have substantial side effects. In the quest for better treatment options, much attention has in recent decades been focused on neuropeptides and their receptors, including galanin. Galanin is a 29 (30 in humans) amino acid neuropeptide that is co-expressed with and modulates noradrenaline and serotonin systems in the rat brain. Animal behavioural experiments and human genetic studies suggest that galanin is involved in the pathophysiology of mood disorders. In this study, we used pyrosequencing and quantitative real-time PCR (qPCR) to analyze DNA methylation changes and transcript expression, respectively, of galanin and its receptors in five different regions of post-mortem human brains. The regions included were Broadmann area (BA) 8/9, BA 24, locus coeruleus (LC), the dorsal raphe nucleus (DRN) and the medullary raphe nucleus (MRN). In total, 212 samples from age and gender matched controls and suicides (with MDD) were processed. Galanin promoter methylation was upregulated in BA8/9 of males, and downregulated in LC and DRN of males and females, and corresponding changes in mRNA levels were found in the same set of samples. With respect to the receptors, GalR1 and GalR2 promoter methylation in the analyzed CpG sites were not significantly changed in depressed suicides versus controls; however, GalR1 mRNA levels were significantly increased in BA8/9 and DRN of males and females and in LC of female brain samples. GalR3 promoter was hypomethylated in female samples of DRN and LC from both genders, with a corresponding upregulation in mRNA levels. These data link changes in mRNA levels and epigenetic alterations in the galanin system to suicidal behavior, making galanin receptors a novel target for development of antidepressant therapeutics.

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Poster

345. Depression: Human Postmortem Studies

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The American Foundation for Suicide Prevention

The Diane Goldberg Foundation

The Brain and Behavior Research Foundation

Title: Increased expression of PARP-1 in granule cells of human hippocampus in major depressive disorder

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Abstract: Based on animal models of depression it have been hypothesized that there is a defect of neurogenesis in the dentate gyrus (DG) in major depressive disorder (MDD). We previously reported that there are fewer granule neurons (GNs) and smaller DG volume selectively in the anterior human hippocampus in MDD compared to control subjects and this seems to be reversed in subjects with MDD who received antidepressant treatment. It is not clear if in MDD there are fewer neural progenitor cells (NPCs), therefore we hypothesized that in MDD there could be an impairment in neuronal differentiation, or in neuronal cell survival. For this reason we assessed the expression of Poly(ADP-ribose) polymerase (PARP-1), a molecule involved in DNA repair, transcription and apoptosis pathway, in DG cells in MDD. To that end, we analyzed the number PARP-1 immunoreactive GNs and glial cells in human hippocampus from subjects: 1) with a history of MDD and no psychopharmacologic treatment in the last three months before death, with clear brain and blood toxicology (n=6); 2) age and gender matched MDDs treated chronically with antidepressants (MDD-SSRI) (n=6); and 3) age and gender matched controls

without any neuropsychiatric disease or treatment (Controls) (n=6). Immunohistochemistry using an anti- PARP-1 antibody (1:1000, Abcam, Cambridge, USA) was performed in 50 μm thick hippocampus sections from post-mortem human brains. PARP-1 immunoreactivity was visualized using diaminobenzidine-peroxidase chromogenic system and sections were stained for Nissl with Cresyl violet to identify GNs and glia. We estimated the number of PARP-1 immunoreactive cells in DG, including subgranular zone (SGZ), granule cell layer (GCL) and molecular layer (ML), using stereology (StereoInvestigator software, MBF, Williston, VT, USA). We analyzed one section every 2 mm throughout the anterior, mid and posterior DG. We found more PARP-1 immunoreactive GNs in the anterior hippocampus in untreated MDD group compared with the Control ($p<.05$). No significant difference was observed between untreated MDD and MDD-SSRI. There was no difference in the number of PARP-1 immunoreactive glial cells between groups. Our results show that MDD is associated with more GNs expressing PARP-1, in the anterior region of the human hippocampus, suggesting a possible role of PARP-1 in cell death pathways of cell of the anterior DG, the region affected by neuroendocrine responses to stress. Glucocorticoids could possibly have a role in the regulation of cell survival through this pathway in MDD. Our findings could explain the observed reduced number of mature neurons in the anterior DG in MDD.

Disclosures: C. Zizola: None. **A. Dwork:** None. **V. Arango:** None. **G. Rosokija:** None. **J. Mann:** None. **R. Hen:** None. **M. Boldrini:** None.

Poster

346. Eating Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: FIRB-RBFR12DELS to CC

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Title: Transcriptional effects on hypothalamic inflammatory mediators in an animal model of binge eating

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[1]Univ. of Camerino, Sch. of Pharmacy, Pharmacol. Unit, Camerino, Italy; [2]Univ. of Modena and Reggio Emilia, Department of Life Sciences, Italy

Abstract: Binge eating episodes are characterized by uncontrollable, distressing eating of a large amount of highly palatable food and represent a central feature of bingeing related eating disorders. Research suggests that inflammation could play a role in the onset and maintenance of eating-related maladaptive behavior. These studies mainly assessed serum levels of pro-inflammatory cytokines in patients suffering from eating disorders. However, markers of inflammation can be altered in the brain while remaining unchanged in the periphery. Indeed, pro-inflammatory cytokines can regulate food intake directly by affecting the hypothalamic neurons implicated in the regulation of eating behavior and appetite or even indirectly via their impact on neuropeptide-neurotransmitter functionality. In the present study we measured the levels of expression of different components of cytokine systems (IL-1, IL-6, IL-18, IL-33, TNF-α and IFN-ɣ) and related molecules (iNOS and COX2) in the preoptic and anterior- tuberal parts of the hypothalamus of a validated animal model of binge eating. In this animal model, based on the exposure to both food restriction and frustration stress, binge-eating behavior for highly palatable food is not shown during the estrus phase. We found decreased levels of IL-1Ra and IL-33 mRNAs in both the hypothalamic regions evaluated in non-estrous rats developing binge-like eating behavior. In the same group of animals, specific differences in the regulation of the expression of components of the IL-18/IL-18R system were found in the anterior-tuberal hypothalamus possibly leading to a general impairment of the functionality of this cytokine in presence of stress-induced binge-eating behavior. Our data suggest that targeting inflammatory markers centrally could be possible to prevent that excessive palatable food consumption turn into bingeing-related eating disorders.

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Poster

346. Eating Disorders

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Program#/Poster#: 346.02/AAA17

Topic: G.07. Other Psychiatric Disorders

Title: Enhanced coupling between salience network and basal ganglia network predicts distorted eating attitude in anorexia nervosa

Authors: ***M. ISOBE**[1], Y. MORI[1], J. MIYATA[1], H. FUKUYAMA[2], S. NOMA[1], T. MURAI[1], H. TAKAHASHI[1];
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Abstract: Introduction
As well as basal ganglia, insula, implicated in regulation of energy intake, has gathered attention as a responsible region for anorexia nervosa (AN) within reward system. Insula also contributes to the processing of external reward-related information. Given that insula, as a node of the salience network (SN), plays a critical role in mediating dynamic interactions between externally oriented and internally related brain states, we hypothesized the functional correlation between SN and reward system can affect decision making in eating. In the current study, we employed food sharing task and resting state functional MRI (rsfMRI) to investigate the neural correlates of eating behaviors in AN patients.
Methods
Twenty-five female AN patients (restricting type: 11, binge-eating/purging type: 14) and 21 healthy controls (HC) were participated in the study. The food version of 'dictator game' task was employed, in which each participant was asked to share the food with an unfamiliar person. Food amounts, which were assumed to be appropriate for themselves and the others, were asked after the task. The ratio of the food amount allocated to oneself over that to the other was calculated for each participant, and was used as the index of eating attitude (meal amount ratio; MR). The rsfMRI data were analyzed by independent component (IC) analysis. Two ICs were identified as the networks of interest: 1 for SN, and 1 for basal ganglia network (BGN). Group differences of intra-network connectivity within each network were tested for contrasts of HC-AN and AN-HC, using a threshold free cluster enhancement (TFCE) correction. Inter-network connectivity between SN and BGN was calculated by partial correlation between the time series of each network.
Results
No differences were seen in understanding of the behavioral experiment and in reaction time. The result of behavioral experiment showed that AN offered the bigger amount of food to the other, compared with HC ($p<0.001$). MR of AN (0.78 ± 0.28) was significantly lower than that of HC (1.00 ± 0.07) ($p<0.01$). In the intra-network connectivity analysis, significantly increased functional connectivity within BGN was shown in AN. Inter-network connectivity between SN and BGN was negatively correlated with MR in AN ($r=0.48$, $p<0.05$).
Discussion
Compared to HC, AN patients offered bigger amount of food, presumably reflecting their distorted belief that small amount of meal was enough for them. This study also showed that the strength of such distortion was correlated with an enhanced coupling between SN and BGN. Excessive dieting of AN may partly derive from abnormal dynamism of these two networks.

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Poster

346. Eating Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: CONACyT Grant 424822

Title: Sex and strain-dependent response to a stress free animal model of binge eating.

Authors: *H. PAPACOSTAS QUINTANILLA[1], V. M. ORTÍZ-ORTEGA[3], C. LÓPEZ-RUBALCAVA[2];
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Abstract: Introduction: The binge eating disorder (BED) was for the first time defined as a specific eating disorder in the DSM-V in 2013. This disorder is characterized by constant binge eating episodes without any inappropriate compensatory behaviours. Today is the most prevalent eating disorder worldwide and very little of its neurobiology is known. There is clear evidence linking BED to anxiety and depression. The Wistar Kyoto (WKY) rat strain, first used as a natural hypertension model, are now recognized for being more prone to develop anxiety and depression like behaviour. In this research we studied the effects of the difference between rat strains with different predisposition to develop anxiety or depression like behaviours in the induction of a binge eating. Methodology: Animals were exposed 3 times a week to a 2 hours period where access to palatable was granted. At all times animals had free access to pellets and water. Water, pellets and palatable food consumption were measured. Results: Both strains developed a strong binge eating like behaviour consuming more volume of palatable food than its theoric gastric capacity, and restricted their caloric intake during the periods were non palatable food was available. The WKY rat strain consumed significantly bigger amounts of palatable food than the Wistar strain, surpassing its theoric gastric capacity by more than 300%, when the binge eating like behaviour was stablished.

Disclosures: H. Papacostas Quintanilla: None. **V.M. Ortíz-Ortega:** None. **C. López-Rubalcava:** None.

Poster

346. Eating Disorders

Location: Halls B-H

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Topic: G.07. Other Psychiatric Disorders

Title: Prefrontal cognitive control over interference by food images in binge eating disorder and bulimia nervosa

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Abstract: Objectives
Binge eating disorder, which is characterized by recurrent episodes of binge eating without inappropriate compensatory behavior, has been accepted as an official diagnosis in DSM-5. The impairment of cortico-striato-thalamo-cortical circuits is known as a contributing factor of development and maintenance of bulimia nervosa, however the difference between bulimia nervosa and binge eating disorder are still under debate. We investigated the neural correlates differentiating binge eating disorder and bulimia nervosa.
Methods
We developed Stroop-Match-to-Sample Task using high calorie food stimuli for use in functional magnetic resonance imaging paradigm to investigate how food stimuli (food pictures) interfered the performances and blood oxygenation level dependent(BOLD) neuronal activity in binge eating disorder, bulimia nervosa, and healthy controls.
Results
Binge eating disorder demonstrated stronger BOLD response to food stimuli in ventral striatum compared to healthy controls. By contrast, bulimia nervosa showed stronger activations in ventral striatum, dorsal prefrontal cortex, temporoparietal junction and middle occipital area compared to not only healthy controls but also binge eating disorder.
Conclusions
Our findings indicate that both bulimia nervosa and binge eating disorder are associated with increased reward sensitivity to food stimuli compared to healthy controls. Bulimia nervosa showed stronger striatal activation than binge eating disorder, which might result in increased cognitive top-down control.

Disclosures: J. Lee: None. **K. Namkoong:** None. **Y. Jung:** None.

Poster

346. Eating Disorders

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Title: Reinforcement learning during a monetary reward task changes with weight restoration in adolescents with anorexia nervosa

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Abstract: Introduction: Understanding neurobiological changes associated with anorexia nervosa (AN) is important in treatment development. Previously, adults with AN showed heightened brain response to unexpected reward receipt or omission in taste reward paradigms, a pattern that improves with recovery. Using a similar paradigm with monetary stimuli, we tested whether this sensitized brain reward function is seen in adolescent AN, generalizes beyond taste, and improves with weight restoration.
Methods: Twenty-four healthy control female adolescents (age = 14.8±2.3 years) and 23 female adolescents (age = 16.0±1.9 years) diagnosed with AN and enrolled in a treatment program underwent functional magnetic resonance imaging (fMRI) before and after treatment (mean time between the two scans = 42±14.3 days, mean BMI increase = 2.13 ±/0.96 kg/m^2). During fMRI, participants learned to associate visual and monetary stimuli. The prediction error evoked when this learned association is violated has been linked with the dopamine function of the brain reward circuit. All images were preprocessed using SPM8. A computational model was used to test the temporal difference model (prediction error)-related brain response. Additional group by condition first level contrast images were analyzed using a general linear model. Whole brain group contrast maps set at $p<0.001$ and 10 voxel cluster threshold and regions-of-interest small volume corrected (family-wise error, FWE $p<0.05$) were used to assess group differences.
Results: Compared to controls, AN displayed greater: 1) dopamine-related reward responses in caudate, dorsal anterior insula, nucleus accumbens, and precuneus when underweight; 2) unexpected reward receipt responses in caudate and anterior cingulum when underweight; and 3) unexpected reward omission responses in medial orbitofrontal cortex, which persisted with weight restoration. Additionally, number of days in treatment predicted BMI change only in AN with low prediction error brain response when underweight.

Discussion: This study has novel results suggesting increased reward system responsiveness in an underweight context that normalizes with weight restoration. However, the sensitivity did not remit for omission of the salient stimulus, presenting a potential obstacle for adolescents undergoing treatment for AN, even after weight restoration. Furthermore, adolescent AN associated with high dopamine-related brain activity may be a phenotype that does not respond well in treatment.

Disclosures: M. Deguzman: None. **M. Shott:** None. **G. Frank:** None.

Poster

346. Eating Disorders

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Topic: G.07. Other Psychiatric Disorders

Title: Reduced serotonin transporter availability in anorexia nervosa: a [^{11}C]DASB PET study.

Authors: *M. YOKOKURA[1], T. TERADA[1], T. BUNAI[1], K. NAKAIZUMI[1], K. TAKEBAYASHI[1], M. FUTATSUBASHI[2], E. YOSHIKAWA[2], Y. OUCHI[1];
[1]Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan; [2]Hamamatsu Photonics KK, Hamamatsu, Japan

Abstract: ***Introduction;*** Anorexia nervosa (AN) is a unique psychiatric disorder characterized by the persistent restriction of energy intake that leads to significantly low body weight, a tremendous fear of gaining weight, and disturbances in normal perception of a body shape. Previous positron emission tomography (PET) studies indicated abnormalities of serotonin (5-HT) system in AN patients by showing a disease-related increase in 5-HT1A receptor activity and a reduction in 5-HT2A receptor activity along with recovery of AN. Although neurocognitive studies showed cognitive impairment in AN patients, any PET study failed to show the association between PET parameters in the 5-HT system and cognitive impairment in AN. While 5-HT transporter (5-HTT) activity did not change in the recovery state of AN, an alteration of 5-HTT during the active state of AN has not been reported. Here, we aimed to clarify the alteration in the 5-HTT availability during the active state of AN using PET with [^{11}C]DASB and find any association between the 5HTT availability and cognitive performance on body perception.
Methods; Twenty female AN patients (mean age±SD, 25.0±6.0 years old, mean BMI±SD, 14.1±1.3) and 20 healthy female subjects (22.8±3.7years old, BMI 20.6±2.4) underwent [^{11}C]DASB PET measurements and neurocognitive tasks. BP_{ND} of [^{11}C]DASB was estimated with a MRTM2 model. We examined the whole brain using a voxel-wise analysis, SPM8

(Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).
Results; The levels of [^{11}C]DASB BP_{ND} in AN patients were significant lower in the medial and parietal cortices and medial frontal cortex than those in healthy subjects. A general intelligence measured by Raven colored progressive matrices (visual and spatial cognition), Stroop test (frontal function), and Iowa Gambling Task (decision making), did not differ between AN patients and healthy subjects. However, a body shape-based perturbation task that was originally made on the basis of the disturbed body perception in AN patients clearly showed significant errors in their performance, the degree of which was correlated to the level of [^{11}C]DASB BP_{ND} in the medial frontal cortex.
Discussion; Our results showed a significant reduction in 5-HTT availability in the particular brain region during the active state of AN, which might be related to the impairment in the body shape-related cognition in the patients. The altered 5-HT system is pathophysiologically implicated in AN.

Disclosures: M. Yokokura: None. **T. Terada:** None. **T. Bunai:** None. **K. Nakaizumi:** None. **K. Takebayashi:** None. **M. Futatsubashi:** None. **E. Yoshikawa:** None. **Y. Ouchi:** None.

Poster

346. Eating Disorders

Location: Halls B-H

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Program#/Poster#: 346.07/AAA22

Topic: G.07. Other Psychiatric Disorders

Title: Psychiatric disorders among diabetic patients attending medical outpatient clinics of abubakar tafawa balewa university teaching hospital, bauchi, nigeria

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Abstract: Patients with diabetes are at higher risk of mental health problems than the general population. This might be as a result of direct metabolic effects of complications of diabetics on the brain. And it could also be psychological response to the discovery by the patient to be living with diabetics & its attendance major live style changes and adjustment required. Psychiatric symptoms, such as irritability, anxiety, depression, suicidal ideas & cognitive deficits have been widely reported among patients especially those on insulin. Studies have been done on the prevalence & types of psychiatric disorders among diabetic patients in different parts of the world. Lustman et al (1986) found a 71% lifetime prevalence of at least one psychiatric disorder

among 57 patients each of type 1 & type 2 diabetes. The commonest lifetime diagnoses were generalized anxiety disorder (41%) & major depressive disorder (33%), while 14% had current major depression, except for simple phobias & agoraphobia (more in type 2). Studies in Nigeria were, Olatunbosun et al (2015) found that among the psychiatric patients with diabetes, depression has the highest prevalence (4.4%) followed by schizophrenia (3.6%), anxiety disorder (1.2%), dementia (1.6%) & so on. Coker et al (2000) found the prevalence of psychiatry diagnosis among diabetics as generalised anxiety (6%), mild depressive disorder (4%) and subjective memory disturbance (2%). Studies have also recognized factors that are associated with the development of psychiatric disorders among this group of patients like low occupational status, duration of illness, sexual dysfunction, impaired quality of life, poor glycemic control, higher frequency of hospitalization. However, there are few studies on this subject in this part of the country, hence the need for this present study. This present study therefore aimed to find the prevalence of psychiatric disorders among diabetic patients attending medical outpatient clinic of ATBUTH, Bauchi, Nigeria. It also examined types of psychiatric disorders among them and factors associated with the presence of psychiatric morbidities among diabetics. Pro formal questionnaire was designed to obtain socio-demographic variables, clinical parameters of the patients including the latest fasting blood sugar, as well as risk factors for development of psychiatric morbidity from the respondents. Mini International Neuropsychiatric Interview (MINI) will be administered to about 400 diabetic patients to assess the prevalence of specific psychiatric disorders among them. The control for this study will be matched non-diabetic patients attending general Medical Outpatient Clinic of the hospital.

Disclosures: Y.M. Mahmud: None. **D. Sulyman:** None.

Poster

346. Eating Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: Price Foundation

Title: Hypersensitivity to pleasant touch in individuals remitted from anorexia nervosa

Authors: *A. BISCHOFF-GRETHE[1], C. E. WIERENGA[1], L. A. BERNER[1], A. N. SIMMONS[1,2], M. OGASAWARA[1], L. J. GREATHOUSE[1], U. BAILER[1], M. P. PAULUS[3], W. H. KAYE[1];
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Abstract: Introduction: Anorexia nervosa (AN) is characterized by puzzling behavioral symptoms, including extreme dietary restriction, elevated anticipatory anxiety, and altered interoceptive awareness. Prediction error (e.g., the mismatch between anticipated and actual outcome) may contribute to food avoidance and observed difficulties in interpreting and responding to physiological, sensory, and affective stimuli in AN. Altered insula response to anticipation or receipt of salient or rewarding stimuli, such as food, has been shown in AN, though few studies have looked at anticipation and receipt in the same paradigm. Touch is a fundamental component of everyday human interaction, and like food, is considered a primary reward and complex interoceptive stimulus that acts on both sensory and emotional systems. Because food may be confounded with AN symptomatology, touch may be a useful, non-symptom-specific probe for examining whether AN individuals show generalized altered interoceptive anticipatory and receipt processing.
Methods: We compared 17 remitted adult AN (RAN) to 24 matched control women (CW) as they received slow brush strokes on the forearm and palm during functional magnetic resonance imaging. Visual cues signaled upcoming stimulation to permit examination of both anticipation and receipt. Subjective ratings to touch were measured with visual analog scales. Our primary focus was to examine group differences to anticipation and receipt of soft touch of the forearm or palm in the bilateral insula.
Results: Groups rated soft touch as equally pleasant. While both RAN and CW showed greater BOLD response to soft touch compared to anticipation (ps < 0.001), RAN responded more to soft touch than CW in the bilateral ventral mid-insula (ps < 0.05). CW with higher trait anxiety scores had higher BOLD response to anticipation of touch in the insula (p = 0.04). In comparison, RAN with higher trait anxiety or harm avoidance had lower BOLD response to anticipation of touch (ps < 0.03). Thus, anxiety may dampen the ability of AN to interpret incoming pleasant stimuli, thereby aiding their ability to restrain from eating.
Discussion: These findings support a small but growing literature showing that AN have altered responses to anticipation and receipt of interoceptive stimuli, consistent with studies of hunger that suggest they may struggle with interpreting changes in homeostatic state. An impaired ability to anticipate changes to the body state, and, once the change occurs, to interpret the experience and update the body state accordingly, may help explain why AN are able to severely restrict their intake.

Disclosures: A. Bischoff-Grethe: None. **C.E. Wierenga:** None. **L.A. Berner:** None. **A.N. Simmons:** None. **M. Ogasawara:** None. **L.J. Greathouse:** None. **U. Bailer:** None. **M.P. Paulus:** None. **W.H. Kaye:** None.

Poster

346. Eating Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 346.09/AAA24

Topic: G.07. Other Psychiatric Disorders

Support: NIH Grant DA006886

NIH Grant DA023641

NIH Grant DA035589

USDA-NIFA Grant NJ06156

Title: Accumbens firing in a dietary-induced model of binge eating

Authors: J. STAMOS[1], A. TAYLOR[2], D. QUINTIN[3], K. COFFEY[2], J. KULIK[2], A. PAWLAK[2], N. BELLO[4], *M. O. WEST[5,2];
[1]Neurosci. & Cell Biol., [2]Psychology, [3]Cell Biol. & Neurosci., [4]Animal Sci., Rutgers Univ., New Brunswick, NJ; [5]Panasonic Corp of North America, Piscataway, NJ

Abstract: The mesolimbic dopamine system is thought to become sensitized by chronic binge eating, a component of certain eating disorders. The present study is the first to investigate single unit activity of dopamine target neurons in the nucleus accumbens (NAc) during behavior in a dietary-induced binge eating model. Naïve, young adult, female Sprague Dawley rats underwent a 6-week pre-treatment of twice-per-week bingeing on sweetened fat (BE group) for comparison to chow controls (CC group). All rats then underwent stereotaxic surgery and had 16 microwires implanted into core and shell subregions of the NAc. Following recovery, subjects individually were trained in a Pavlovian task in which a tone cue predicted a 32% sucrose reward, with 56 trials per day for 10 days. All rats learned the task and exhibited positive affect as indicated by elevated 50 kHz ultrasonic vocalizations. BE rats tended to exhibit greater elevation of positive affect than CC rats. Frame-by-frame video analysis identified discrete behavioral events, in particular, the exact time of onset of cue-evoked approach to the sucrose port on each trial. NAc neurons acquired responsiveness to the tone CS, differentially between BE vs CC animals. These findings are consistent with anatomical data suggesting that limbic signals regarding biologically relevant cues affect NAc firing, and moreover, provide the first evidence of its kind that such firing is indeed altered by a history of binge eating. Moreover, using behaviorally equivalent measures, standardized change scores of firing rates of NAc neurons relative to baseline were found to be consistently different during consumption of the sucrose reward when compared to a motorically similar state. This observation was enabled only by comparing firing rates between motorically similar states, thus emphasizing the need for employing “behavioral equivalence” in

comparisons of neural activity. The present studies have established a protocol capable of revealing important neural and behavioral consequences of chronic binge-like eating, which may inform strategies for treating disorders in which it is involved.

Disclosures: **J. Stamos:** None. **A. Taylor:** None. **D. Quintin:** None. **K. Coffey:** None. **J. Kulik:** None. **A. Pawlak:** None. **N. Bello:** None. **M.O. West:** None.

Poster

346. Eating Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 346.10/AAA25

Topic: H.02. Human Cognition and Behavior

Title: Glucose effect on memory is modulated by stress

Authors: ***C. FOX**[1], J. DOYLE[2];
[1]Psychology, [2]Holy Cross Col., Notre Dame, IN

Abstract: Numerous research studies have examined the effect of carbohydrates in the form of a glucose or a sucrose drink on cognitive abilities in human subjects of various ages (Messier et al. 2010). Few of those studies measured the effect of glucose or sucrose on mood with limited and inconsistent results (Van der Zwaluw et al., 2014). To our knowledge only one recent study by Smith et al. 2011 took a different approach and investigated the interaction between glucose and anxiety on cognitive performance. The study found that glucose enhancement of memory is modulated by anxiety in adolescent males. Therefore, the current research study aimed to further this finding by looking at the effect of a sucrose load and self-reported stress on episodic memory in male college students with the hypothesis that sucrose will enhance recall only in stressed subjects. As part of a pilot study, eight male college students between the ages of 18 and 22 years old were randomly assigned to sucrose (n=4) group or saccharin (n=4) group. Subjects in both groups were asked to fast from midnight the night before testing. Subjects were given 240 ml lemon flavored drinks containing either 50g of sucrose or 50 mg of saccharin. Blood glucose levels were measured at baseline and at various intervals post drink. Five minutes following drink consumption, subjects were given the Stress Indicators Questionnaire measuring physical, sleep, behavioral, emotional and personal stress indicators. Following the stress questionnaire, subjects were presented a slideshow of 50 pictures. Memory recall testing was done immediately following the pictures presentation and at a 20 min and a 40 min delay. Using a median split, subjects were divided into low stress group and high stress group for each of the stress indicators. Multivariate statistical analysis revealed a statistically significant interaction between the type of drink and the physical stress indicator on the immediate memory recall test

and a marginally significant significant interaction between type of drink and physical stress indicator on the 20 min delayed memory recall test. The results support our hypothesis and are in agreement with Smith et al. 2011 by showing that sucrose enhanced memory performance only in subjects with high stress physical indicators. No interaction was found between drink type and other stress indicators but more data will be collected and analyzed prior to the SFN conference. Understanding the interaction between stress/anxiety and the effect of sugar on memory sheds light on the mechanism by which carbohydrates modulate cognition.

Disclosures: **C. Fox:** None. **J. Doyle:** None.

Poster

346. Eating Disorders

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 346.11/AAA26

Topic: G.07. Other Psychiatric Disorders

Support: NSFC Grant 81271549

NSFC Grant 81470816

NSFC Grant 61131003

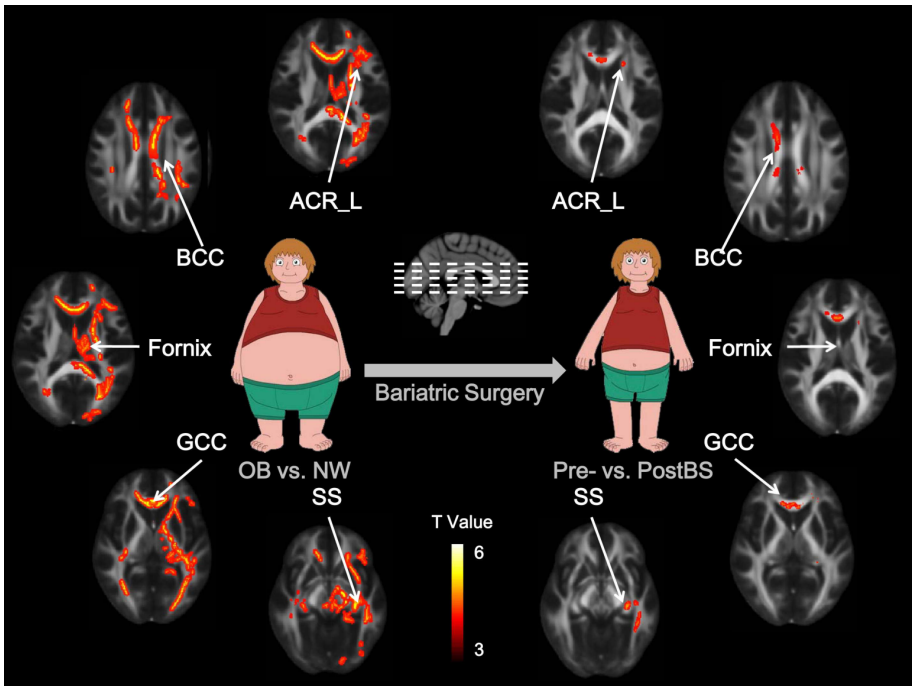
NSFC Grant 61431013

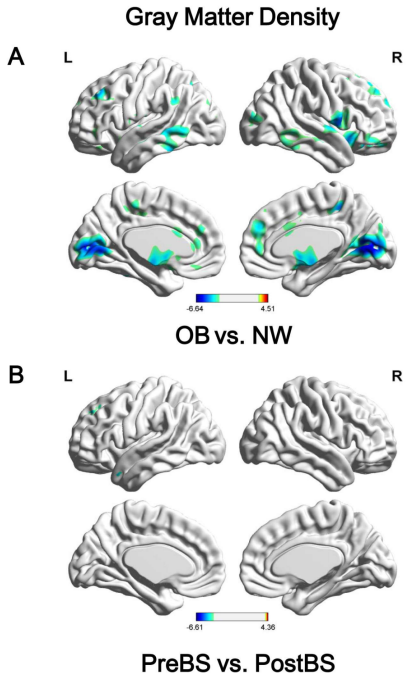
Title: Recovery of brain structural abnormalities in morbidly obese patients after bariatric surgery

Authors: ***Y. ZHANG**[1], W. CAI[2], Q. ZHU[2], G. LI[2], Q. MENG[2], G.-J. WANG[3];
[1]Sch. of Life Sci. and Technol., Xidian Univ., Shaanxi, China; [2]Sch. of Life Sci. and Technol., Xidian Univ., Xi'an, China; [3]Lab. of Neuroimaging, NIAAA, Bethesda, MD

Abstract: Obesity-related brain structural abnormalities have been reported extensively, and bariatric surgery (BS) is the most effective intervention to produce sustained weight reduction in obese (OB) people. It is unknown if BS can repair brain circuitry abnormalities, concomitantly with weight loss. In order to investigate if BS promotes neuroplastic structural recovery in OB patients, we quantified fractional anisotropy (FA), mean diffusivity (MD), and gray (GM) and white (WM) matter densities in 15 OB patients and in 18 normal weight (NW) subjects. OB patients were studied at baseline and also one month after BS. Two sample *t*-test between OB and NW groups showed decreased FA (Fig 1), GM/WM densities (Fig 2) and increased MD in brain regions associated with food intake control (caudate, orbitofrontal cortex and corpus

callosum) and cognitive-emotion regulation (inferior frontal gyrus, hippocampus, insula, external capsule) ($P_{FWE} < 0.05$). Paired *t*-test in OB group between before and after surgery showed that BS generated partial neuroplastic structural recovery in OB group, but the differences had relative less strength and smaller volume ($P < 0.001$). This study provides the first anatomical evidence for BS-induced acute neuroplastic recovery that might mediate the long-term benefit of BS in weight loss. It also highlights the importance of gut-brain axis research employing the combined BS and neuroimaging model for identifying longitudinal changes in brain structure correlates with obesity status.





Disclosures: **Y. Zhang:** None. **W. Cai:** None. **Q. Zhu:** None. **G. Li:** None. **Q. Meng:** None. **G. Wang:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.01/BBB1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant R01 AA013983

Title: Social defeat stress and escalated ethanol drinking by C57BL/6J mice: Modulation by CRF-R1 antagonism

Authors: ***J. F. DEBOLD**[1], P. ANDREW[1], J. G. AULD[1], E. L. NEWMAN[1], E. Y. ZHANG[1], K. A. MICZEK[1,2];
[1]Psychology, Tufts Univ., Medford, MA; [2]Neurosci., Tufts Univ., Boston, MA

Abstract: Stressful experiences can increase ethanol (EtOH) consumption in the human population. We have previously demonstrated that a history of social defeat stress can also

increase EtOH drinking in mice. The present experimental work aimed to identify critical targets for pharmacological interventions in either hypothalamic or extra-hypothalamic regions associated with stress-escalated drinking. To do so, experimental male C57BL/6J (B6) mice were subjected to ten consecutive days of social defeat during which a larger, dominant mouse inflicted approximately thirty bites onto the subordinate B6 male. Daily confrontations were terminated after thirty bites or at five minutes. Ten days after the final social confrontation, experimental mice received continuous or intermittent two-bottle choice access to 20% EtOH and water. Four weeks of EtOH access was sufficient to observe escalated drinking by defeated mice as compared to non-defeated controls. Once baseline drinking levels were established, defeated and non-defeated control mice received the CRF-R1 antagonist, CP 376,395 (CP; 0, 10, 17, 30 mg/kg IP), the 5-alpha-reductase inhibitor, finasteride (0, 10, 30, 100 mg/kg IP) or the 11-beta-hydroxylase inhibitor, metyrapone (0, 10, 30, 50 mg/kg IP). The highest dose of CP selectively reduced continuous access EtOH intake (g/kg) by mice that were subjected to social defeat stress. In contrast, this effect was absent in socially defeated mice that were maintained on an intermittent access protocol. Mice that received continuous or intermittent access to EtOH showed dose-dependent reductions in drinking following finasteride or metyrapone administration. While the effect of finasteride was most evident after 2- and 4-hour EtOH access, diminished EtOH intake was observed up to 24-hours after metyrapone treatment. Our interpretation of these findings is that the 24-hour periods of forced abstinence during the intermittent access protocol may reduce the potentially therapeutic effects of CP. While CRF-R1 antagonism may diminish the positive reinforcing effects of EtOH, CRF-R1 may not serve as the underlying site of action for the negative reinforcing effects of EtOH withdrawal. Unlike CP, reduced EtOH intake after finasteride or metyrapone injections was not specific to defeated mice. These findings suggest that central CRF receptors and/or hypothalamic CRF release are involved in stress-escalated EtOH drinking by mice. Future investigations will probe for the effect of CRF-GABA interactions on stress-escalated drinking by targeting the central and basolateral amygdala.

Disclosures: **J.F. DeBold:** None. **P. Andrew:** None. **J.G. Auld:** None. **E.L. Newman:** None. **E.Y. Zhang:** None. **K.A. Miczek:** None.

Poster

347. Alcohol: Behavioral Studies I

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Program#/Poster#: 347.02/BBB2

Topic: G.08. Drugs of Abuse and Addiction

Support: R37AA010422

Title: Paternal chronic variable stress reduces ethanol drinking behavior selectively in male offspring

Authors: *G. ROMPALA, G. HOMANICS;
Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: We have previously shown that paternal vapor ethanol (EtOH) exposure decreases EtOH drinking behavior, increases sensitivity to an anxiolytic injection of EtOH, and blunts HPA axis responsivity selectively in male offspring. Interestingly, paternal chronic variable stress (CVS) has also been shown to similarly blunt HPA axis responsivity in the next generation. Since EtOH is a physiologic stressor, paternal EtOH exposure and paternal CVS may have similar effects on behavior in offspring. Here, we tested the hypothesis that paternal CVS impacts EtOH-related behaviors in the next generation. To test this hypothesis, we exposed adult male mice to six weeks of CVS. This entailed random daily exposure to one of seven stressors (i.e., restraint, novel object, predator odor, wet cage, constant light, white noise, and multiple cage changes). CVS- and control (C)- males were bred with stress naïve females to produce male and female offspring to be tested for EtOH-related behaviors. For EtOH drinking tasks, adult offspring were tested for two bottle choice EtOH drinking at concentrations of 3, 6, 9, 12, and 15% (w/vol) and for binge-like EtOH consumption (20% w/vol) in a limited access paradigm. Sensitivity to an anxiolytic injection of EtOH (1.0 g/kg) was tested in the elevated plus maze. HPA axis responsivity was tested by collecting tail blood at time points 0, 15, 30, and 90 min from the onset of a 15 min restraint stress and measuring plasma corticosterone levels using an ELISA assay. In the two bottle choice EtOH drinking task, CVS-sired male offspring exhibited reduced EtOH preference at concentrations of 3, 6, and 9% and reduced EtOH consumption at concentrations of 9 and 12% vs C-sired males. Moreover, when CVS-sired male offspring were tested for binge-like EtOH consumption in the limited access assay, there was similarly a significant reduction in EtOH consumption vs C-sired male offspring. In contrast, CVS-sired female offspring showed no difference in EtOH drinking behaviors vs C-sired females in either EtOH drinking paradigm. We did not find a difference in EtOH sensitivity or HPA axis responsivity to acute stress for CVS-sired males or females vs C-sired groups. These results show that paternal CVS attenuates intergenerational EtOH drinking behavior in mice. This suggests that paternal environmental exposures, such as to alcohol or stress, can lead to heritable changes in alcohol drinking behavior. Ongoing studies are exploring possible epigenetic mechanisms in sperm.

Disclosures: **G. Rompala:** None. **G. Homanics:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

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Program#/Poster#: 347.03/BBB3

Topic: G.08. Drugs of Abuse and Addiction

Support: The Alcohol Research Council of the Swedish Alcohol Retail Monopoly

The Swedish Research Council (K2012-61X-22090-01-3)

Title: No maternal separation- or supplier-dependent effect on basal corticosterone or alcohol intake and preference in female Wistar rats

Authors: *S. LUNDBERG, I. NYLANDER, E. ROMAN;
Dept. of Pharmaceut. Biosci., Uppsala Univ., Uppsala, Sweden

Abstract: Background: Maternal separation (MS) is an umbrella term for different manipulations of the early environment of rodent pups. Short separation time simulate naturalistic conditions and is used as an ethologically based control group. Prolonged separation time is associated with early-life stress and negative effects in adolescent and adult male rats. Dissimilarities among adult male rats of the same strain from different suppliers have been observed for several alcohol-related parameters. Studies on female animals are underrepresented concerning both MS- and supplier-dependent effects.
Objective: To study how short or prolonged MS affect basal corticosterone levels in adolescence and adulthood, and subsequent alcohol intake and preference in female Wistar rats from two suppliers.
Methods: Eight cross-fostered litters, four RccHan:WI (Harlan Laboratories, Horst, the Netherlands) and four HanTac:WI (Taconic Bioscience, Ejby, Denmark), were randomized into one of two MS conditions: daily 15- or 360-minute separation from the day after birth until weaning on postnatal day (PND) 22. Forty weaned female offspring continued in the study. Basal serum corticosterone levels were measured on PND 25/26 (adolescence) and 76/77 (adulthood). Starting on PND 87-90 the animals had access to 20% alcohol in a two-bottle, free choice modified intermittent paradigm were alcohol was available for three consecutive days per week. The animals had access to alcohol for twelve weeks with an alcohol deprivation period of two weeks between week six and seven of access. Alcohol intake and preference was measured for every 24-hour session and weekly averages were calculated.
Results: There were no effects or interactions of MS or supplier on basal corticosterone levels or alcohol intake and preference but there were effects of time. Basal corticosterone levels increased from adolescence to adulthood by 50%. Alcohol intake and preference decreased over the whole alcohol access period. However, after the two-week alcohol deprivation period alcohol intake and preference increased by 40-50% compared to the week directly before the deprivation

period.
Conclusion: Female offspring from Harlan or Taconic are not sensitive to early-life stress and do not exhibit any supplier-dependent effects on basal corticosterone levels or alcohol intake and preference. Nonetheless, after two weeks of alcohol deprivation the females reinstated their alcohol consumption at a higher level than before the deprivation period, a so-called alcohol deprivation effect.

Disclosures: S. Lundberg: None. **I. Nylander:** None. **E. Roman:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.04/BBB4

Topic: G.08. Drugs of Abuse and Addiction

Title: The effect of stress on ethanol self-administration in Wistar rats.

Authors: *C. J. HEYSER[1], B. HOFF[1], R. E. BLASER[2];
[1]Neurosciences, Univ. of California San Diego, La Jolla, CA; [2]Psychoolgical Sci., Univ. of San Diego, San Diego, CA

Abstract: The hypothesis that stressful life experiences may contribute to an individual's vulnerability to drug and alcohol abuse has received considerable attention. However, to date the results in animal models has been mixed, with many studies reporting a reduction in ethanol intake following stress. Clearly this is a complex issue as the impact of stress can depend on the magnitude of stress, its predictability, the control an organism has over it and the social environment (context) and proximity to the stressor. Therefore, the present study was conducted to examine the effects of unpredictable stressor exposure on the acquisition of ethanol self-administration in male Wistar rats. All rats were given two-bottle (ethanol and water) access (1 hr or 24 hr) to gradually increasing sweetened (saccharin) ethanol concentrations over a 12-week period. The final concentration of ethanol was 10% w/v. The rats were either individually housed or group housed in pairs during their access to ethanol. Animals assigned to the stress condition were exposed to unpredictable mild stressors daily (e.g., cage crowding, forced swim, cage tilt, strobic light, etc) throughout the period of the experiment. Overall, the results showed that animals exposed to stressful conditions drank significantly less ethanol than control animals, however this result was specific to the social environment. More specifically, the stress-induced reduction in ethanol intake was greatest in animals that were group housed during their access to ethanol. In contrast, stress had little to no effect on ethanol consumption in individually housed animals. Our working hypothesis is: 1) group housed rats form a fairly stable social structure

(dominance hierarchy), 2) exposure to unpredictable stressors may alter the behavior of the rats, resulting in perturbations to the established dominance hierarchy, 3) instability in the social structure along with continued stressor presentation reduces ethanol intake during acquisition. These results strengthen the hypothesis that stress can modify ethanol consumption and that this influence is dependent on the organism's history of ethanol intake and its social environment.

Disclosures: C.J. Heyser: None. **B. Hoff:** None. **R.E. Blaser:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.05/BBB5

Topic: G.08. Drugs of Abuse and Addiction

Title: Stress and chronic ethanol interactions on drinking and cognitive control

Authors: *E. M. RODBERG[1], C. R. DEN HARTOG[1], D. E. MOORMAN[2], E. M. VAZEY[1]; [1]Biol., [2]Psychology, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Stress is a risk factor implicated in the transition from initial alcohol drinking to dependence, as well as relapse following abstinence. Ethanol consumption is influenced by the intersection of genetic and environmental factors. In particular, a history of repeated cycles of intoxication and withdrawal reliably escalates drinking in rodents. Additionally, chronic stress increases ethanol consumption in rodents with a history of ethanol dependence (Anderson et al., 2016). Chronic exposure to stress or alcohol can drive neuroadaptations in areas such as prefrontal cortex (PFC) and locus coeruleus (LC), disrupting cognition and contributing to alcohol use disorders. Here we examined interactive effects of chronic ethanol exposure and stress on volitional ethanol drinking and prefrontal dependent cognition.
Adult male C57BL/6J mice were trained to drink ethanol (15%, v/v) on a 1hr/day 1-bottle choice schedule until a baseline was established. Mice were exposed to weekly cycles of chronic intermittent ethanol (CIE) or air-control vapor exposure (Air), followed by test cycles of 1hr/day ethanol drinking (Becker & Lopez, 2004). Mice were split into non-stress control (NS) or stress groups. Stress groups received 10 minutes of forced swim stress (FSS) 4 hours before each drinking test. This schedule produced four experimental groups: control, Air/NS; ethanol-dependent no stress, CIE/NS; non-dependent stress, Air/FSS; or ethanol-dependent stress, CIE/FSS. Prefrontal dependent cognition was assessed using object/context recognition and attentional set shifting. After testing brains were collected for histological analysis of neuronal activity using immunohistochemistry for c-Fos and DeltaFosB in PFC and LC.
CIE/FSS mice escalated ethanol intake faster than CIE/NS during CIE cycles one and two.

CIE/FSS mice consumed more ethanol than Air/NS across all test cycles. In the object/context recognition task, CIE/FSS mice performed at chance, indicating that stress and ethanol interactions disrupted context specific recognition memory. In the attentional set shifting task, CIE/FSS mice required more trials to reach criterion during the extradimensional shift than all other groups, indicating that combined chronic ethanol and stress impaired behavioral flexibility. We are currently analyzing neuronal signaling in PFC and LC to characterize circuit changes underlying this transition. Together, these findings show that previous ethanol exposure and stress escalates drinking and disrupts normal cognitive functions. This "double-hit" may facilitate transitions to alcohol dependence.

Disclosures: E.M. Rodberg: None. **C.R. den Hartog:** None. **D.E. Moorman:** None. **E.M. Vazey:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.06/BBB6

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA Division of Intramural Clinical and Biological Research (Z1A AA000466)

Title: Risk and severity of alcohol dependence are associated with the fatty acid amide hydrolase C385A missense variant

Authors: *M. E. SLOAN[1], J. YAN[2], J. L. GOWIN[3], M. L. SCHWANDT[3], H. SUN[3], C. HODGKINSON[3], D. GOLDMAN[3], V. A. RAMCHANDANI[3];
[1]Natl. Inst. on Alcohol Abuse and Alcoholism, NIH, Washington, DC; [2]Johns Hopkins, Baltimore, MD; [3]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Even though alcohol use represents the sixth leading cause of disability and premature death worldwide, the neurochemical underpinnings of alcohol consumption remain poorly understood. Recently the endocannabinoid system, one of the most widespread neurotransmitter systems in the brain, has been implicated in alcohol consumption. Problem drug and alcohol use in humans has been linked to the C385A single nucleotide polymorphism of the gene encoding fatty acid amide hydrolase (*FAAH* rs324420), an enzyme which terminates the signaling of anandamide, one of the main endocannabinoid neurotransmitters. We sought to further investigate the effects of this polymorphism in our sample, which consisted of 1434 Caucasian and African American participants who underwent a comprehensive assessment at the National Institutes of Health Clinical Center. Assessment included genotyping for fatty acid amide

hydrolase rs324420, standardized clinical interview for DSM-IV axis I disorders, and timeline followback interview. The sample was divided into two distinct groups, participants who had never met criteria for alcohol dependence when assessed at our center (control group, n=482, 70.5% Caucasian) and participants who had met the criteria during at least one assessment (lifetime alcohol dependent group, n=952, 56.4% Caucasian, 888 of whom had timeline followback interviews). Due to significantly different minor allele frequencies in Caucasian and African American participants, groups were subdivided by ethnicity and all comparisons were done between individuals of the same ethnicity. Two-proportion Z-tests revealed that *FAAH* C385A minor allele frequency was significantly higher in Caucasians (cases 0.2263, controls 0.1853; p=0.037) but not African Americans (cases 0.3771, controls 0.3803; p=0.944) with lifetime alcohol dependence. Mann-Whitney U tests revealed that dependent Caucasian A-allele carriers demonstrated significantly higher median total drinks (860 drinks $FAAH^{AA/AC}$, 753 drinks $FAAH^{CC}$; p=0.022), drinking days (81 days $FAAH^{AA/AC}$, 71 days $FAAH^{CC}$; p=0.006), and binge drinking days (77 days $FAAH^{AA/AC}$, 64 days $FAAH^{CC}$; p=0.003) over the 90 days preceding assessment; there were no significant differences in African Americans (ps > 0.05). These findings provide evidence that the *FAAH* C385A missense variant is associated with both risk and severity of alcohol dependence in Caucasians. Furthermore, our results provide the first evidence that the *FAAH* C385A polymorphism may have differential effects on alcohol consumption in Caucasian and African American individuals.

Disclosures: **M.E. Sloan:** A. Employment/Salary (full or part-time): National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, United States. **J. Yan:** None. **J.L. Gowin:** None. **M.L. Schwandt:** None. **H. Sun:** None. **C. Hodgkinson:** None. **D. Goldman:** None. **V.A. Ramchandani:** None.

Poster

347. Alcohol: Behavioral Studies I

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.07/BBB7

Topic: G.08. Drugs of Abuse and Addiction

Support: CRC 950-229982

Title: Longitudinal analysis of GPR88 knockout mice behavior under ethological conditions.

Authors: ***G. MAROTEAUX**[1], S. BEN HAMIDA[1], T. AREFIN[2], B. KIEFFER[1,3], L.-A. HARSAN[2];
[1]Douglas Res. Ctr., Verdun, QC, Canada; [2]Advanced Mol. Imaging Ctr. (AMIR), Med. Physics,

Univ. Med. Ctr., Freiburg, Germany; [3]IGBMC, Inst. Génétique Biologie Moléculaire Cellulaire, Illkirch, France

Abstract: GPR88 is an orphan G protein coupled receptor highly expressed in striatum, present in hippocampus and amygdala and involved in several traits of psychiatric disorders (Del Zompo et al., 2014; Ghate et al., 2007). Mice lacking *Gpr88* displayed hyperactivity, motor coordination impairment and reduced anxiety-like behaviors (Meirsman et al., 2015). Moreover, these mice showed an alteration of alcohol taking and seeking behavior possibly due to the decreased level of dopamine in the nucleus accumbens after alcohol treatment (SFN 2016 poster Ben Hamida S). In order to strengthen $GPR88^{-/-}$ behavioral phenotyping, we used a novel approach combining automated behavioral monitoring and social housing conditions, the Intellicage. We designed a long lasting protocol of sucrose and alcohol operant self-administration followed by a corner avoidance task. Continuous monitoring affirmed the previously described hyperactivity trait, no alteration in sucrose preference but alteration in the motivation to drink alcohol. In addition, longitudinal analysis revealed that $GPR88^{-/-}$ mice hyperactivity in refined tasks (nosepoke and licks) corresponds to the activity peak during the dark phase of $GPR88^{+/+}$ without modification of the diurnal rhythm. During the corner avoidance task $GPR88^{-/-}$ learned normally but exhibited faster extinction. In combination with previous classical tests data, these data strengthen our understanding of $GPR88^{-/-}$ behavioral alteration. Automated home cage behavioral monitoring allows an efficient screening by reducing stress for the animal in an ethologically valid environment. Moreover, the longitudinal aspect of this paradigm facilitates the study of spontaneous daily behavior and offers the possibility to study the progression of behaviors (habituation, baseline and challenged responses to different task), which is of utmost relevance to study models of psychiatric disorders.
Del Zompo, et al., 2014 . Mol. Genet. Genomic Med. 2, 152–159. doi:10.1002/mgg3.54
Ghate, A., et al., 2007. 146, 1182–1192. doi:10.1016/j.neuroscience.2007.02.040
Meirsman, et al., 2015. doi:10.1016/j.biopsych.2015.05.020
Ben Hamida, et al.,2016. SFN 2016 Poster.

Disclosures: G. Maroteaux: None. **S. Ben Hamida:** None. **T. Arefin:** None. **B. Kieffer:** None. **L. Harsan:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.08/BBB8

Topic: G.08. Drugs of Abuse and Addiction

Support: Research Foundation for New York and NIH (1R01DA035923 and 1R01DA035949)

Title: FABP5/7 deficiency decreases ethanol consumption in female but not male mice

Authors: *B. H. CLAVIN[1], J. A. HAMILTON[1], J. O'ROURKE[1], D. DEUTSCH[2], S. HAJ-DAHMANE[1], M. KACZOCHA[3], P. K. THANOS[1];
[1]Res. Inst. on Addictions, Univ. At Buffalo, Buffalo, NY; [2]Dept. of Biochem., [3]Dept. of Anesthesiol., Stony Brook Univ., Stony Brook, NY

Abstract: The endocannabinoid (ECB) system is recognized as being involved in the mechanism of a wide range of diseases including alcoholism. ECBs are inhibitory retrograde messengers that bind to the cannabinoid type 1 (CB1) receptor, which is abundant in many areas of the brain including those related to reward. Previous research has shown that the ECB system influences ethanol reward. More recently ECB signaling has been investigated and inhibition of fatty acid amide hydrolase (FAAH), which degrades the ECB anandamide (AEA), has been shown to significantly increase ethanol consumption and preference, particularly in female mice. Similarly, fatty acid binding proteins (some expressed in the brain), which transport AEA to FAAH for degradation, increase AEA levels. Recent work from our group has implicated FABP5/7 inhibition (via the novel drug SBFI26) or deletion in mediation of pain and inflammation. Our objective was to examine if disruption of FABP5 and FABP7 signaling would potentiate alcohol intake comparable to FAAH inhibitors. In one experiment, male mice were split into four treatment groups and received vehicle, 5 mg/kg, 20 mg/kg, or 40 mg/kg SBFI26 throughout a restricted-access ethanol two-bottle choice paradigm. Results showed that FABP5/7 inhibition did not significantly affect ethanol consumption in male wild-type (WT) mice. Similar pharmacological studies in females are in progress. In a second experiment, male and female FABP5/7-/- mice underwent the same drinking paradigm along with their wild-type littermates. Results showed that alcohol consumption in FABP5/7 knockout (KO) male mice was not different from WT. However, female FABP5/7 KO mice drank significantly less alcohol as compared to WT females. Based on our results, targeting of FABPs appears to play a role in alcohol consumption that is differentially regulated in males and females. Additional experiments are in progress to further elucidate these findings.

Disclosures: B.H. Clavin: None. **J.A. Hamilton:** None. **J. O'Rourke:** None. **D. Deutsch:** None. **S. Haj-Dahmane:** None. **M. Kaczocha:** None. **P.K. Thanos:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R15 AA022506

Douglas K. Candland Undergraduate Research Fund

Title: Alcohol consumption in response to exercise access is modulated by gonadal hormones

Authors: *C. E. MCGONIGLE, L. P. ERCOLANO, Z. J. KOZICK, T. B. NENTWIG, J. E. GRISEL;
Bucknell Univ., Lewisburg, PA

Abstract: Sex differences in alcohol (EtOH) consumption may relate to sex differences in stress-sensitivity. For instance, males of all species are less sensitive to stress and male rodents self-administer lower levels of alcohol than females, phenomena that may be dependent on sex hormones. Our goal is to understand the potential contributions of estrogen and testosterone from gonadal sources to the relationship between voluntary EtOH consumption and stress. We measure free choice oral EtOH self-administration by C57BL/6J mice in a modified drinking in the dark paradigm, in which running wheels may or may not be available for voluntary activity. Previous studies indicated that females, but not males, increased drinking in response to blocked access of the running wheel. We tested male and female naïve, sham, and gonadectomized mice in our paradigm. For the duration of a four-day habituation period and ten-day experimental period, 20% alcohol is available for two-hours each day, along with 24-hour access to water and food. In the experimental period for some mice, the running wheel is locked every other day beginning one hour prior to alcohol presentation and lasting for a total of three hours, but is otherwise always available in the home cage. We hypothesized that estrogen would promote and testosterone would buffer stress-induced drinking. Our preliminary results suggest that gonadal hormones appear to interact with voluntary activity to modulate EtOH consumption. In general, males lacking testosterone appear to be more at risk for high drinking and in the presence of stress, females lacking estrogen may drink less. Better understanding of sex-dependent neuroendocrine influences on drinking may facilitate more appropriate individualized treatment and prevention of alcoholism.

Disclosures: **C.E. McGonigle:** 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; NIAAA R15 AA022506. **L.P. Ercolano:** None. **Z.J. Kozick:** None. **T.B. Nentwig:** None. **J.E. Grisel:** 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; NIAAA R15 AA022506.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIAAA R03AA022479

European Regional Development Fund and project of specific research at the Masaryk University MUNI/A/1284/2015 (JR-K) and funds from the Faculty of Medicine MU to junior researcher

Title: The AMPA antagonist NBQX reduces alcohol intake in sham-operated and olfactory bulbectomized rats

Authors: J. RUDA-KUCEROVA[1], Z. BABINSKA[1], B. GETACHEW[2], *Y. TIZABI[2]; [1]Dept. of Pharmacology, Fac. of Med., Masaryk Univ., Brno, Czech Republic; [2]Dept. of Pharmacol., Howard Univ. Col. of Med., Washington, DC

Abstract: Recently, a major role for the glutamatergic system in alcohol addiction has been suggested. Indeed, we have observed that ketamine, an NMDA receptor antagonist can lower alcohol intake in olfactory bulbectomized (OBX) as well as sham-operated control animals (Kucerova et al. SFN abst 2015). On the other hand, although ample evidence suggests a potential role for AMPA receptors in the adaptive responses to chronic alcohol in discrete brain areas, the role of these receptors in alcohol consumption has not been adequately investigated. This study was carried out to explore the effects of NBQX (2,3-dihydroxy-6-nitro-7 sulfamoylbenzo(~quinoxaline), an AMPA receptor antagonist on voluntary alcohol intake in OBX as well as sham-operated control animals. Adult male Wistar rats in both groups were trained in drinking-in-dark paradigm with the sucrose fading procedure for over 3 weeks (23 days). Drinking sessions lasted 90 minutes daily and started at 10 am (3 hours after the lights went off). A single dose of NBQX (10 mg/kg) was administered intraperitoneally (i.p.) 25 min before the alcohol drinking session on day 24 and the drinking behavior during the 90 min period was recorded. Controls received saline. Although no difference in alcohol intake between the OBX and sham-operated animals were noted, NBQX pretreatment significantly attenuated alcohol consumption in both groups by approximately 50 %. These studies further confirm a role for AMPA receptors in alcohol consumption and suggest potential therapeutic application of AMPA antagonists in alcoholism or alcohol use disorders. Supported by: NIH/NIAAA R03AA022479 (YT), European Regional Development Fund and project of specific research at the Masaryk University MUNI/A/1284/2015 (JR-K) and funds from the Faculty of Medicine MU to junior researcher JR-K.

Disclosures: **J. Ruda-Kucerova:** None. **Z. Babinska:** None. **B. Getachew:** None. **Y. Tizabi:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

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Program#/Poster#: 347.11/BBB11

Topic: G.08. Drugs of Abuse and Addiction

Support: NHMRC APP1061979

Title: The 5-HT1A partial agonist Tandospirone reduces long term binge-like alcohol drinking and prevents the subsequent deletious effects on anxiety and neurogenesis

Authors: ***A. BELMER**, O. PATKAR, S. BARTLETT;
QUT-IHBI-TRI, Woolloongabba, Australia

Abstract: Long term binge-like alcohol exposure has been shown to induce various deleterious effects such as stress, anxiety and depression as well as alterations in hippocampal neurogenesis. As the serotonin (5-Hydroxytryptamine, 5-HT) pathway has been shown to play an important role in alcohol drinking, stress, anxiety, depression and neurogenesis, we investigated the role of the 5-HT1A receptors in alcohol drinking, withdrawal-induced anxiety and alcohol-induced reductions in neurogenesis. We show that the 5-HT1A partial agonist Tandospirone reduces binge ethanol drinking following 12 weeks of exposure in the Drinking In the Dark as (DID) well as withdrawal induced-anxiety in the marble burying test and elevated plus maze. Furthermore, we show that chronic treatment with Tandospirone reduces the impairment in hippocampal neurogenesis and novel-object recognition. Together, these results suggest that Tandospirone is a potential candidate for the treatment of alcohol drinking and the subsequent alterations in mood, neurogenesis and working memory.

Disclosures: **A. Belmer:** None. **O. Patkar:** None. **S. Bartlett:** None.

Poster

347. Alcohol: Behavioral Studies I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA021233

Title: Circadian dysregulation exacerbates alcohol induced tissue injury and mortality

Authors: *L. C. LYONS, A. K. DE NOBREGA, A. P. MELLERS, E. J. NOAKES;
Dept. of Biol. Science, Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Circadian regulation of metabolic, physiological and behavioral outputs is highly conserved across species. However, well-functioning circadian clocks are also necessary for protection of cells and tissues from damage by external or internal factors. The circadian clock modulates alcohol sensitivity and toxicity across species from insects to human; however, the mechanism through which this occurs remains unclear. Recent research using cell cultures and a rodent model suggests that the lack of functional circadian oscillators aggravates chronic alcohol-induced tissue damage potentially worsening disease pathologies (Swanson et al., 2011; Sunma et al., 2013). Previously, we found that the circadian clock modulates the loss of motor control, sedation and recovery following acute alcohol exposure in *Drosophila*, with the greatest alcohol sensitivity observed at night (Van der Linde & Lyons, 2011, De Nobrega & Lyons, 2016). The highly conserved neurological and behavioral effects of alcohol as well as the conserved nature of the circadian clock make *Drosophila* a valuable model for understanding the interaction between the circadian clock and alcohol-induced toxicity. We rendered flies arrhythmic either environmentally using constant light or genetically using circadian mutants and then exposed them to a single binge-type alcohol exposure. Per^{01} flies or wild-type flies in constant light exhibit significantly increased behavioral sensitivity to alcohol with shorter alcohol exposures needed to induce sedation and significantly longer times needed for recovery of motor reflexes. Flies with a dysfunctional circadian clock also exhibit significantly increased mortality compared to age matched control flies, particularly following alcohol exposures during the subjective day. We found similar results using a repeat binge model of 3 alcohol exposures separated by 24 h. We also are investigating the effects of a dysfunctional circadian oscillator on tissue damage and mortality following chronic alcohol exposure. Based on our results, we hypothesize that the circadian clock phase specifically buffers alcohol toxicity with the time of greatest protection corresponding to the temporal windows when flies would normally be exposed to alcohol.

Disclosures: L.C. Lyons: None. **A.K. De Nobrega:** None. **A.P. Mellers:** None. **E.J. Noakes:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

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Program#/Poster#: 347.13/BBB13

Topic: G.08. Drugs of Abuse and Addiction

Title: Differential rearing alters ethanol preference during adolescence but not operant responding for ethanol in adulthood

Authors: *T. J. WUKITSCH[1], K. PARKS[2], M. E. CAIN[2];
[1]Psychological Sci., [2]Kansas State Univ., Manhattan, KS

Abstract: Isolation rearing increases operant responding for ethanol (ETOH) (e.g. Deehan et al., 2007). While previous research suggests that intermittent ETOH exposure during adolescence increases operant responding for ETOH in adulthood (Alaux-Cantin et al., 2013), a recent study exposed isolated and social housed rats to adolescent ETOH exposure and observed no differences in operant responding for ETOH in adulthood (Lesscher et al., 2015). Our previous research has demonstrated that isolation conditions can alter glutamate function in rats responding for amphetamine (Arndt et al., 2015). Given the role of the glutamatergic system for ETOH responding (Bäckström & Hyytiä, 2005), isolation-induced deficits in glutamatergic function may alter the effects of adolescent ETOH exposure. In the current study, we determined if isolation rearing paired with voluntary ETOH exposure during adolescence increased ETOH preference and operant responding for ETOH in adulthood when compared to standard rearing. Male Long Evans rats arrived in the lab on postnatal day (PND) 21 and were randomly assigned to either the IC or a standard condition (SC). After 1 week, rats began 6 weeks of Intermittent Adolescent Ethanol Exposure (IAEE) in a 2-bottle choice paradigm (Simms et al. 2008). During the IAEE, the rats received 24-hour access to two bottles. The contents of one bottle every other day switched between 20% (v/v) ETOH solution and water while the other bottle was always water. The control group was always offered water. Analyses indicate IC rats preferred ETOH more than SC rats. IC rats consumed significantly more ETOH during the middle portion of the exposure period while SC rats escalated their intake towards the end of the exposure period. Following the IAEE phase, rats were trained to lever press on an FR1 schedule for 20% ETOH. There were no significant differences in active lever responding between SC and IC rats. Operant responding was then extinguished and there were no significant differences in responding during extinction between SC and IC rats. These results suggest that while isolation increases preference for ETOH during voluntary ETOH exposure, it does not alter subsequent operant responding for ETOH. We are currently testing cue and ETOH-induced reinstatement following injections of LY379268 or saline to explore the effects of $mGluR_{2/3}$ activation on reinstatement of ETOH-seeking. We hypothesize that $mGluR_{2/3}$ activation will result in a greater attenuation of

reinstatement in IC rats than SC rats due to isolation-induced deficiencies in glutamate homeostasis.

Disclosures: **T.J. Wukitsch:** None. **K. Parks:** None. **M.E. Cain:** None.

Poster

347. Alcohol: Behavioral Studies I

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Topic: G.08. Drugs of Abuse and Addiction

Support: F32AA023434

R37AA008757

Title: Preconception alcohol increases offspring vulnerability to stress

Authors: *L. G. CHASTAIN, S. JABBAR, O. GANGISETTY, M. A. CABRERA, K. SOCHACKI, D. K. SARKAR;
The Endocrine Program, Dept. of Animal Sci., Rutgers The State Univ. of New Jersey, New Brunswick, NJ

Abstract: While the detrimental effects of drinking during pregnancy are known, the effect of preconception drinking by the mother on the life-long health outcomes of her children is unknown. In this study using an animal model, we determined the impact of preconception alcohol drinking of the mother on offspring stress response during adulthood. In our preconception alcohol exposure model, adult female Fischer rats were fed with 6.7% alcohol in their diet for four weeks, went without alcohol for three weeks and were bred with untreated male rats to generate male and female offspring. Preconception alcohol exposed offsprings' birth weight, body growth, stress response, anxiety-like behaviors and changes in stress regulatory gene and protein hormone levels were evaluated. Additionally, roles of epigenetic mechanisms in preconception alcohol effects were determined. Alcohol feeding three weeks prior to conception significantly affected pregnancy outcomes of female rats, in respect to delivery period and birth weight of offspring, without affecting maternal care behaviors. Preconception alcohol negatively affected offspring adult health, including increased stress hormone response (corticosterone and ACTH) to an immune challenge (lipopolysaccharides, 0.1 mg/kg body weight) and increased anxiety behaviors in the open field and elevated plus maze tests. These health abnormalities were associated with changes in expression and methylation profiles of stress regulatory genes in various brain areas. Specifically, offspring of pre-conception alcohol exposed (PCAE) dams

showed an increase in hypothalamic corticotrophin releasing factor (*Crf*) expression and a decrease in proopiomelanocortin (*Pomc*) expression. In addition, offspring of PCAE rats had suppressed hypothalamic *Crf* methylation of the CpG dinucleotides at -232 in the proximal promoter as determined by pyrosequencing methods, and increased hypothalamic *Pomc* gene methylation as determined by pyrosequencing and methylation-specific PCR. These changes in stress regulatory genes and anxiety behaviors were normalized following treatment with a DNA methylation blocker (5-azadeoxycytidine, 5 mg/kg) during the postnatal period. These data highlight the novel possibility that preconception alcohol affects the inheritance of stress-related diseases possibly by epigenetic mechanisms. (Supported by NIH grants F32AA023434 and R37AA008757)

Disclosures: L.G. Chastain: None. **S. Jabbar:** None. **O. Gangisetty:** None. **M.A. Cabrera:** None. **K. Sochacki:** None. **D.K. Sarkar:** None.

Poster

347. Alcohol: Behavioral Studies I

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Ulm Foundation

David Lehr Research Award from American Society for Pharmacology and Experimental Therapeutics

National Institute on Alcohol Abuse and Alcoholism (AA018779)

Title: Gender differentially responds to disruption of circadian rhythm in alcohol drinking and mood behaviors via adenosine transporter 1

Authors: *Y.-F. JIA[1], C. VADNIE[1], N. CARNEIRO[2], H. DAVID[1], D.-S. CHOI[1];
[1]Dept. of Mol. Pharmacol. and Exptl. Therapeut., Mayo Clin. Dept. of Mol. Pharmacol. A, Rochester, MN; [2]Univ. Federal de Viçosa, Viçosa, Brazil

Abstract: The disruption of circadian rhythm contributes to alcohol use disorder (AUD). Conversely, excessive alcohol drinking dampens circadian activity accompanied by the reduction of circadian genes. Adenosine signaling is implicated in several aspects of circadian rhythm and AUD. Mice lacking ENT1 consume more alcohol compared to wild-type (WT) littermates.

However, it remained unclear whether circadian disruption affects alcohol drinking and/or there is a gender difference in mice lacking ENT1. Here, utilizing constant light (LL), we investigate the effects of circadian disruption in male and female ENT1 knockout (KO) and WT mice. We investigated real-time circadian activity during LL and its effect on alcohol drinking using ClockLab. Our results showed that LL dramatically increased alcohol drinking in male ENT1 KO compared to WT mice. Interestingly, while we observed a LL-induced free-run pattern of circadian activity, alcohol drinking abolished this pattern in male WT mice. In contrast, the male ENT1 KO mice displayed free-running activity during LL with alcohol drinking, indicating that the mice may become more tolerant the effect of alcohol on LL-induced circadian disruption. On the other hand, female mice during LL did not show any differences between WT and KO in alcohol drinking or circadian activity, which suggested that the female mice may be more resilient to the effect of alcohol during LL than male. Taken together, these results revealed a gender difference in alcohol drinking and circadian activity pattern during LL. ENT1 appears to be an important player in this gender difference, which may provide a novel insight to understand the underlying mechanisms of ENT1 signaling-regulated alcohol drinking.

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Poster

347. Alcohol: Behavioral Studies I

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Support: NIAAA Grant 1R21AA023078

B&BRF 2014 NASRAD Young Investigator #22646

Title: Longitudinal effects of alcohol on sleep distribution using a novel murine model of alcohol abuse: repeated drinking-in-the-dark alternating with two-bottle choice paradigm

Authors: *S. PERREAU-LENZ, J. VAZQUEZ-DEROSE, M. D. SCHWARTZ, W. POLGAR; Ctr. for Neuroscience, Biosci. Div., SRI Intl., Menlo Park, CA

Abstract: Alcohol abuse is associated with reduced sleep quality or insomnia in alcoholic patients. Disruption of sleep may in turn promote the development of alcohol dependence. However, the neurobiological mechanisms linking alcohol abuse and sleep remain mostly unknown. To further understand the reciprocal interaction of alcohol abuse development and

sleep, we developed a novel longitudinal model of chronic alcohol binge drinking while simultaneously characterizing associated sleep distribution. Our model consisted of repeated binge drinking week-cycles of 4-days restricted drinking-in-the-dark exposure (DID) alternating with 3-days free-access exposure using the 2-bottle choice paradigm (2BC). Concurrently, sleep parameters electroencephalogram/electromyogram (EEG/EMG), locomotor activity and body temperature were continuously recorded. 7 week old male C57BL/6J mice (n=12) were surgically implanted with tethered EEG/EMG implants and a telemetry device. After recovery, mice were subjected to the longitudinal binge drinking paradigm. Our results showed that tethered mice consumed high and intoxicating levels of ethanol during binge drinking sessions (4.8g/kg/4h intake; 20% v/v ethanol solution) with a blood ethanol content range of 100mg/dl post-session. Over the course of the 7-week experiment, alcohol intake significantly increased during the 4h DID weekly sessions ($p<0.0001$). Interestingly, 2BC free-choice alcohol intake (10% ethanol) was also significantly enhanced ($p<0.01$). During binging, sleep/wake was incrementally fragmented with increasing number of bouts ($p<0.001$) and decreasing bout durations for wake and non-rapid eye movement (NREM) sleep ($p<0.001$). Of note, the number of rapid-eye movement (REM) sleep bouts decreased gradually ($P<0.05$). During the 2BC exposure, sleep time was specifically altered during the 12h of the dark phase (phase during which DID sessions occurred). NREM sleep time then increased after 3 weeks but returned to baseline after 7 weeks. Interestingly, REM/NREM ratio significantly declined after 7 weeks ($P<0.05$). In summary, the present study reveals that chronic alcohol binge drinking (1) enhances intermittent free-choice consumption, (2) incrementally fragments sleep distribution during binging, and (3) alters the subsequent 24h sleep state distribution with a significant change of REM/NREM ratio, revealing dynamic impact on sleep/wake during chronic heavy alcohol exposure.

Disclosures: **S. Perreau-Lenz:** None. **J. Vazquez-DeRose:** None. **M.D. Schwartz:** None. **W. Polgar:** None.

Poster

347. Alcohol: Behavioral Studies I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R03AA023096

Title: Intermittent two-bottle choice (I-2BC) chronic alcohol exposure alters REM sleep in a rodent model of alcoholism.

Authors: *J. VAZQUEZ-DEROSE, S. PERREAU-LENZ, A. NGUYEN;
Ctr. for Neuroscience, Biosci. Div., SRI Intl., Menlo Park, CA

Abstract: Alcohol-related sleep disturbances are well-documented (Brower, 2003; Gillin, 2000). Alcohol abuse impacts sleep even after long periods of abstinence in humans, and the EEG could be a sensitive biomarker of the long-term effects of alcohol abuse. Rapid eye movement (REM) sleep is particularly vulnerable to the effects of alcohol; clinical studies have shown a variety of REM sleep abnormalities occur in alcoholics including suppressed REM when drinking and increased REM following abstinence (Drummond, 1998; Colrain, 2009). Alcohol's effects on arousal state in rats have been conflicting and depend on strain, sleep parameters used, method of ethanol (EtOH) delivery, and duration of EtOH presentation (Ehlers, 2000; Mukherjee, 2008). However, little is known about alcohol's impact on the mechanisms of REM sleep. We therefore characterized the effects of chronic alcohol consumption on wake (W), non-rapid eye movement (NREM) and REM sleep in the rat using an I-2BC design (Carnicella, 2014) that efficiently induces high levels of voluntary EtOH consumption with high validity and translation to the clinic. EEG/EMG data were collected from rats (n=15) prior to EtOH exposure and then subjected to I-2BC for 5 months. EEG was assessed at several time points during the study (Baseline; 1st EtOH presentation, 8 wks EtOH, and 12 wks EtOH). Preliminary analyses of I-2BC alcohol intake showed that EtOH drinking significantly increased (r^2=0.7; n=11) in most of the animals while a subset of rats showed low EtOH drinking (r^2=0.3; n=3) behavior throughout the study. Repeated measures ANOVA identified a significant interaction of EtOH drinking on % W, NREM and REM sleep over the 24 h period ($F_{3,69} = 25.6$; $P < 0.0001$). No change in % W occurred relative to baseline during the 1st EtOH presentation, but % W in low-EtOH drinkers at 8 weeks was significantly different from baseline ($P < 0.05$). High-EtOH drinkers at 8 wks and 12 wks also showed significant differences in %W, most notably in the dark period ($P<0.05$). % NREM in low- and high- EtOH drinkers was also significantly different from baseline at 8 wks and 12 wks ($P < 0.05$). % REM sleep was particularly vulnerable to I-2BC. % REM sleep was increased during the 1st EtOH presentation compared to baseline. By 8 wks, REM sleep in high-EtOH drinkers was significantly elevated in the dark period ($P<0.05$); however, by 12 weeks, REM was subsequently suppressed compared to pre-EtOH sleep. These results using I-2BC, a voluntary, chronic EtOH consumption model, provide new evidence that sleep-wakefulness and, in particular, REM sleep undergo dynamic and plastic changes over the course of escalating EtOH abuse and addiction.

Disclosures: J. Vazquez-Derose: None. **S. Perreau-Lenz:** None. **A. Nguyen:** None.

Poster

347. Alcohol: Behavioral Studies I

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: UT Genetics and Alcohol Research Fund

Title: Effects of acute, mild sleep deprivation on alcohol-induced effects in mice: analysis of strain, sex and age

Authors: *K. M. HAMRE[1], J. A. BAKER[2], N.-P. VO[3], A. AGARWAL[4], K. DONOHUE[4], B. F. O'HARA[4];
[2]Dept. of Anat. & Neurobio., [1]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]Christian Brothers Univ., Memphis, TN; [4]Signal Solutions LLC, Lexington, KY

Abstract: Sleep deprivation is a common throughout the population particularly in adolescents. Moreover, sleep and exposure to alcohol are complex interacting phenotypes leading to the hypothesis that sleep disruption may alter behavioral responses to alcohol. A corollary to this is that these effects may be modulated by a number of variables including genetic background, sex, and/or the age of exposure. To test this, adult (over 90 days of age) and adolescent (35 $\pm$3 days) male and female DBA/2J (D2) and C57BL/6J (B6) mice were examined. Because 1) mice acclimate to most automated sleep deprivation paradigms, 2) mice sleep in micro-bouts rather than one long period, and 3) mild sleep deprivation is more applicable to the human condition, a 4-hour mild sleep deprivation paradigm was used where sleep deprivation was induced by a host means including placing each mouse in a new cage, introducing a novel object, gentle touching and moving the bedding. Immediately after sleep deprivation, mice were injected i.p. and behaviorally tested for anxiety in the elevated plus maze followed by testing in an activity chamber to monitor anxiety and locomotor activation. Four groups of mice were tested from each strain, sex and age (n = 6 per group): 1) Ethanol/Deprived (E/D) given 1.5 g/kg ethanol with sleep deprivation, 2) Saline/Deprived (S/D) given isovolumetric saline with sleep deprivation, 3) Ethanol/Non-Deprived (E/N) controls given ethanol as in group 1 without sleep deprivation, and 4) Saline/Non-Deprived (S/N) controls. There was no difference between the S/D and S/N groups showing that the sleep deprivation itself did not alter any phenotypes. Further, the sleep deprivation did not alter the effects of ethanol on anxiety-related phenotypes as measured in the elevated plus maze. In contrast, there were significant differences between the E/D and E/N groups on a number of measures in the activity chamber with sleep deprivation enhancing ethanol's locomotor stimulating effects. Moreover, these results occurred in a strain- and sex-specific manner. Age also was a significant factor in these results with more pronounced effects observed in adult animals than in adolescents, consistent with previous results that show

that adolescents are less sensitive to many of alcohol's effects. These results suggest that ethanol and mild sleep deprivation interact to alter responses to ethanol in a strain-, sex- and age-specific manner. Further implications from these results are that even mild sleep disruption could be a contributing factor in determining whether or not an individual progresses from social drinking to alcohol abuse or alcoholism.

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Poster

347. Alcohol: Behavioral Studies I

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Topic: G.08. Drugs of Abuse and Addiction

Support: Funding provided from the University of Minnesota

Title: Sazetidine-A reduces alcohol but not nicotine consumption in a mouse model of alcohol and nicotine co-addiction

Authors: *J. C. TOUCHETTE, K. Y. LEE, E. C. HARTELL, E. J. BADE, R. PEARSON, A. M. LEE;
Pharmacol., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Alcohol and nicotine addiction are highly co-morbid. The α4β2 nicotinic acetylcholine receptors (nAChRs) are implicated in both alcohol and nicotine addiction, however the mechanism by which they contribute to co-addiction is unclear. Recently, desensitization of α4β2 nAChRs has been proposed as a new strategy to treat alcohol and nicotine addiction as sazetidine-A, a drug that primarily desensitizes α4β2 nAChRs, reduces nicotine and alcohol consumption in rats. However, sazetidine-A has not been tested in mice or in alcohol and nicotine co-consumption procedures. In this study, we determined the effect of sazetidine-A in voluntary oral co-consumption of alcohol and nicotine in mice, using consumption models developed in our lab in which alcohol, nicotine, and water are presented concurrently. We found that male and female C57BL/6 mice readily co-consumed unsweetened alcohol and nicotine, and female mice consumed more alcohol than male mice in all procedures. In our continuous co-consumption procedure where access to drugs is available 24-hours a day, we found that varying

the nicotine concentration available during an alcohol abstinence period affected compensatory nicotine consumption and alcohol reinstatement. Chronic, intermittent access to alcohol and nicotine, which incorporates repeated abstinence periods, resulted in higher alcohol, but not nicotine, consumption compared with the continuous access procedure. Chronic intermittent alcohol and nicotine co-consumption also resulted in physical dependence, with males and females exhibiting different withdrawal signs. We then tested the effect of a single injection of 1.0mg/kg i.p. sazetidine-A and found that sazetidine-A reduced alcohol, but not nicotine, consumption in both male and female mice. When alcohol was reintroduced after a period of abstinence, sazetidine-A reduced rebound alcohol consumption but not concurrent nicotine consumption. In summary, our data suggest that sazetidine-A has different effects on alcohol and nicotine consumption, and therefore, prolonging α4β2 nAChR desensitization via sazetidine-A treatment may be a useful strategy to treat alcohol, but not nicotine, addiction.

Disclosures: **J.C. Touchette:** None. **K.Y. Lee:** None. **E.C. Hartell:** None. **E.J. Bade:** None. **R. Pearson:** None. **A.M. Lee:** None.

Poster

347. Alcohol: Behavioral Studies I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 347.20/BBB20

Topic: G.08. Drugs of Abuse and Addiction

Support: AA016849

Title: Microarchitecture of self-administration patterns during concurrent alcohol and nicotine access in mice: temporal overlap in drug intakes amplifies bout size and extends bout duration

Authors: *M. M. FORD, S. S. OSWALD, A. D. MCCRACKEN, N. L. DAVIS;
Div. of Neurosci. (L-584), Oregon Hlth. and Sci. Univ., Beaverton, OR

Abstract: Epidemiological evidence indicates that smokers are more likely to engage in binge drinking than non-smokers, and that smokers will consume up to 3-fold more cigarettes when co-engaged in a drinking episode. These findings point to a co-abuse vulnerability in which the escalation in intake of both alcohol and nicotine are observed when the drugs are used concurrently. While preclinical animal models have begun to investigate this phenomenon, a convincing demonstration of escalated drug intakes with concurrent use has yet to be shown. This absence of additive or synergistic effects may be attributable to experimenter-imposed dose units or response demands and the inability of the animal to self-regulate amount and rate of intake. The goal of this work was to explore a simple concurrent access procedure with separate

oral solutions of alcohol (10% v/v) and nicotine (75 μg/ml) in which male C57BL/6 mice (n=32) were permitted to spontaneously self-administer both drugs during daily 22-hr sessions. Drug access was provided in custom lickometer chambers to facilitate the collection of cumulative drinking records and bout microarchitecture. Self-administration interactions were examined during the fifth week of access, a time point when daily alcohol and nicotine intakes were stable at 9.8 ± 0.6 g/kg and 10.7 ± 0.6 mg/kg, respectively. Bouts of each drug were categorized based on their temporal relationship to bouts of the other drug as follows: 1) no overlap, 2) starts before and ends during, 3) starts during and ends after, 4) starts and ends during, and 5) starts before and ends after. Bout analyses revealed that mice consumed 51% of alcohol bouts and 52% of nicotine bouts in temporal isolation from one another (i.e., no overlap). Average bout sizes for alcohol and nicotine were significantly amplified by 1.5- to 2.5-fold and 1.8- to 3.2-fold, respectively, when animals were co-engaged with both drugs simultaneously versus self-administering each drug independently. Similarly, mice remained actively engaged for longer periods of time in alcohol bouts by 4.2- to 8.2-fold and in nicotine bouts by 4.0- to 7.6-fold when both drugs were simultaneously self-administered. Thus, observations from our simple concurrent access model are congruent with recent reports in human smokers and drinkers documenting that concurrent nicotine and alcohol use augments bout size of both drugs. Identification of the neural mechanisms underlying this synergism will permit the subsequent uncoupling of this behavioral interaction, and may prove to be a useful strategy for the treatment of alcohol and nicotine co-abuse. Supported by NIH grant AA016849.

Disclosures: **M.M. Ford:** None. **S.S. Oswald:** None. **A.D. McCracken:** None. **N.L. Davis:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.01/BBB21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA029635

NIH Grant DA039168

NIH Grant F31 DA040324

Title: Increased methamphetamine self-administration in hnrnph1 heterozygous mice directly implicates this rna binding protein in genetic susceptibility to methamphetamine addiction.

Authors: *K. K. SZUMLINSKI[1], J. SHAHIN[1], E. K. FULTZ[1], C. N. BROWN[1], N. YAZDANI[2], C. D. BRYANT[2];
[1]Univ. California-Santa Barbara, Santa Barbara, CA; [2]Boston Univ. Sch. of Med., Boston, MA

Abstract: *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) has been identified as a quantitative trait gene for methamphetamine (MA)-induced hyper-locomotion with mice heterozygous for a frameshift deletion in *Hnrnph1 (Hnrnph1*$^{+/-}$*)* exhibiting reduced MA locomotor-responsiveness [PLOS Genetics, 1(12):e1005713]. Very recent extension of the initial locomotor results for *Hnrnph1*$^{+/-}$ mice to place-conditioning procedures indicated that gene deletion shifted to the right the dose-response function for MA-induced place-conditioning. Herein, we examined oral MA reinforcement and intake in *Hnrnph1*$^{+/-}$ mice and their *Hnrnph1*$^{+/+}$ littermates. Male and female mice were trained to nose-poke for an unadulterated 10 mg/L MA solution initially under an FI20 schedule of reinforcement and then the response requirement was progressively increased to an FI20/FR5 schedule across weeks. While female mice tended to exhibit less MA reinforcement during this training phase of the study, we observed no genotypic differences in either active hole-responding or in MA intake. As MA intake dropped precipitously in all mice with increasing response requirement, we next established the dose-response function for MA intake under the initial FI20 schedule. Interestingly, the dose-response function for active hole-responding did not vary by genotype. However, *Hnrnph1*$^{+/-}$ mice exhibited greater MA intake than *Hnrnph1*$^{+/+}$ controls at the highest dose tested (80 mg/L), which is consistent with an increase in MA-induced conditioned reward in *Hnrnph1*$^{+/-}$ mice at higher doses that are no longer rewarding to wild-type mice. These results are a crucial extension of the MA-insensitive phenotype reported for *Hnrnph1*$^{+/-}$ mice and provide novel evidence that hnRNP H1 protein regulates the neural circuitry mediating high-dose MA-taking behavior that is directly relevant to MA abuse and addiction.
Funding provided by NIDA grants R00 DA029635 (CDB), R01 DA039168 (CDB,KKS) and F31 DA040324 (NY).

Disclosures: K.K. Szumlinski: None. **J. Shahin:** None. **E.K. Fultz:** None. **C.N. Brown:** None. **N. Yazdani:** None. **C.D. Bryant:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.02/BBB22

Topic: G.08. Drugs of Abuse and Addiction

Title: Manipulation of extracellular glutamate in the prelimbic and infralimbic cortices during the incubation of cocaine craving

Authors: *C. B. SHIN, T. J. TEMPLETON, E. S. GABLE, A. S. CHIU, T. E. KIPPIN, K. K. SZUMLINSKI;
Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Cue-elicited drug-craving increases in a time-dependent manner during drug abstinence - a phenomenon termed "incubation of craving". Our recent neurochemical studies suggest the neural substrates of this phenomenon involve glutamate within the ventromedial prefrontal cortex (vmPFC). Prior evidence posits an inhibitory role for the infralimbic cortex and a potentiating role for the prelimbic cortex subregions of the vmPFC during drug-seeking. As no study has directly examined the role for vmPFC glutamate in the incubation of cocaine-seeking, the present study aimed to fill this gap. It was hypothesized that if the IL inhibits incubated drug-seeking, then decreasing endogenous glutamate in this subregion, using the mGlu2/3 receptor agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC), will potentiate, while increasing endogenous glutamate, using the excitatory amino acid transporter inhibitor threo-β-benzyloxyaspartate (TBOA), will attenuate, incubated drug-seeking. As the PL plays a potentiating role in drug-seeking, it was hypothesized that APDC would inhibit, while TBOA would attenuate incubated drug-seeking. Male Sprague-Dawley rats were trained for 10 consecutive days to lever-press for cocaine (0.25 mg/infusion; 6 h/day), delivery of which was signaled by 20 second tone-lights cues and then subdivided into 3 or 30-day withdrawal groups. On their appropriate withdrawal day, rats were microinjected with vehicle, 50 μM APDC, or 300 μM TBOA (0.5μl/min/side) into either the IL or PL, and then given a 30-minute extinction-like test, during which responding resulted in presentation of the cues but no cocaine. As expected, animals exhibited a time-dependent intensification of cue-reinforced responding on the lever previously paired with cocaine. Microinjection of APDC did not alter behavior at either time-point in either brain region, but TBOA in the IL attenuated incubated drug-seeking during protracted withdrawal. These results argue that glutamate in the IL is functionally relevant in the incubation of cocaine-seeking and may be a potential pharmacotherapeutic strategy for curbing cue-induced craving.

Disclosures: C.B. Shin: None. **T.J. Templeton:** None. **E.S. Gable:** None. **A.S. Chiu:** None. **T.E. Kippin:** None. **K.K. Szumlinski:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.03/BBB23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA grant R01DA039168

Title: Neuroanatomically specific role of Homer2 expression in NAC regulation of methamphetamine reward sensitivity

Authors: *C. N. BROWN, E. K. FULTZ, T. E. KIPPIN, K. K. SZUMLINSKI;
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Abstract: Despite the prevalence of methamphetamine (MA) abuse, only a small percentage of MA abusers develop addiction. Understanding the genetic and neural underpinnings of individual variance in addiction susceptibility opens the possibilities for treating those who do develop addiction. Homer2 is a post-synaptic scaffolding protein involved in regulating glutamate receptor function with implications for MA-induced excitatory neurotransmission and therefore addiction plasticity. Homer2 plays a role in glutamate-dependent drug reward processing within the nucleus accumbens (NAC) and its expression is upregulated by MA within both the core and shell subregions of the NAC. As such, Homer2 plays a likely role in regulating the motivational valence of MA and sensitivity to MA reward and therefore vulnerability to MA addiction. To test this hypothesis, Homer2b expression was knocked down within the NAC core or shell of C57BL/6J mice using an adeno-associated viral vector (AAV) carrying a short hairpin RNA (shRNA). MA-induced place-conditioning procedures were employed to first gauge the effects of Homer2 knock-down upon initial MA-liking/disliking. Then operant-conditioning procedures were employed to examine shRNA effects upon MA reinforcement and oral intake. The effects of intra-NAC shRNA-Homer2b upon MA's motivational valance and intake was neuroanatomically selective, with core infusions promoting and shell infusions attenuating MA reward sensitivity. These data argue a complex regulation of MA reward sensitivity by Homer2 scaffolding within the NAC that warrants greater experimental attention.

Disclosures: C.N. Brown: None. **E.K. Fultz:** None. **T.E. Kippin:** None. **K.K. Szumlinski:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Support: K99/R00DA029635

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TTPAS Burroughs Wellcome Fund

F31DA040324-01A1

Title: Transcriptomic and neuroanatomical mechanisms of Hnrnph1 in methamphetamine reward

Authors: *N. YAZDANI[1], Q. T. RUAN[1], M. CHAU[1], E. R. REED[4], F. MORTAZAVI[2], D. ROSENE[2], J. GRANT[5], W. JOHNSON[3], C. D. BRYANT[1];
[1]Pharmacol. & Exptl. Therapeut., [2]Anat. & Neurobio., [3]Dept. of Medicine, Div. of Computat. Biomedicine, Boston Univ. Sch. of Med., Boston, MA; [4]Bioinformatics, Boston Univ., Boston, MA; [5]The Univ. of New Orleans, New Orleans, LA

Abstract: Using fine mapping and gene editing, we recently identified *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) as a quantitative trait gene for the locomotor stimulant properties of methamphetamine (**MA**). To extend the contribution of *Hnrnph1* to MA reward, we conducted conditioned place preference (**CPP**) which consisted of one day of baseline preference assessment followed by four alternating saline and MA training days with floor textures as cues, two consolidation days, and final assessment of MA drug preference on Day 8. *Hnrnph1*$^{+/-}$ mice were less sensitive to the rewarding properties of MA at 0.5 mg/kg (i.p.) and conversely, more sensitive at 2 mg/kg. Transcriptome analysis via RNA-seq of *Hnrnph1*$^{+/-}$ striatal tissue followed by Ingenuity Pathway Analysis revealed significant perturbations in EIF2 signaling, Calcium signaling, Dopamine-DARPP32 signaling, and Corticotropin Releasing Hormone signaling. Diseases and biofunctions analysis ranked Neurological and Psychiatric Disorders, Cell Morphology, and Nervous System Development and Function as top categories, which include addiction-relevant Anxiety, Depression, Drug Dependence, neuron growth, and synaptic transmission disease/function annotations. Spliceome analysis of *Hnrnph1*$^{+/-}$ and a congenic line possessing a 112kb congenic interval from the DBA/2J strain (encompassing solely *Hnrnph1* and *Rufy1* genes) revealed differential splicing of *Hnrnph1* as a molecular mechanism that bridges one or more functional *Hnrnph1* polymorphisms with behavior. Notably, *Ppp3ca* (Calcineurin catalytic subunit) was the top differentially spliced gene and interestingly, SNPs affecting splicing of *PPP3CA* in humans have been associated with addiction vulnerability. To further dissect the neurobiological mechanisms underlying differential MA sensitivity and reward in *Hnrnph1*$^{+/-}$ mice, immunohistochemical (**IHC**) analysis of tyrosine hydroxylase indicated a more pronounced increase in dopaminergic innervation in the rostral striatum. Additionally, hnRNP H was expressed pan-neuronally throughout the prefrontal cortex and striatum. Finally, thionin counterstaining revealed exclusion of hnRNP H in glia. These studies demonstrate that *Hnrnph1* is crucial for neurodevelopment and function of midbrain dopaminergic neurons and the behavioral response to psychostimulants.

Disclosures: **N. Yazdani:** None. **Q.T. Ruan:** None. **M. Chau:** None. **E.R. Reed:** None. **F. Mortazavi:** None. **D. Rosene:** None. **J. Grant:** None. **W. Johnson:** None. **C.D. Bryant:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.05/BBB25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA024044

Title: The effects of mGlu5 blockade within the nucleus accumbens shell on alcohol withdrawal-induced anxiety in mice

Authors: ***K. M. LEE**, M. A. COELHO, M. A. CLASS, K. R. SERN, M. D. BOCZ, M. SUZUKI, K. K. SZUMLINSKI;
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Abstract: It is well established in both human and animal literature that a history of alcohol abuse is capable of dysregulating glutamatergic signaling within key brain regions implicated in the etiology of addiction, such as the nucleus accumbens. Previous work from our lab found a significant increase in mGlu5 expression within the nucleus accumbens shell (AcbSh) of adult binge-drinking animals during early withdrawal. This increase in mGlu5 coincided with behavioral manifestations of hyperanxiety. Subsequently, we've demonstrated that systemic treatment with an mGlu5 antagonist successfully reverses this hyperanxious state. Therefore, the present study sought to determine if the anxiolytic effects of mGlu5 antagonism during early withdrawal was specific to the AcbSh using an intracranial approach. Adult male C57BL/6 mice (N=40) were bilaterally implanted with indwelling guide cannulae positioned above the AcbSh. After recovery, half of the animals were subjected to 14 consecutive days of binge drinking under modified Drinking in the Dark (DID) procedures: beginning 3hrs into the dark cycle, animals were given access to 10%, 20% and 40% (v/v) alcohol and allowed to drink for 2hrs. Control animals received only water. Approximately 24hrs following the final alcohol presentation, all animals underwent behavioral testing consisting of the light/dark box test, marble burying test, and forced swim test (FST). Fifteen minutes prior to the start of testing, half of the animals from each drinking group received 10ug/side bilateral infusions of 3-((2-Methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP) into the AcbSh. The remaining animals received vehicle. Consistent with our previous findings, alcohol-drinking animals showed increased anxiety relative to water controls in the light/dark box test and marble burying test. Alcohol drinkers also showed a significant decrease in immobility in the FST compared to water drinkers.

We have seen this reduction in immobility repeatedly across our previous studies and interpret this to reflect hyper-reactivity to an acute stressor, predictive of anxiety. However, there was no significant effect of MTEP treatment relative vehicle. Based on evidence suggesting that the dose-response curve for MTEP may have an inverted-U shape, we speculate that our dose may have been too high and thus nullified any therapeutic effects. Therefore, these data are insufficient to discredit the role of mGlu5 within the AcbSh in withdrawal-induced hyperanxiety. Further investigation is warranted and we are in the process of replicating this experiment with a lower dose.

Disclosures: **K.M. Lee:** None. **M.A. Coelho:** None. **M.A. Class:** None. **K.R. Sern:** None. **M.D. Bocz:** None. **M. Suzuki:** None. **K.K. Szumlinski:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.06/BBB26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA Intramural Research Program

Title: Increased expression of AMPA glutamate receptors in the prefrontal cortex distinguishes abstinent rats from compulsive methamphetamine takers

Authors: *J. L. CADET[1], I. KRASNOVA[2], B. LADENHEIM[2], M. MCCOY[2], N. TERRY[2], D. WALTHER[2];
[1]Mol. Neuropsychiat Br., [2]NIH/NIDA, Baltimore, MD

Abstract: Methamphetamine addiction is a common psychiatric disorder associated with loss of control over drug use. In addition, many medical and neurological complications are pervasive during the clinical course of this brain disorder. In the present study, we used footshocks as adverse consequences to distinguish rats that continue to take methamphetamine compulsively (footshock-resistant) from those that become abstinent (footshock-sensitive) in the presence of increasing footshocks. Male Sprague-Dawley rats were trained to self-administer methamphetamine (0.1 mg/kg/injection, i.v.) or saline during twenty-two 9-h sessions. During training, all rats escalated their intake of methamphetamine. Following the training phase, rats were subjected to incremental footshocks for 10 additional sessions. During the footshock phase, the rats were split into shock-resistant and shock-sensitive groups. Animals were euthanized 2 hours after the last shock sessions and brain regions were dissected and processed to measure mRNA levels. We found significant increases in the expression of AMPA glutamate receptors in

the prefrontal cortex (PFC), but not in the dorsal striatum, of shock-sensitive rats. The expression of NMDA GluN1 and GluN2A receptors was also increased in the PFC of sensitive rats. These results suggest that increased expression of glutamate receptors in the PFC may play a significant role in promoting abstinence from methamphetamine self-administration in the presence of footshocks. These observations are consistent with the accumulated evidence that proposes significant roles of glutamatergic systems in addiction.

Disclosures: **J.L. Cadet:** None. **I. Krasnova:** None. **B. Ladenheim:** None. **M. McCoy:** None. **N. Terry:** None. **D. Walther:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.07/CCC1

Topic: G.08. Drugs of Abuse and Addiction

Title: The dorsomedial striatum is critical for incubation of methamphetamine craving after voluntary abstinence

Authors: *D. CAPRIOLI, M. VENNIRO, M. ZHANG, A. LI, B. L. WARREN, Y. SHAHAM; Behavioral Neurosci., Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Objectives and rationale: We recently introduced an animal model of incubation of methamphetamine craving after prolonged voluntary abstinence. Here, we studied the role of dorsal striatum dopamine in this form of relapse, which more closely mimics the human condition. Methods: We trained rats with free access to food and water to self-administer palatable food pellets (6 days, 6-h/d) and intravenous methamphetamine (12 days, 6-h/d, 0.1 mg/kg/infusion). We then assessed methamphetamine seeking in extinction after 1 and 21 abstinence days. Between tests, the rats underwent voluntary abstinence (achieved via a discrete choice procedure between methamphetamine and palatable food; 20 trials per day) for 19 consecutive days. Next, we used RNAscope® in situ hybridization to measure co-labeling of the neuronal activity marker *Fos* with dopamine *Drd1* and *Drd2* in dorsomedial (DMS) and dorsolateral (DLS) striatum after the relapse tests. Based on the RNAscope® results, we injected D1 or D2-family receptor antagonists (SCH39166 or raclopride, respectively) into the DMS prior to the extinction tests to determine a causal role of DMS dopamine in incubation of methamphetamine craving after voluntary abstinence. Results: Methamphetamine seeking was higher after 21 days of voluntary abstinence than after 1 day (incubation of methamphetamine craving) and was associated with increased *Fos* expression in DMS but not DLS; *Fos* was co-labelled with both *Drd1* and *Drd2*. DMS injections of SCH39166 or raclopride decreased

'incubated' methamphetamine seeking after 21 abstinence days. Conclusions: Results demonstrate a critical role of DMS dopamine D1 and D2-receptors in incubation of methamphetamine craving after voluntary abstinence. This research was supported by the Intramural Research Program of the NIH

Disclosures: D. Caprioli: None. **M. Venniro:** None. **M. Zhang:** None. **A. Li:** None. **B.L. Warren:** None. **Y. Shaham:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.08/CCC2

Topic: G.08. Drugs of Abuse and Addiction

Title: A critical role of the central amygdala nucleus in relapse to methamphetamine seeking after voluntary abstinence

Authors: *M. VENNIRO[1], M. ZHANG[1], C. CIFANI[2], B. L. WARREN[1], J. M. BOSSERT[1], N. J. MARCHANT[1], C. CHIAMULERA[3], D. CAPRIOLI[1], Y. SHAHAM[1];
[1]Natl. Inst. On Drug Abuse, Baltimore, MD; [2]Univ. of Camerino, Camerino, Italy; [3]Univ. of Verona, Verona, Italy

Abstract: Rationale and Objective: We recently developed a rat model of relapse to methamphetamine seeking after voluntary abstinence. The mechanisms underlying this form of relapse are unknown. Here, we studied the role of central amygdala (CeA) and its forebrain projections in relapse after voluntary abstinence. Methods: We trained rats to self-administer palatable food (6 d, 2-h/d) and intravenous methamphetamine (15 d, 2-h/d). We then assessed methamphetamine seeking in extinction tests after 14 voluntary abstinence days (achieved via a discrete choice procedure between methamphetamine and palatable food). Results: Relapse to methamphetamine seeking after voluntary abstinence was associated with increased expression of the activity marker Fos in CeA but not basolateral amygdala (BLA). Systemic injections of SCH39166 (D1-family receptor antagonist [20 µg/kg]) decreased relapse to methamphtemaine seeking and Fos expression in CeA. Using RNAscope in-situ hybridization we found that the majority of Fos positive neurons co-labelled with dopamine Drd1, but not Drd2 in CeA. CeA injections of SCH39166 (0.5-1.0 µg/side) decreased relapse to methamphetamine seeking after voluntary abstinence; CeA injection of raclopride (D2-family receptor antagonist [1.0 µg/side]) or BLA injections of SCH39166 (1.0 µg/side) had no effect. Double-labeling analysis of Fos with the retrograde tracer cholera toxin subunit-B (CTb, injected into CeA) showed that relapse after voluntary abstinence was associated with selective activation of ventral anterior insula

(AIV) neurons projecting to CeA.Conclusions: Results demonstrate a critical role of CeA Drd1 in relapse to methamphetamine seeking after voluntary abstinence and further suggest a role of AIV→CeA projection in this form of relapse. Supported by the NIDA-IRP

Disclosures: **M. Venniro:** None. **M. Zhang:** None. **C. Cifani:** None. **B.L. Warren:** None. **J.M. Bossert:** None. **N.J. Marchant:** None. **C. Chiamulera:** None. **D. Caprioli:** None. **Y. Shaham:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.09/CCC3

Topic: G.08. Drugs of Abuse and Addiction

Title: Incubation of methamphetamine but not heroin craving after voluntary abstinence in male and female rats

Authors: *M. ZHANG, M. VENNIRO, Y. SHAHAM, D. CAPRIOLI;
Natl. Inst. on Drug Abuse, NIH, Baltimore, MD

Abstract: Rationale and Objective: We recently introduced an animal model of incubation of methamphetamine craving after prolonged voluntary abstinence in male rats. Here, we determined whether incubation of drug craving after voluntary abstinence generalizes to rats with a history of heroin self-administration. We also determined whether there are sex differences in this incubation. Methods: We trained male and female rats with free access to food and water to self-administer palatable high carbohydrate 45-mg food pellets for 6 days (6-h/day, 5 pellets per lever-press; FR1 reinforcement schedule) and then to self-administer either methamphetamine (0.1 mg/kg/infusion) or heroin (0.1 mg/kg/infusion) for 12 days (6-h/day, FR1 schedule). We then assessed cue-induced drug seeking in extinction tests after 1 and 21 abstinence days. Between tests, the rats underwent voluntary abstinence (achieved via a discrete choice procedure between drug and palatable food; 20 trials per day) for 19 days. Results: There were no sex differences in methamphetamine or heroin self-administration or in the strong preference for the palatable food over the drugs during the voluntary abstinence period. In both sexes, cue-induced methamphetamine seeking in the extinction tests was higher after 21 days of voluntary abstinence than after 1 day (incubation of methamphetamine craving). In contrast, in both sexes, cue-induced heroin seeking after 21 days of voluntary abstinence was not statistically higher than after 1 day. Conclusions: Our results show that incubation of methamphetamine craving after voluntary abstinence generalizes to female rats. Unexpectedly, prolonged voluntary abstinence

prevented the emergence incubation of heroin craving in both sexes. This work was supported by NIDA IRP.

Disclosures: **M. Zhang:** None. **M. Venniro:** None. **Y. Shaham:** None. **D. Caprioli:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.10/CCC4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA/NIH

Title: Role of afferents into dorsal striatum in incubation of methamphetamine craving

Authors: ***X. LI**, F. SURJONO, T. ZERIC, J. BOSSERT, Y. SHAHAM;
Behavioral Neurosci. Res. Br., Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Background: We previously reported that methamphetamine (Meth) seeking progressively increases after withdrawal from extended access Meth self-administration (incubation of Meth craving). Here we studied the role of the dorsal striatum (a brain region previously implicated in cue- and context-induced drug relapse) and its afferent projections in incubation of Meth craving.
Methods: In Exp. 1, we trained rats to self-administer intravenous Meth for 10 days (6-9 h/d; 0.1 mg/kg/infusion); infusions were paired with a tone-light cue. After 1-month withdrawal from Meth self-administration, we injected a D1-family receptor antagonist (SCH23390) into the dorsolateral or dorsomedial striatum, and then tested the rats for Meth seeking under extinction conditions. In Exp. 2, we injected Cholera toxin b (CTb, a retrograde tracer) into dorsomedial striatum on withdrawal day 15. Two weeks later, we either tested or did not test rats for "incubated" Meth seeking and performed immunohistochemistry for CTb and Fos (a neuronal activity marker).
Results: We found that injections of SCH23390 into dorsolateral or dorsomedial striatum decreased 'incubated' Meth seeking after 1-month withdrawal. Furthermore, we found that "incubated" Meth seeking was associated with increased neurons double-labeled for CTb and Fos in central lateral thalamus, but not in prefrontal cortex, basolateral amygdala, or substantia nigra.
Conclusions: Our results demonstrated a critical role of dorsal striatum in incubation of Meth craving. In addition, this incubation is associated with selective activation of projections from central lateral thalamus to dorsomedial striatum. We currently explore the casual role of central

lateral thalamus in incubation of Meth craving.
This work was supported by NIDA/NIH.

Disclosures: **X. Li:** None. **F. Surjono:** None. **T. Zeric:** None. **J. Bossert:** None. **Y. Shaham:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.11/CCC5

Topic: G.08. Drugs of Abuse and Addiction

Support: CNPq

CAPES

FAPERJ

FINEP

Title: Diazepam inhibits phasic dopamine release in the nucleus accumbens and reverses the increase of phasic dopamine release induced by amphetamine

Authors: A. GOMEZ-A.[1], A. M. FIORENZA[2], S. L. BOSCHEN[3], A. H. SUGI[3], D. BECKMAN[4], S. T. FERREIRA[4], K. LEE[5], C. D. BLAHA[5], *C. DA CUNHA[3];
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Abstract: Diazepam is a benzodiazepine receptor agonist widely used to treat anxiety, muscle spasms, seizures, insomnia, and restless legs syndrome. It is intriguing that, although diazepam is also used to treat alcohol withdrawal syndrome, long term use of diazepam can result in dependence. Furthermore, while most addictive drugs increase tonic dopamine in the nucleus accumbens (NAc), acute administration of diazepam causes the opposite effect. In the current study we confirmed the effect of diazepam on tonic dopamine in the NAc by using 20 min sampling *in vivo* microdialysis and tested whether an acute administration of diazepam also affects phasic dopamine release in the NAc. In addition, we studied whether diazepam can reverse the enhancing effect of amphetamine on phasic release of dopamine. These experiments were carried out in urethane (1.5 gm/kg i.p.) anesthetized adult male Swiss mice. Phasic release

of dopamine in the NAc was evoked by electric stimulation (20 biphasic pulses, 0.5 ms per pulse, 600 μA, 60 Hz) of the ventral tegmental area (VTA) and measured by fast-scan cyclic voltammetry in combination with carbon fiber microelectrodes. Diazepam (1, 2, or 3 mg/kg i.p.) administration dose-dependently inhibited VTA stimulation-evoked dopamine release. Administration of the benzodiazepine receptor antagonist flumazenil (2.5 mg/kg i.p.) 17 min before or 17 min after the administration of 2 mg/kg diazepam blocked the attenuating effect of 2 mg/kg diazepam on VTA stimulation-evoked dopamine release. Administration of diazepam (2 mg/kg i.p.) 3 min after amphetamine (5 mg/kg i.p.) injection reversed the increase of VTA stimulation-evoked dopamine release caused by amphetamine. These results suggest that the anxiolytic effects of benzodiazepines have a dopaminergic component and that benzodiazepines may be useful not only to treat alcohol withdrawal syndrome, but also to treat addiction to drugs of abuse which augment dopamine release in the NAc.

Disclosures: **A. Gomez-A.:** None. **A.M. Fiorenza:** None. **S.L. Boschen:** None. **A.H. Sugi:** None. **D. Beckman:** None. **S.T. Ferreira:** None. **K. Lee:** None. **C.D. Blaha:** None. **C. Da Cunha:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020919

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Title: Methamphetamine induces striatal dopamine efflux through interactions between VMAT2 and sigma receptors

Authors: ***D. HEDGES**[1], E. Y. JANG[2], J. T. YORGASON[3], C. CARR[1], J. SKIDMORE[1], V. K. WEERASEKARA[1], F. P. BELLINGER[4], J. D. UYS[5], S. STEFFENSEN, 84602[1];
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Abstract: The main objective of this study was to determine the mechanism of methamphetamine (METH)-induced dopamine (DA) release in the nucleus accumbens (NAc). Methamphetamine enhanced phasic (stimulated) DA release and caused an electrically-

independent efflux of DA. The sodium channel blocker lidocaine abolished phasic DA release, but did not affect METH-induced DA efflux, indicating action-potential dependent and independent mechanisms behind METH's effects. The sigma-1 receptor antagonist BD 1063 significantly attenuated METH's effect on DA release. Depletion of intracellular calcium (Ca^{2+}) reserves also attenuated METH-enhancement of DA release. Reduced glutathione (the substrate for glutathione peroxidase) and 4-hydroxy-TEMPOL (a superoxide dismutase mimetic) blocked METH's effect on DA release, suggesting that a reactive oxygen species (ROS), most likely superoxide, is necessary for METH-induced DA efflux. We were also able to determine that oxidative stress, as well as acute METH, impair the vesicular monoamine transporter 2 (VMAT2) by S-glutathionylation modification of Cys-488, highlighting VMAT2 as a likely regulator of METH's effects on electrically independent DA release. Taken together, METH induces DA release in the NAc through a cascade involving the sigma receptor and ROS signaling molecules, subsequent inhibition of VMAT2, cytoplasmic accumulation of DA and release through the DA transporter.

Disclosures: **D. Hedges:** None. **E.Y. Jang:** None. **J.T. Yorgason:** None. **C. Carr:** None. **J. Skidmore:** None. **V.K. Weerasekara:** None. **F.P. Bellinger:** None. **J.D. Uys:** None. **S. Steffensen:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: Loma Linda University School of Pharmacy

Title: Methylphenidate significantly alters functional connectivity between the prefrontal cortex and ventral tegmental area dopamine neurons

Authors: *I. DELA PENA[1], W.-X. SHI[2];
[1]Pharmaceut. Sci., [2]Pharmaceut. and Administrative Sci., Loma Linda Univ., Loma Linda, CA

Abstract: The neural mechanisms underlying the therapeutic and reinforcing effects of methylphenidate are not fully understood. Deciphering the effects of methylphenidate on the functional connectivity between the prefrontal cortex (PFC) and the ventral tegmental area (VTA) dopamine neurons may provide important insights into the drug's mechanism(s) of action. We made dual site recordings from the PFC and dopamine neurons in the VTA, and studied the effect of methylphenidate on their functional connectivity using both time and

frequency domain correlational analysis methods. Methylphenidate drove PFC local field potentials (LFPs) to the UP states suggesting increased activity of PFC neurons. It also inhibited dopamine neurons through activation of D2-like receptors. Importantly, methylphenidate shifted the functional coupling between the PFC and dopamine neurons from negative (i.e., dopamine neurons fired spikes preferentially during cortical DOWN states) to positive (dopamine neuron fired spikes more frequently during UP states) as revealed by dopamine cell spike-triggered averages of PFC LFPs. This change was further confirmed using the fast Fourier transformation-based phase analysis, a frequency-domain method, and the Hilbert transformation-based phase analysis, a mixed time and frequency-domain method. We conclude that the observed change in phase in PFC-dopamine neuron coupling induced by methylphenidate would significantly alter the relative timing between glutamate release from PFC terminals and DA release from DA neurons and thus modify dopamine-glutamate interaction in brain areas innervated by both PFC and dopamine terminals, e.g., the nucleus accumbens (NAc). As we erstwhile suggested, the timing between glutamate and DA release is crucial for certain forms of synaptic plasticity in the NAc. This change induced by methylphenidate may, thus, contribute critically to the drug's therapeutic and reinforcing effects.

Disclosures: I. dela Pena: None. **W. Shi:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: FONDECYT N° 1141088

Beca de Doctorado Nacional CONICYT

Concurso de Apoyo para el Desarrollo de Tesis de Post-grado 2016 PMD-05/16

Title: Effect of amphetamine sensitization on single unit activity in the rat dorsolateral striatum

Authors: *R. I. GATICA[1,2,3], M. I. AGUILAR-RIVERA[4], J. A. FUENTEALBA[1,3];
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Abstract: Drug addiction is a cluster of cognitive, behavioral and physiological symptoms associated with compulsive drug intake. The transition from voluntary drug use to its compulsive and repetitive use characterizes the development of this habit. The dorsolateral striatum (DLS)

has a key role in habit acquisition. The increase of dopamine levels in the DLS, after psychostimulant administration, is correlated with the establishment of habitual drug seeking behavior. The repeated and chronic exposure to psychostimulants like amphetamine (AMPH) has been associated with the development of locomotor sensitization. However, knowledge about changes in DLS neural electrical activity that could correlate with AMPH locomotor sensitizations is lacking. To understand this association, rats were injected once daily with amphetamine (1mg/kg) for five consecutive days. After four days of withdrawal, rats were injected with an acute AMPH injection to assess the expression of locomotor sensitization. Twenty-four hours after the expression of locomotor sensitization, rats were anesthetized with urethane and an array of eight tetrodes was lowered into the DLS for acute neuronal recording. Our data showed a non-significant increase of basal firing rate in AMPH group relative to control group with a median of 0.117 Hz vs 0.0458 Hz respectively (Mann-Whitney test, $P > 0.05$). However, we found a bigger proportion of DLS neurons in AMPH treated rats that showed a decrease of their firing rate in response to AMPH in comparison to control rats (40% v/s 19% respectively, Fisher exact test, $p<0.05$). This data show that AMPH locomotor sensitization is accompanied by changes in DLS neural activity.

Disclosures: **R.I. Gatica:** None. **M.I. Aguilar-Rivera:** None. **J.A. Fuentealba:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSC 102-2410-H-431-005-MY3

Title: Roles of nucleus accumbens core and shell for methamphetamine-induced behavioral sensitization in rats

Authors: ***C.-N. CHENG**, A. C. W. HUANG;
Fo Guang University, Psychology, Fo Guang University, Psychology, Taipei, Taiwan

Abstract: To our knowledge, nucleus accumebns mediates drug addiction and reinforcement learning. Nucleus accumbens core and shell regions have different roles for drug addiction and dependence needs to be scrutinized. The present study conducted behavioral sensitization paradigm to address this issue whether different involvements of nucleus accumbens core and shell in behavioral sensitization. On the experimental procedure, rats were intraperitoneally given a high dose of NMDA to destroy nucleus accumbens shell or core, respectively. Following

7 days recovery, all of rats were continuously injected with methamphetamine (MAMPH, 1mg/kg) or vehicle normal saline, respectively. Rats were assigned into Saline and MAMPH groups one trial a day for 7 days. On the withdrawal phase, they were conducted with no treatments for 7 days. Later, a low dose of methamphetamine (0.5mg/kg) was challenged to measure the locomotor activity for behavioral sensitization. The results showed that: (a). MAMPH induced hyperactivity for behavioral sensitization in drug addiction. However, lesion of nucleus accumbens core with NMDA decreased locomotor activity, indicting behavioral sensitization reduction only on the acquisition phase. However, during testing, NMDA did not affect locomotor activity for behavioral sensitization. (b). During acquisition phase, NMDA injection in the nucleus acumens shell exhibited increases in locomotor activity for behavioral sensitization. During testing, NMDA lesions of the nucleus accumebns shell actually increased locomotor activity. The present data provide some insights for drug addiction and dependence in clinic. **Keywords**: nucleus accumbens shell, nucleus accumbens core, dopamine, behavioral sensitization, methamphetamine NSC 102-2410-H-431-005-MY3

Disclosures: C. Cheng: None. **A.C.W. Huang:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Program#/Poster#: 348.16/CCC10

Topic: G.08. Drugs of Abuse and Addiction

Title: Effects of TMEM168 overexpression on methamphetamine-induced hyperlocomotion and place preference, and anxiety in mice via regulating dopaminergic and GABAergic neuronal systems in the nucleus accumbens of mice

Authors: *K. FU[1], Y. MIYAMOTO[1], E. SAIKA[1], S.-I. MURAMATSU[2], K. UNO[1], A. NITTA[1];
[1]Univ. of Toyama, Toyama-Shi, Japan; [2]Jichi Med. Univ., Shimotsuke-Shi, Japan

Abstract: Transmembrane protein 168 (TMEM168) is a novel molecule that overexpressed in the nucleus accumbens (NAc) of mice after repeated administration of methamphetamine (METH). The protein consists of 697 amino acid residues, including some putative transmembrane domains, and might be related to the pathological mechanism of METH dependence. However, the neuropsychological functions were not be clarified. In this study we investigated the effects of accumbal overexpressed TMEM168 on METH-induced behaviors in mice and then tried to elucidate the physiological function of TMEM168 *in vivo*.
Adeno-associated virus vector containing TMEM168 cDNA was injected to the NAc of

C57BL/6J mice (TMEM mice), and then the accumbal TMEM168 mRNA in TMEM mice was overexpressed, increasing approximately seven fold of the control mice (Mock mice). TMEM mice showed no change in spontaneous locomotor activity, but the METH-induced hyperlocomotion was attenuated compared with Mock mice. Similarly, in the conditioned place preference test, METH-induced place preference was comparatively attenuated in TMEM mice. Although basal levels of extracellular dopamine in the NAc of TMEM mice were not different with that of Mock mice, METH-induced dopamine elevation was suppressed. Furthermore, high K^+ -stimulated dopamine efflux was also inhibited in the NAc of TMEM mice. As a series of behavioral test, TMEM mice showed no change in cognitive ability, social interaction and depression-like behavior. But in the light/dark box and elevated-plus maze tests, TMEM mice exhibited anxiety and this increased anxiety was reversed by anti-anxiety drug diazepam. Although total contents of GABA in the NAc of TMEM mice were not changed, basal levels of accumbal extracellular GABA levels were decreased in TMEM mice and high K^+ -stimulated GABA efflux was also inhibited. The present results suggest that accumbal TMEM168 overexpression inhibits METH-induced hyperlocomotion and place preference, but induces anxiety by affecting dopaminergic and GABAergic neuronal system in the NAc of mice.

Disclosures: K. Fu: None. **Y. Miyamoto:** None. **E. Saika:** None. **S. Muramatsu:** None. **K. Uno:** None. **A. Nitta:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: the University at Buffalo-SUNY

Title: Methamphetamine-induced dysfunction of NE signaling in the bed nucleus of the stria terminalis of the rat brain

Authors: *J. PARK, R. V. BHIMANI, K. T. WAKABAYASHI;
Univ. At Buffalo, Buffalo, NY

Abstract: Norepinephrine (NE) in the bed nucleus of the stria terminalis (BNST) known to integrate information between the stress and reward systems plays a critical role in the mechanisms contributing to the stress induced drug seeking, aversion related to drug withdrawal, and the reinforcing effects of drugs. Despite this, not much is known about how NE signaling in the BNST is regulated by addictive stimulant drugs due to the comparatively diffuse distribution

of NE neurons. Furthermore, these terminals are localized to the very small BNST in the rat brain. Accumulating evidence shows that methamphetamine (METH), a powerful and highly addictive stimulant drug, has stronger, more potent effects on NE transmission than dopamine. Therefore, NE in the BNST may be critically implicated in METH-induced effects including mediating its withdrawal symptoms.
In the present study, we investigated the characteristic features of NE regulation in the BNST of both urethane-anesthetized and awake rats during withdrawal after repeated exposure to METH. For these studies, we employed fast-scan cyclic voltammetry with carbon-fiber microelectrodes to study subsecond changes of NE signaling in the BNST. We will demonstrate the METH-induced dysregulation of NE transmission in the BNST and its effect on behaviors. These results will extend our understanding of BNST-NE mechanisms in METH abuse and addiction.

Disclosures: J. Park: None. **R.V. Bhimani:** None. **K.T. Wakabayashi:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: JPB foundation

PDF

NIH NS095053

Title: Time correlated single photon counting *In vivo* reveals enhanced activation of D1-expressing medium spiny neurons in the dorsal striatum following acute amphetamine administration in mice

Authors: *T. H. CHEUNG, R. M. MIKOFSKY, T. ZERIC, S. D. CLARK, U. J. KANG, D. L. SULZER;
Neurol., Columbia Univ., New York, NY

Abstract: Amphetamine (administered as Dexedrine or Adderall) is a stimulant with both therapeutic and high abuse potential. The abuse liability of amphetamine is in part determined by its action on dopaminergic synapses in the striatum. There is much evidence supporting the role of the nucleus accumbens (ventral striatum) in addiction-like behaviors. However, less is known about the role of dorsal striatum in mediating amphetamine's effects in these behaviors. We used time correlated single photon counting (TCSPC) (custom machine ChiSquare Bioimaging) to

optically record activity in D1-expressing medium spiny neurons (D1MSN) in the dorsal striatum that project to the substantia nigra pars reticulata and the internal segment of the globus pallidus (termed the direct pathway). Mice expressing cre recombinase in D1-receptor-expressing neurons were injected with AAV9 Flex GCaMP6f virus into the dorsal striatum, and imaging fibers were also implanted in the same site. We recorded D1MSN calcium transients from mice in their home cage following either an acute amphetamine injection (5 mg/kg, IP) or saline as a control. We find that enhanced locomotion induced by acute amphetamine administration was correlated with increased calcium transients from D1MSN, implicating amphetamine modulation of the dorsal striatal direct pathway.

Disclosures: T.H. Cheung: None. **R.M. Mikofsky:** None. **T. Zeric:** None. **S.D. Clark:** None. **U.J. Kang:** None. **D.L. Sulzer:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015351

Title: Medial prefrontal cortex is not required for amphetamine to produce acute-withdrawal related hypoactivity in rats

Authors: *W. WHITE, H. L. HOWARD, Z. S. ABBOTT, K. M. HAGER, K. L. EVERMAN, I. M. WHITE;
Psychology, Morehead State Univ., Morehead, KY

Abstract: Rats administered 2.0 mg/kg amphetamine or 5.0 mg/kg morphine show a reduction in activity 12 to 24 hours later (longer-term hypoactivity), an aspect of an acute withdrawal or “hangover” state. A dopamine D1 receptor antagonist (SCH 23390) given 30 minutes after drug, blocks the reduction in activity, suggesting that amphetamine and morphine work through a common pathway to initiate the cascade of events resulting in aspects of acute withdrawal. One possibility is that amphetamine and morphine produce aspects of acute withdrawal by modifying dopaminergic output regions of the ventral tegmental area (VTA), such as the medial prefrontal cortex (mPFc), the amygdala, and the nucleus accumbens. This study assessed the role of the mPFc in the elicitation of longer-term hypoactivity by amphetamine. Adult male Wistar rats received NMDA (10 mg, 0.5 microliter/site) or sham lesions in the mPFc. The animals then received a series of seven-day tests. At the beginning of Day 1 of a test, near light onset, each

animal was given a saline administration and was placed in a separate open field arena for 24 hours, and activity was monitored. At the beginning of Day 4 of a test, each animal was given either amphetamine (2.0 mg/kg) or amphetamine followed 30 minutes later by SCH 23390 (0.05 mg/kg). Again, each animal was placed in an open field for 24 hours, and activity was monitored. Activity following drugs was compared to activity following saline. In both the sham and lesioned rats, amphetamine produced longer-term hypoactivity, and in both groups D1 antagonist blocked longer-term hypoactivity. The results suggest that the longer-term hypoactivity produced by amphetamine does not depend on the mPFc and on dopaminergic changes that occur within the mPFc.

Disclosures: W. White: None. **H.L. Howard:** None. **Z.S. Abbott:** None. **K.M. Hager:** None. **K.L. Everman:** None. **I.M. White:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSC 102-2410-H-431-005-MY3

Title: The medial prefrontal cortex-basolateral amygdala pathway mediates methamphetamine-induced conditioned saccharin suppression: Evaluation of the reward comparison hypothesis

Authors: *A. C.-W. HUANG, A. B. H. HE;
Fo Guang Univ, Psychology, Yilan County, Taiwan

Abstract: Abused drugs have been shown to produce the similar conditioned taste aversion effect to be resulted from the outweighing reward value of the unconditioned stimulus (US) than that of the conditioned stimulus (US), in termed of the reward comparison hypothesis. Until now, it is unknown how the medial prefrontal cortex (mPFC)-the basolateral amygdala (BLA) pathway mediates abused drugs-induced conditioned CS suppression and whether the reward comparison hypothesis needs to be scrutinized in an aspect of neural mechanisms. The purpose of the present study used immunohistochemical c-Fos and pERK staining to address the mPFC-BLA neural circuits on methamphetamine (MAMPH)-induced conditioning, extinction, and reinstatement phases. The prelimbic cortex (PrL) and infralimbic cortex (IL) of the mPFC was determined whereas the BLA was chosen using the immunohistochemical staining with c-Fos and p-ERK proteins. The present results showed that MAMPH-induced conditioned suppression occurred conditioning over 3 trials, extinction over 5 trials, and reinstatement for 1 trial,

respectively, in behavior level. On conditioning phase, the c-Fos and p-ERK overexpressions occurred at the IL, PrL, and BLA. On extinction, the c-Fos and p-ERK overexpressions were found at the IL and PrL, but not BLA. On the reinstatement phase, the c-Fos was active at the BLA but not IL and PrL. The p-ERK was active at the IL, PrL, and BLA. Some data would challenge the reward hypothesis and some would be supportive of the reward comparison hypothesis.The present findings may provide some insights for examining the reward comparison hypothesis. The reward comparison hypothesis is considered to be revised further. Key word: the medial prefrontal cortex, amygdala, methamphetamine, the reward comparison hypothesis NSC 102-2410-H-431-005-MY3

Disclosures: **A.C. Huang:** None. **A.B.H. He:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.21/CCC15

Topic: G.08. Drugs of Abuse and Addiction

Title: Chemogenetic inhibition of CaMKII neurons in the rat dorsal medial prefrontal cortex attenuates methamphetamine addiction following concurrent sexual behavior

Authors: *L. B. KUIPER[1], L. M. COOLEN[2];
[1]Neurosci., Univ. of Mississippi Med. Ctr., Madison, MS; [2]Physiol., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Drug addiction is influenced by social factors and psychostimulant use is associated with increased sexual reward and risk behavior. We previously showed that male rats that experienced concurrent methamphetamine (Meth) and mating experience (Meth/sex) displayed compulsive sex and drug seeking behavior. Specifically, males that experienced concurrent Meth/sex displayed significantly more drug seeking behavior in extinction and reinstatement sessions, compared to males that experienced Meth and sex experience non concurrently. Moreover, we showed that Meth and sex co-activated CaMKII-expressing pyramidal neurons in the anterior cingulate area (ACA) of the medial prefrontal cortex (mPFC), an area involved in inhibitory control of behavior. Here we tested the hypothesis that ACA CaMKII neurons play a key role in the effects of the concurrent Meth/sex experience on drug seeking behavior using chemogenetic silencing (DREADD) of CaMKII neurons. Male Sprague Dawley rats received bilateral injections of AAV5-CaMKII-HM4D(Gi)-mCherry into the ACA. In the first experiment, sexually experienced animals received either CNO (1 mg/kg, s.c.) or vehicle 30 minutes prior to receiving either Meth (1 mg/kg s.c.) or saline, and locomotor activity was

measured. 45 minutes later, males mated (sex) or were left undisturbed (no sex) for 10 minutes before perfusion. Brain sections were immunoprocessed for Meth-induced cFos and sex-induced pERK, and mCherry expression in CAMKII cells was verified. CNO did not affect Meth-induced locomotion or mating. But, CNO attenuated both Meth-induced cFos and sex-induced pERK in mPFC subregions and nucleus accumbens, confirming the inactivation of ACA CAMKII neurons. In the second experiment, males received CNO (1 mg/kg) or vehicle 30 minutes prior to each of 5 daily Meth self-administration sessions. Males self-administered a total of 1 mg/kg Meth (FR1, 25 active lever presses) and mated immediately after each session. CNO did not affect the acquisition of Meth self-administration or mating. Next, males were tested for extinction and CNO-treated Meth/sex males responded significantly less on the active lever compared to vehicle-treated males during several of the 10 daily sessions. Finally, cue-induced reinstatement was tested and shown to be significantly attenuated in CNO-treated Meth/sex males. These results indicate that activation of ACA CaMKII neurons during concurrent Meth/sex experience mediate the development of compulsive drug seeking behavior. In conclusion, ACA CaMKII neurons may play a key role in mediating effects of interactions between drug and social reward behavior on vulnerability for addiction.

Disclosures: L.B. Kuiper: None. **L.M. Coolen:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIGMS P30GM103328

Title: Glutamatergic output from the medial prefrontal cortex modulates the daily rhythm in amphetamine reward.

Authors: *I. C. WEBB[1], G. G. WILSON[1], N. N. NEMATI[1], L. M. COOLEN[2,1];
[1]Dept. of Neurobio. and Anatom. Sci., [2]Dept. of Physiol. and Biophysics, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Behavioral responses to drugs of abuse vary by time-of-day. We have previously shown that psychostimulant reward, as assessed via the conditioned place preference (CPP) paradigm, exhibits a daily rhythm with peaks in the late dark and early light periods, and a nadir near the light-to-dark transition. Moreover, we have shown that inhibition of the ventral medial prefrontal cortex (mPFC), using lesions or pharmacological inactivation, increases drug reward

at the nadir time, thereby attenuating the daily rhythm. These findings led to the hypothesis that glutamatergic output from the mPFC attenuates amphetamine reward during the late light period. To specifically inhibit mPFC pyramidal neurons, male Sprague-Dawley rats received bilateral injections of an adeno-associated virus that induces the expression of an inhibitory DREADD receptor (AAV5-CAMKIIa-hM4D(Gi)-mCherry) in these cells. Three weeks following the surgery, separate groups of rats were tested for amphetamine (2.5 mg/kg) CPP at previously observed peak (zeitgeber time [ZT] 23) or nadir times (ZT11). During the expression phase of CPP testing, males were systemically administered vehicle or clozapine-N-oxide (CNO; 1 mg/kg). CNO administration significantly increased drug-paired chamber dwell time in rats tested at ZT11, thereby eliminating the daily rhythm in amphetamine reward. In contrast, CNO administration in males tested at ZT23 did not significantly affect amphetamine CPP as compared to vehicle. Analysis of hM4D expression revealed virtually exclusive expression in CAMKII cells. These results indicate that glutamatergic output from the mPFC modulates the diurnal rhythm in amphetamine CPP during the expression of learned reward-context associations. Moreover, as the loss of rhythmicity occurs via an increase at the nadir point, these results suggest that glutamatergic output from the mPFC normally inhibits context-elicited reward seeking prior to the light-to-dark transition.

Disclosures: I.C. Webb: None. **G.G. Wilson:** None. **N.N. Nemati:** None. **L.M. Coolen:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Program#/Poster#: 348.23/CCC17

Topic: G.08. Drugs of Abuse and Addiction

Title: Prelimbic α1-adrenergic receptors modulate extinction of both appetitive and aversive conditioned memories

Authors: *E. LATAGLIATA[1], G. CHIACCHIERINI[3], M. SANCANDI[3], S. PUGLISI-ALLEGRA[3,2];
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Abstract: Prelimbic norepinephrine (NE) transmission has been shown involved in the attribution of motivational salience to both appetitive and aversive stimuli (Ventura et al., 2003 J Neurosci. Mar 1;23(5):1879-85; Ventura et al., 2007 Proc Natl Acad Sci U S A. Mar 20;104(12):5181-6; Ventura et al., 2008 PLoS One.Aug 22;3(8):e3044.) as well as in the extinction of conditioned place preference (CPP) induced by amphetamine (Latagliata et al.,

2015 Psychopharmacology (Berl). Mar;233(6):973-82.). Recent studies on the modulation of drug-associated memories has pointed on PL β-adrenergic receptors, showing their critical role in mediate retrieval of cocaine-associated memory (Otis et al., 2013 J Neurosci. Jan 16;33(3):1271-81a; Fitzgerald et al., 2016 Behav Brain Res Jan 1;296:94-9.). However, recent results have suggested a potential contribution on the extinction of conditioned response also by α1-adrenergic receptors (Bernardi and Lattal 2010 Behav Neurosci Apr;124(2):204; Bernardi and Lattal 2012 Neuroreport. Dec 19;23(18):1048-51), although their role in PL cortex has not yet fully investigated. Here, we assessed the role of α1-adrenoceptors in the PL cortex on the extinction of appetitive and aversive conditioned memories. Thus, we investigated the effects of the α1-adrenoceptor antagonist Prazosin infusion in PL cortex of C57BL/6J mice on the extinction of both amphetamine-induced CPP and conditioned passive avoidance. Prazosin treated mice show an anticipation of the extinction of appetitive and aversive conditioned memories in comparison with the Vehicle groups. These results confirm the involvement of PL NE transmission on the extinction of conditioned responses, pointing on a critical role of α1-adrenergic receptors in this area.

Disclosures: E. Latagliata: None. **G. Chiacchierini:** None. **M. Sancandi:** None. **S. Puglisi-Allegra:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.24/CCC18

Topic: G.08. Drugs of Abuse and Addiction

Support: NSF grant DGE-1144086

NIDA grant DA022473

NIH grant DA034140

Title: Inhibition of withdrawal induced neurogenesis in the dentate gyrus Inhibition of withdrawal-induced neurogenesis in the dentate gyrus blocks methamphetamine relapse

Authors: *M. H. GALINATO[1], J. LOCKNER[2], M. C. STAPLES[3], S. S. SOMKUWAR[3], J. SOBIERAJ[3], S. CHAING[3], M. FANNON[3], A. GHOFRANIAN[3], A. JOEA[3], A. I. NAVARRO[3], B. W. LUIKART[4], K. JANDA[2], C. MANDYAM[3,1];
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Abstract: *Purpose of study*: Adult hippocampal neurogenesis is affected by both drug taking and withdrawal from drugs of abuse. We hypothesized that the observed increases in hippocampal neurogenesis during withdrawal would contribute to reinstatement of drug seeking behavior, a hippocampus-dependent task, in rats that experienced extended-access to methamphetamine self-administration. This study used isoxazole-9, a synthetic small molecule compound, to reverse withdrawal-induced changes in hippocampal neurogenesis and to test whether manipulations to neurogenesis could reduce reinstatement of drug seeking behavior.
Animal behavior and neurogenesis: Male adult Wistar rats were trained to self-administer methamphetamine intravenously (0.05 mg/Kg/infusion, 6h/session) for 17 sessions in the same operant chamber (context A). Animals were then kept in the home cage during withdrawal and received daily i.p. Injections of either isoxazole-9 or vehicle for the first 12 days of withdrawal. The day after last isoxazole-9 injection, a subset of animals received one i.p. injection of BrdU (150 mg/kg). Animals remained in their home cages for an additional 13 days followed by 6 extinction sessions in a different operant chamber (context B) and 2 reinstatement sessions in context A. Our results showed that isoxazole-9 reduced expression of BrdU and Ki-67 in the dentate gyrus (DG) and therefore reduced hippocampal neurogenesis after 28 days of withdrawal from methamphetamine self-administration. As a result, isoxazole-9 reduced context-driven reinstatement of drug seeking behavior.
Hippocampal plasticity: These changes in drug seeking behavior could be mediated by cellular and molecular mechanisms of hippocampal plasticity important for learning and memory. To understand changes in hippocampal plasticity that could be associated with changes in drug associated memory, we measured changes in cFos expression for neuronal activation, dendritic branching and spine density for structural plasticity, and protein expression for activation of synaptic plasticity proteins. We found that isoxazole-9 also reduced neuronal activation of granule cell neurons (GCNs) in the DG, enhanced structural plasticity of GCNs, and enhanced activation of synaptic proteins associated with learning and memory in the DG. These findings identify a subset of GCNs within the DG that contribute to drug-seeking behavior. Taken together, these results support a direct role for the importance of adult neurogenesis during withdrawal in compulsive-like drug reinstatement.

Disclosures: **M.H. Galinato:** None. **J. Lockner:** None. **M.C. Staples:** None. **S.S. Somkuwar:** None. **J. Sobieraj:** None. **S. Chaing:** None. **M. Fannon:** None. **A. Ghofranian:** None. **A. Joea:** None. **A.I. Navarro:** None. **B.W. Luikart:** None. **K. Janda:** None. **C. Mandyam:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.25/CCC19

Topic: G.08. Drugs of Abuse and Addiction

Support: Regis University Undergraduate Research & Scholarship Grants

Title: Contributions of neurotrophic factors to exercise-induced attenuation of methamphetamine-induced neurotoxicity

Authors: *M. F. MURRAY, A. E. SIMPSON, A. N. FRICKS-GLEASON;
Regis Univ., Denver, CO

Abstract: Abuse of methamphetamine (METH) in the Unites Stated has increased significantly in the past 15 years, and use is now endemic in the Western states. Colorado currently ranks 7th in the nation for total number of METH users over the age of 25. Overall, the economic cost of drug abuse is high. METH abuse alone costs the U.S. $23.4 billion annually due to crime, lost workplace productivity, foster care, and other social problems stemming from abuse. In addition to the known health risks associated with psychostimulant abuse, METH use carries the additional danger of permanent brain injury. One well-known animal model of METH abuse utilizes binge METH administration, where repeated doses of METH are given to rats in a single day. This dosing regimen has been shown to cause long-lasting damage to dopaminergic nerve terminals in the striatum and serotonergic nerve terminals in the prefrontal cortex similar to that seen in human METH abusers. In humans, it has been suggested that METH-induced monoaminergic damage may lead to the development of Parkinson's disease. Exercise is a non-pharmacological treatment, known for its beneficial physiological effects and cognition-enhancing properties, being explored for use in treating Parkinson's disease. Recently, this work has been extended to the study of METH-induced monoaminergic neurotoxicity. It has been shown that when rats exercised for 3 weeks before and 3 weeks after a binge treatment of METH, this exercise significantly attenuated METH-induced decreases in striatal dopamine. Interestingly, if the exercise regimen was limited to only 3 weeks before a binge treatment of METH, it did not protect against striatal dopamine damage, suggesting that pre-METH exercise does not help with prevention of neurotoxicity, but perhaps post-METH exercise aids in recovery. Recently, we've shown that 3 weeks of exercise after a METH binge resulted in significant attenuation of neurotoxicity, suggesting that exercise may provide a novel, non-pharmacological treatment for METH-induced neurotoxicity. In an effort to identify a potential mechanism for this attenuation, we've begun examining the contributions of the neurotrophic factors BDNF (brain-derived neurotrophic factor) and GDNF (glial cell line-derived neurotrophic factor). Voluntary exercise is known to induce production of neurotrophic factors and an extensive literature details the beneficial role of neurotrophins in neurodegenerative disease. The study presented herein examined not only the expression levels of these neurotrophins, but also the correlation between neurotrophin expression and attenuation of neurotoxicity.

Disclosures: M.F. Murray: None. **A.E. Simpson:** None. **A.N. Fricks-Gleason:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

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Program#/Poster#: 348.26/CCC20

Topic: G.08. Drugs of Abuse and Addiction

Support: NSC 102-2410-H-431 -005 -MY3

Title: Neural substrates of conditioning and extinction on methamphetamine-induced conditioned place preference paradigm: an immunohistochemistry c-Fos and p-ERK staining

Authors: *B. H. HE, A. HUANG;
Fo Guang Univ., Yilan County, Taiwan

Abstract: The important symptoms of methamphetamine addiction include psychological dependence and physical dependence. However, psychological dependence involves in rewarding memory through classical conditioning, and thereby it is difficult for treatments compared to physical dependence. The intoxification approach cannot reduce psychological dependence although the treatment is a better way that it can antagonize the toxic effect of the plasma for drug abusers. A growing body of data has shown that the neural mechanism research regarding extinction might provide some insights for a novel treatment to drug addiction, particularly in psychological dependence. Accordingly, the present study investigated the neural substrates of conditioned place preference (CPP) extinction including the insular cortex (IC), perhirihal cortex (PR), central amygdala (CeA), prelimbic cortex (PrL), and infralimbic cortex (IL) using the immunohistochemical staining with c-Fos and p-ERK proteins. The present results indicated that (a). on conditioning phase, the c-Fos and p-ERK overexpressions occurred at the IL, PrL, CeA, and PR; however, the c-Fos of the IC was not active. (b). On extinction, the c-Fos overexpressions were found at the IL, PrL, CeA, IC, and PR. The p-ERK was active in IL, PrL, CeA, and PR, but the p-ERK was not active in IC on extinction. The present findings may provide some implications for the clinic treatments of psychological dependence for drug addiction and reduce the drug relapse.
Key word: methamphetamine addiction, conditioned place preference, extinction

Disclosures: B.H. He: None. **A. Huang:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

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Program#/Poster#: 348.27/CCC21

Topic: G.08. Drugs of Abuse and Addiction

Support: Regis University Undergraduate Research & Scholarship Grant

Title: Voluntary exercise attenuates methamphetamine-induced monoaminergic neurotoxicity

Authors: *A. SIMPSON[1], M. F. MURRAY[2], A. N. FRICKS-GLEASON[2];
[1]Regis Univ., Arvada, CO; [2]Regis Univ., Denver, CO

Abstract: Methamphetamine (METH) abuse continues to be a major public health concern. As many as 60 million people worldwide report using amphetamine-type stimulants, especially METH. Use is endemic in the western United States; Colorado currently ranks 7th nationally in METH use among people over the age of 25. The use of METH is highly problematic, not only due to the acute effects of the drug which can include psychosis and aggressive behavior, but also due to the well documented long-term consequences of the drug on the structure and function of the central nervous system, and concomitant cognitive deficits. METH-induced toxicity to central monoamine systems has been modeled in numerous species. In rodents, partial monoamine loss has been reported to exist for at least 6 months and is associated with impaired cognitive function. Imaging studies in humans show similar long-lasting decreases in markers of dopamine (DA) innervation in the caudate-putamen of METH abusers. In fact, recent studies have shown that METH abusers are more likely to develop Parkinson's disease, suggesting enduring and possibly progressive DA loss as a consequence of METH abuse. Exercise is well known to have myriad positive effects on the CNS. Multiple studies using rodent models of Parkinson's disease have demonstrated a beneficial role of exercise on neurochemical and behavioral outcomes. Recently, this work has been extended to the study of METH neurotoxicity; with data showing that wheel running ameliorates METH-induced monoaminergic loss. Importantly, that study employed an exercise regimen consisting of both pre- and post-METH exercise. While these results are encouraging, the study was not designed to elucidate whether this effect was the result of protection against the neurotoxic insult, or was due to accelerating the known neurochemical recovery seen after METH administration. Here we show that 3 weeks of post-METH exercise significantly attenuates monoaminergic neurotoxicity. Having established that voluntary exercise beginning immediately post-METH attenuates drug-induced monoamine system neurotoxicity, we now turn our attention to the timing of this intervention. By delaying the start of exercise for 7 or 30 days post-METH, we can target the therapy to a time when the bulk of the neurotoxicity has already taken place. As a result, we can

begin to investigate whether post-METH exercise is disrupting the mechanism that lead to neurotoxicity or, rather, is reversing the neurotoxic effects post-hoc.

Disclosures: **A. Simpson:** None. **M.F. Murray:** None. **A.N. Fricks-Gleason:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIGMS grant T32 GM081741

Title: Regulator of G Protein Signaling-12 (RGS12) in the action of amphetamine and related drugs of abuse

Authors: ***J. D. GROSS**[1], A. SCHROER[1], K. WIX[1], D. P. SIDEROVSKI[1], V. SETOLA[1,2]; [1]Physiol. & Pharmacol., West Virginia Univ. Sch. of Med., Morgantown, WV; [2]Behavioral Med. and Psychiatry, West Virginia Univ., Morgantown, WV

Abstract: Substance abuse remains a national crisis that presents substantial health-care and socio-economic costs. Among the most commonly abused substances, amphetamine (AMPH) and AMPH-like psychostimulants are of considerable concern as they exert considerable neuro- and cardio-toxicity. Moreover, a recent surge in the generation and consumption of novel AMPH-like congeners that circumvent legal prohibitions has precipitated new, major public health concerns. Recent findings from our group suggest that Regulator of G Protein Signaling-12 (RGS12) may be a critical molecular component underlying the mechanisms by which these drugs exert their effects. We found that RGS12 knockout mice exhibit attenuated locomotor responses to AMPH and AMPH-like psychostimulants that induce monoamine transporter (MAT)-mediated neurotransmitter release, but not to psychostimulants that act as MAT reuptake inhibitors. We also have preliminary evidence that this effect involves the dopamine transporter (DAT), and not the serotonin (SERT) and norepinephrine (NET) transporters. The rewarding and locomotor-activating effects of AMPH and AMPH-like psychostimulants are dependent on mesolimbic, nigrostriatal, and mesocortical brain structures, such as the ventral tegmental area, nucleus accumbens, substantia nigra pars compacta, dorsal striatum, and prefrontal cortex -- all regions where *Rgs12* mRNA and RGS12 protein are robustly expressed and dopaminergic neurotransmission is critical. At a molecular level, we have demonstrated that RGS12 affects DAT expression and function in cell culture and mouse brain synaptosomal preparations using radioligand-based assays. RGS12 contains a complex domain architecture that is capable of

modulating several of the signal transduction pathways involved in AMPH-like psychostimulant action. Specifically, RGS12 exhibits canonical GTPase-accelerating activity on GTP-bound Galpha-i subunits via its RGS domain, N-terminal PDZ and phosphotyrosine binding (PTB) domains, two Ras-binding domains (RBDs), and a C-terminal GoLoco motif, which has guanine dissociation inhibitor (GDI) activity on GDP-bound Galpha-i subunits. Future studies will seek to identify the mechanisms underlying the effects of RGS12 on AMPH and AMPH-like drugs of abuse.

Disclosures: J.D. Gross: None. **A. Schroer:** None. **K. Wix:** None. **D.P. Siderovski:** None. **V. Setola:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.29/CCC23

Topic: G.08. Drugs of Abuse and Addiction

Title: Sex differences in neural activation patterns within HPA axis associated brain regions following repeated methamphetamine exposure

Authors: *J. JACOBSKIND, Z. J. ROSINGER, D. G. ZULOAGA;
SUNY Albany, Albany, NY

Abstract: There are striking sex differences in abuse patterns of methamphetamine (MA) and its subsequent effects. Women spiral into MA dependence faster than men and tend to be more dependent on and committed to MA. In rodents, females show greater MA seeking and reinstatement to MA use after withdrawal. Stress and the hypothalamic-pituitary-adrenal (HPA) axis are key components in psychostimulant addiction that may contribute to sexually dimorphic patterns of abuse. Our previous studies indicate that females show elevated MA-induced glucocorticoid release and altered activation of HPA axis associated brain regions following an acute exposure. However, little is known about potential sex specific effects of chronic MA on activation of HPA axis associated brain regions. In this study we administered MA (5 mg/kg) or saline to C57BL/6J mice for 10 consecutive days. Ten days after the chronic treatment period, mice were injected with a final dose of MA (5 mg/kg) or saline. Brains were collected and immunohistochemically (IHC) labeled for detection of the immediate early gene c-Fos, and dual-label c-Fos/glucocorticoid receptor (GR). HPA axis associated brain regions including the paraventricular nucleus of the hypothalamus (PVH), central amygdala (CeA), bed nucleus of the stria terminalis (BNST), and hippocampus (CA1, CA3) were assessed. Final injection with MA increased c-Fos positive cells in all investigated regions. Chronic exposure to MA attenuated c-

Fos expression following a final MA dose in the PVH, BNST, CeA, CA1, and CA3. In hippocampal regions, this reduction was greater in males. In the PVH, MA increased c-Fos to a greater extent in females compared to males regardless of prior exposure. The number of c-Fos/GR positive cells decreased following chronic MA in CeA, BNST, and PVH. We also hypothesized that chronic exposure to MA would alter activation of HPA axis associated brain regions to a subsequent stressor. To test this, mice treated with MA or saline for 10 days were placed in a Forced Swim Test 48 hours following the last injection. IHC revealed decreased c-Fos in chronic MA-treated mice, specifically within CA3, BNST, and CeA. In the BST, MA-induced suppression of c-Fos expression was found only in males. Together our findings demonstrate that chronic MA can suppress subsequent activation of HPA axis associated brain regions by MA and stress. In specific regions, MA-induced alterations in neural activation were sex specific and may contribute to sex differences in MA abuse patterns reported in humans and rodents.

Disclosures: J. Jacobskind: None. **Z.J. Rosinger:** None. **D.G. Zuloaga:** None.

Poster

348. Amphetamines: Neurocircuitry and Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 348.30/CCC24

Topic: G.08. Drugs of Abuse and Addiction

Title: Dopamine transporter-inhibiting psychostimulants increase exocytotic dopamine release

Authors: P. CHALWADI[1], S. H. WALTERS[2], A. C. MICHAEL[2], *P. A. GARRIS[1];
[1]Illinois State Univ., Normal, IL; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Psychostimulants like amphetamine and cocaine elevate brain extracellular dopamine (DA) by inhibiting DA uptake. More recently, increases in exocytotic DA release have also been proposed to contribute to DA elevations. While converging evidence supports this new mechanism for cocaine, it remains highly controversial for amphetamine, which is historically thought to deplete vesicular DA stores. Psychostimulant-induced increases in exocytotic DA release were demonstrated by fitting electrically evoked DA signals measured by fast-scan cyclic voltammetry (FSCV) to the diffusion gap (DG) model, which describes extracellular DA as a balance between the opposing actions of DA release and uptake. In the DG model, DA must diffuse across a gap between release sites and the FSCV microsensor to be measured. Calculated responses are altered to reflect this diffusion prior to comparison with data. However, features regularly observed in evoked DA signals, such as “hangup”, the slow return of DA to baseline, and incongruous changes in “lag”, the time for DA to increase after stimulus initiation, and

"overshoot", the continued DA increase after stimulus cessation, are not described by the DG model. These insufficiencies thus question the finding of psychostimulant-induced increases in DA release with the DG model. Here we analyze the *in vivo* effects of amphetamine and another psychostimulant, modafinil, used to treat sleep-related disorders, on exocytotic DA release and DA uptake. Parameters describing these mechanisms and previously determined with the DG model were compared to those obtained by the restricted diffusion (RD) model. Developed to address insufficiencies of the DG model, the RD model divides extracellular space into inner and outer compartments. DA is initially released into the inner compartment and is transported by restricted diffusion to the outer compartment, where it is measured by the microsensor and cleared by DA uptake. Hangup, attributed to DA adsorption to the microsensor, is removed from data prior to comparison with calculated responses. Although the RD model has unique parameters for transport between compartments and modification of initial DA release, both DG and RD models have comparable parameters for DA release elicited per stimulus pulse and DA uptake. We found that both models demonstrated an increase in exocytotic DA release and inhibition of DA uptake for both psychostimulants. These results support the hypothesis that psychostimulants elevate extracellular DA by targeting both exocytotic DA release and DA uptake. Further studies are needed to identify the mechanism by which psychostimulants enhance exocytotic DA release.

Disclosures: P. Chalwadi: None. **S.H. Walters:** None. **A.C. Michael:** None. **P.A. Garris:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: 5R01DA014339-14 to RMC

Title: Neural activity in the anterior insula tracks cocaine-induced devaluation of natural rewards

Authors: *T. M. MOSCHAK, E. A. WEST, R. M. HAAKE, X. WANG, R. M. CARELLI; Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstract: In individuals suffering from drug addiction, negative reinforcement can be a powerful motivator for repeated drug use. From this perspective, addicts continue to take drug not for the positive reinforcing aspects of the compound, but to circumvent negative affective states such as dysphoria or anxiety typically associated with drug withdrawal. Our lab has

developed a preclinical model to investigate drug-induced natural reward devaluation and associated negative affect in rats. On each day, rats receive 45 intraoral infusions of a saccharin solution (3.5 s/inf; ~1 inf/min), followed by access to self-administered cocaine for 2 hr. Rats initially exhibit appetitive responses to the saccharin. However, as the animals form the association between the tastant and delayed cocaine access, they come to exhibit aversive responses to the saccharin, suggesting a change in affective state. We have previously shown that neural activity in the nucleus accumbens tracks this shift, but the involvement of other brain regions in this phenomenon is unknown. One neural substrate that may play an important role in this process is the anterior insula (AI), which is well integrated with reward circuitry and has been implicated in craving, negative affect, and drug addiction. Thus, we sought to examine neural activity in the AI during our model of drug-induced natural reward devaluation. Over the course of 14 days, rats (n = 6) received infusions of saccharin paired with delayed cocaine access as mentioned above. On Day 1 and Day 14 of the task, neural activity in the AI was recorded and orofacial behavior during saccharin delivery was measured and quantified as appetitive (e.g. lateral tongue protrusions) or aversive (e.g. gaping). Replicating previous work in our lab, our preliminary findings show that rats primarily exhibited appetitive behavior on Day 1 and aversive behavior on Day 14. Neural activity in the AI tracked this shift. On Day 1, average AI activity (n = 38 neurons) exhibited an excitatory peak approximately 1.5 s after initiation of saccharin delivery. However, by Day 14 (n = 43 neurons), this was replaced by a small inhibitory trough at 1.5 s followed by a delayed excitatory peak approximately 4 s after initiation of saccharin delivery. The AI also reflected aspects of cocaine self-administration. AI activity exhibited a small excitatory peak around the lever press for cocaine. This was followed by a delayed (approximately 15 s) but long lasting (5 min) decrease in activity. These preliminary results show that the AI tracks cocaine-induced devaluation of natural rewards, and that it may be an important substrate in the negative affective state associated with drugs of abuse.

Disclosures: **T.M. Moschak:** None. **E.A. West:** None. **R.M. Haake:** None. **X. Wang:** None. **R.M. Carelli:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Program#/Poster#: 349.02/CCC26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA031747

Title: Neuronal correlates of motivational sensitivity to natural and drug rewards

Authors: *B. O'DONOVAN[1], P. HASHEMI[2], S. SAMARANAYAKE[2], R. ROBKE[2], P. I. ORTINSKI[1];
[1]Pharmacology, Physiol. and Neurosci., [2]Chem. and Biochem., Univ. of South Carolina, Columbia, SC

Abstract: Individual differences in motivation for natural rewards may predict future response to psychostimulants. Regulation of motivated behavior is known to be dependent on dopamine signaling in the nucleus accumbens (NAc) shell. However, the neuronal mechanisms underlying motivation remain largely unexplored. This study evaluates the mechanisms underlying individual differences in motivation for natural reward and the effect of motivational state on neuronal response to cocaine. Rats were identified as high (HighS) and low (LowS) motivated responders based on their performance on a sucrose self-administration task. Following the final behavioral session, dopamine levels in the NAc shell were measured using fast scan cyclic voltammetry (FSCV) and fast scan adsorption controlled voltammetry (FSCAV), or slices containing NAc were prepared and whole-cell patch clamp recordings were performed from NAc shell medium spiny neurons (MSNs). A separate group of HighS and LowS rats were subjected to 5 days of 'binge' cocaine treatments (3 daily injections at 1 hour intervals, 15mg/kg i.p.). 2 days after the termination of treatment whole-cell patch clamp recordings were performed from NAc shell MSNs. HighS rats had elevated levels of phasic and tonic dopamine and slower dopamine clearance in the NAc shell when compared to LowS rats. HighS rats also had more spontaneous dopamine transients. NAc shell MSNs in LowS rats are significantly more excitable than MSNs in HighS rats. The 'binge' treatment of cocaine resulted in suppressed firing of NAc shell MSNs in the LowS, but not the HighS, group. Individual differences in motivation for a sucrose reward are linked to alterations in dopamine signaling in the NAc shell. The greater excitability of MSNs in LowS rats may be due to the lower level of dopamine signaling in this group. Differences in motivation are also associated with susceptibility to cocaine-induced intrinsic plasticity. We are now exploring whether motivation for natural reward predicts a pattern of cocaine self-administration, and the mechanisms via which dopamine signals may modulate MSN excitability.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 349.03/DDD1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA035322

Title: Altered encoding of motivational stimuli in the basolateral and central amygdala in cocaine-experienced rats

Authors: *K. J. STANSFIELD, K. L. AGSTER, K. S. MCCONOMY, C. N. BROWN, M. R. PAYNE, M. P. SADDORIS;
Univ. of Colorado Boulder, Boulder, CO

Abstract: The reinforcing properties of cocaine act via dopamine-related signaling in the nucleus accumbens (NAc). Consequently, our lab and others have shown that chronic experience with cocaine self-administration alters neural signaling properties in the NAc to the extent to which subjects experience a variety of behavioral deficits in motivated learning that normally depend on NAc processing such as conditioned approach. However, the NAc is part of a network involved in reward-related learning and receives projections from other nuclei in this circuit including a direct glutamatergic connection from the basolateral amygdala (BLA), and possible indirect connections from the central nucleus of the amygdala (CN) via the ventral tegmental area (VTA). In our lab cocaine-experienced rats display both impoverished neural encoding and abnormal phasic dopamine (DA) signals in the NAc. However it is not known whether this is due to neuroadaptations at the site of cocaine action, or whether drug experience alters associative encoding in afferent regions like the BLA and CN. Rats were assigned to either self-administer iv cocaine (Cocaine), receive passive iv infusions of cocaine (Yoked) or self-administer water to a food receptacle (Control) for 2h sessions over 14d. Following 30d of abstinence, we recorded neural activity from electrodes aimed at the BLA and CN neurons while subjects learned a first-order Pavlovian discrimination (FOC; CS+/food, CS-/nothing) followed by a second-order discrimination (SOC; SOC+/CS+, SOC-/CS-). Cocaine-experienced rats (both Cocaine and Yoked) showed greater conditioned approach during FOC sessions than Controls, but failed to show appropriate learning during SOC sessions. At the neural level, Cocaine groups showed altered neural signaling to task stimuli relative to Controls. During FOC, peak signaling for cue neurons was similar between Cocaine and Controls, but the proportion of cue-selective cells in Cocaine rats was reliably lower. Further, reward encoding in the BLA was enhanced in Cocaine groups. In contrast, CN neurons displayed both fewer cue-selective neurons and significantly lower peak activity following cue onset. During SOC sessions, both BLA and CN neurons failed to appropriately signal information about the SOC cues. These findings argue that repeated cocaine experience alters important limbic inputs to the NAc and may thus contribute to persistent neural and motivational impairments well after the cessation of drug taking activities.

Disclosures: **K.J. Stansfield:** None. **K.L. Agster:** None. **K.S. McConomy:** None. **C.N. Brown:** None. **M.R. Payne:** None. **M.P. Saddoris:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA025679

NIH Grant DA038114

Title: Reduced dopamine as a substrate of aversive motivation

Authors: ***M. G. SPRING**[1], R. C. TWINING[1], M. A. ROBBLE[1], S. M. CONWAY[2], D. S. WHEELER[1], M. G. BLACKMORE[1], M. F. ROITMAN[2], R. A. WHEELER[1];
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Abstract: Stressful events promote maladaptive behaviors such as compulsive drug use by acting, in part, on the mesolimbic dopamine (DA) system. While the effects of aversive stimuli on DA neuron firing rates are mixed, studies of terminal DA release in the nucleus accumbens, commensurate with the experience of the aversive stimulus, indicate that such stimuli decrease DA signaling. Previously, using fast-scan cyclic voltammetry, we determined that a primary aversive stimulus that decreases DA is sufficient to promote drug-seeking. Further, we reported that blocking the actions of corticotropin releasing factor (CRF) in the ventral tegmental area (VTA) prevented both the aversion-induced decrease in DA and drug seeking. These findings suggest that, just as increased DA signaling promotes approach behavior, reduced DA signaling may promote avoidance. The reduction in DA could serve as a motivating signal in response to aversive stimuli, and subsequent motivated behavior could be an attempt to correct this state. In order to directly test the contribution of reduced DA signaling to aversive motivation, we induced the expression of the CRE-dependent viral construct, AAV-hSyn-DIO-hM4d(Gi)-mCherry, selectively in VTA DA neurons by transfecting cells in rats expressing CRE exclusively in tyrosine hydroxylase-producing neurons. Following recovery, rats were trained to self-administer cocaine during daily 2-hour sessions. Once cocaine intake stabilized, we used an ABA design to determine the role of reduced DA in promoting drug seeking. Rats were injected with the hM4d(Gi) ligand, clozapine N-oxide (CNO; 2 mg/kg, i.p.) on test days to selectively activate the inhibitory DREADD, and saline on control days thirty minutes prior to self administration sessions. Compared with saline-injected control sessions, CNO administration significantly increased early-session drug intake. Subsequently, rats were trained in a free choice paradigm in which responses on one lever delivered cocaine and responses on the other lever delivered a sucrose pellet during daily 2-hour sessions. In this design, CNO administered prior to the session significantly decreased the latency to obtain the first cocaine infusion and increased the number of cocaine infusions during the session. CNO administration did not affect any aspect

of operant behavior for sucrose pellets, either within the choice paradigm or when sucrose was exclusively available. Taken together, the results of these studies indicate that the reduction in DA signaling that accompanies the experience of an aversive stimulus is sufficient to motivate drug taking.

Disclosures: **M.G. Spring:** None. **R.C. Twining:** None. **M.A. Robble:** None. **S.M. Conway:** None. **D.S. Wheeler:** None. **M.G. Blackmore:** None. **M.F. Roitman:** None. **R.A. Wheeler:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA012512

DA003906

DA015369

Title: Dissecting the role of the ventral pallidum in cocaine seeking

Authors: ***J. A. HEINSBROEK**, D. N. NEUHOFER, A.-C. BOBADILLA, P. W. KALIVAS; Med. Univ. of South Carolina, Charleston, SC

Abstract: The ventral pallidum is essential for linking motivational states to motor action, and is critically involved in relapse to drug seeking behavior. It receives input from nucleus accumbens and several other limbic brain regions, and drives motivated behavior by means of projections to the habenula, subthalamic nucleus, ventral tegmental area and mediodorsal thalamus. In contrast to the segregated dorsal pallidal regions whose striatal inputs arise almost exclusively from either direct pathway dopamine D1 receptor expressing, or indirect pathway D2 receptor expressing medium spiny neurons, the ventral pallidum receives inputs from both D1 and D2 receptor expressing neurons. Further, both D1 and D2 projections to the ventral pallidum give rise to direct and indirect basal ganglia circuits (Kupchik, 2015). However, despite this convergence of circuitry at the level of the ventral pallidum D1 inputs are hypothesized to increase, and D2 inputs to decrease motivated behavior. Since both projections release the inhibitory transmitter GABA, exactly how specificity is achieved within the circuit remains unknown. To generally assess the role of ventral pallidum neurons in relapse to drug seeking, we expressed designer receptors exclusively activated by designer drugs (DREADDs, activated by clozapine-N-oxide)

in the ventral pallidum of mice to bi-directionally modulate neuronal activity during extinction and cue induced reinstatement of cocaine seeking. Activation of the ventral pallidum using the Gq coupled DREADD hM3D strongly augmented cue induced cocaine seeking. In addition, stimulation of the ventral pallidum caused reinstatement in extinguished animals. Conversely, chemogenetic inhibition of the ventral pallidum using the inhibitory DREADD hM4D inhibited cue-induced cocaine seeking. Future experiments are set out to dissect cell type specific, and projection specific contributions of ventral pallidum neurons in these behaviors, investigate cell type specific responses to D1 and D2 input, and to monitor the activity of different ventral pallidum neurons during drug-seeking behavior.

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Poster

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Title: Social defeat stress augments economic demand for cocaine via CRF in the rat VTA

Authors: *M. Z. LEONARD, D. STEIN, J. F. DEBOLD, K. A. MICZEK;
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Abstract: Exposure to intermittent social defeat stress can lead to persistent neuroadaptations that induce escalated cocaine taking-behavior in rats. During both stress and repeated cocaine exposure, corticotropin releasing factor (CRF) is elevated in the ventral tegmental area (VTA), and induces long-term modulation of mesocorticolimbic activity. These adaptations are associated with an intense cocaine-taking phenotype which can be prevented by intra-VTA infusion of CRF receptor antagonists (CRF-R1 or CRF-R2) prior to social defeat. The present studies examine the extent to which infusion of CRF into the VTA is sufficient to augment cocaine-taking behavior, in the absence of social defeat experience. Additionally, we aimed to quantitatively characterize changes in consummatory behavior that may promote binge-like cocaine intake in certain animals. To that end, we used a behavioral economics approach to assess 1) whether economic demand determines cocaine consumption during a 24-hour continuous “binge”, and 2) whether defeat stress and/or intra-VTA CRF microinfusion persistently alters cocaine valuation. Long-Evans rats were microinjected with CRF (50 or 500

ng/side), vehicle (aCSF), or subjected to social defeat stress, intermittently over 10 days (on days 1, 4, 7, 10). Animals were subsequently implanted with an I.V. catheter and trained to self-administer cocaine (0.75mg/kg/infusion) on a fixed-ratio (FR5) schedule of reinforcement. Following acquisition, economic demand was evaluated using a within-session threshold paradigm, where demand parameters (Q_0, Pmax, α) were determined as a function of consumption in response to increasing price (responses per unit dose cocaine) across a single session. Performance on the threshold procedure was measured for at least 6 sessions prior to a 24-hour extended-access "binge" (0.3mg/kg/infusion, FR5). Rats that experienced social defeat or received 50ng CRF microinfusions into the VTA took significantly more cocaine than non-defeated controls over the 24-hour "binge". Behavioral economic analysis revealed that individual demand for cocaine is predictive of total consumption during extended access, and that these parameters (notably, the derived "essential value") are augmented by intra-VTA CRF microinjection or social defeat. Together, these results support the idea that repeated exposure to social stress may persistently alter the reinforcing effects of cocaine in drug-experienced animals, which might contribute to increased abuse liability. Moreover, we demonstrate that CRF activity within the VTA is a critical mediator of these behavioral consequences.

Disclosures: **M.Z. Leonard:** None. **D. Stein:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: The impact of cocaine self-administration on value signals in ventral striatum

Authors: ***A. C. BURTON**[1], G. B. BISSONETTE[2], K. C. HEATLEY[2], E. M. BLUME[2], M. L. DONNELLY[2], M. R. ROESCH[2];
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Abstract: Drug addiction is commonly associated with maladaptive decision-making and habit-like behaviors. The development of addiction is thought to reflect a transition from response-outcome (goal-directed) to stimulus-response (habit) driven behavior, functions which are thought to be under the control of ventral (VS) and dorsolateral striatum (DLS), respectively. Previously, we have shown that VS dysfunction due to lesion, irrespective of drug use, enhanced stimulus-response encoding in DLS while rats performed a reward-guided decision-making task

where rats follow directional odor cues to obtain liquid sucrose rewards. The value of reward was independently manipulated by changing the delay to or size of reward across different blocks of trials in order to determine how this influenced the flexibility of decision-making both behaviorally and at the single-neuron level. In a follow-up experiment, we implemented a two-week cocaine exposure protocol and then recorded from single neurons in DLS while rats performed the same task. As expected from previous work we found that after going through withdrawal, rats that had self-administered cocaine were more impulsive. However, quite unexpectedly, we found that changes in behavior were not accompanied by simple increases in stimulus-response correlates, but instead, an over-representation of contextual response-bias encoding in DLS. This suggests that drug use might not simply reduce VS function (as observed after lesions), but alter it in some other way that promotes impulsive choice and miscoding in DLS. Here, we examine how previous cocaine self-administration in rats alters neural encoding in VS during performance of the same task.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Increased limbic connectivity strength and impaired social interaction, recognition memory, and ultrasonic vocalizations, 24 hours after single MDPV exposure

Authors: *M. FEBO[1], M. POMPILUS[1], J. A. PINO-REYES[2], S. E. KAPLITZ[1], N. T. CHOUDHURY[1], G. E. TORRES[2], L. M. COLON-PEREZ[1];
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Abstract: Our prior work showed that high doses of the bath salt drug 3,4-methylenedioxypyrovalerone (MDPV) significantly disrupt resting state functional connectivity at 1 hr following exposure. This previous study did not address the re-establishment of resting state networks and did not determine if MDPV effects are still observable at longer delays after exposure. The present study assessed functional connectivity at 24 hr following exposure to MDPV and determined the effects of MDPV on social interactions, novel object recognition memory, and ultrasonic vocalizations (USVs) at this time point. Adult male Long Evans rats

(250-400g) were treated with a single saline of saline (n = 12), cocaine (15 mg kg^{-1}, i.p; n = 12), or MDPV (1 and 3 mg kg^{-1}, i.p; n = 12 each). Twenty-four hr later resting state scans were collected on a 4.7 Tesla MRI. Data were processed for functional connectivity and then analyzed using graph theory based network analyses (e.g., node degree, strength, efficiency/path length, clustering coefficient, modularity, small worldness, and rich club index). A separate group of saline (n = 8) and MDPV (1 and 3 mg kg^{-1}, i.p; n = 8 each) treated rats underwent behavioral assays for locomotor activity, social interaction/recognition, novel object recognition, and USV recordings. Behavioral results show that MDPV produces behavioral sensitization by the 2^{nd} injection. At this time (24 hr after initial exposure), rats also show impaired social recognition memory and object recognition memory, and show significantly lower USV calls than saline controls. Our functional imaging results show interesting and novel network topology changes at 24 hr after MDPV. First, while global indices of node strength, path length, clustering coefficients, and small worldness are significantly suppressed at 1 hr after MDPV or cocaine, these are no longer suppressed at 24 hr. Instead we observed that with the highest dose of MDPV (3 mg kg^{-1}) there is a significant increase in clustering coefficients (number of neighboring nodes that are connected with each other) and path length (less efficient network). Interestingly, both doses of MDPV showed greater rich club index values than saline or cocaine, suggesting the emergence of a strongly interconnected set of structures. Analysis of clustering coefficients showed that the strongly connected structures are part of a network including areas of the prefrontal cortex (orbital and infralimbic), dorsal striatum, amygdala and hypothalamic regions. The described neuroadaptations last 24 hr after MDPV administration and are possibly associated with the adverse effects of MDPV intake.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

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T32 DA007244

Title: Enhancement of negative affect by abstinence from cocaine in a preclinical model

Authors: *R. M. HAAKE, E. A. WEST, X. WANG, E. L. THOMAS, R. M. CARELLI; Psychology and Neurosci., Univ. of North Carolina, Chapel Hill, NC

Abstract: We have previously demonstrated that an initially palatable saccharin solution that predicts impending, but delayed, cocaine availability elicits a negative affective state as evidenced by the emergence of aversive taste reactivity (Wheeler at al., 2008). This shift in affective state is dynamically encoded by nucleus accumbens (NAc) neurons. Specifically, NAc neurons shift from exhibiting predominately inhibitory to excitatory cell firing during intraoral infusion of the drug-paired tastant. It is not known, however, whether the negative affective state in our model is enhanced by prolonged cocaine abstinence. Importantly, abstinence from cocaine leads to heightened motivation to obtain the drug, as well as numerous forms of neuroplasticity including an increase in the percent of NAc cells that encode cocaine-associated stimuli and cocaine-seeking behavior (Hollander and Carelli, 2007). Here, we determine the effects of prolonged abstinence on aversive behavioral responses to saccharin following 14 days of saccharin-cocaine pairings. On each day, saccharin (0.15%; 0.2 ml delivered over 4 s/infusion; ~1 inf/min for 45 trials) was intraorally infused, followed by a 2 h cocaine self-administration session (0.33 mg/inf). Orofacial movements were video recorded to examine taste reactivity for the cocaine-predictive tastant during the first (day 1) and final (day 14) taste-drug pairing sessions. Following 1 or 30 days of abstinence (1-day group, n=11; 30-day group, n=11), rats were tested for their affective responses to the drug-paired tastant and cocaine seeking behavior during three phases: 1) intraoral saccharin delivery 2) extinction and 3) cocaine self-administration. We found that rats in the 30-day abstinence group exhibited a greater number of aversive taste reactivity behaviors (i.e., gapes) on the test day compared to the last day of taste-drug pairing (day 14). Importantly, no such differences in aversive taste reactivity were observed in the 1-day abstinence group. These preliminary findings indicate that 30 days of cocaine abstinence leads to a heightening of the aversive, or negative affective, state elicited by the cocaine-predictive taste cue. Further, preliminary data indicate that this enhancement of negative affect was accompanied by an increase in cocaine seeking during extinction, consistent with an incubation of cocaine craving. Ongoing electrophysiological analyses are examining whether the encoding of this aversive state and subsequent cocaine seeking by NAc neurons is altered by prolonged abstinence.

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Poster

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Title: Role of anterior dorsal lateral hypothalamic area perineuronal nets in the acquisition of cocaine-induced conditioned place preference and self-administration

Authors: *J. M. BLACKTOP[1], R. P. TODD[1], L. CHURCHILL[2], M. SLAKER[1], B. A. SORG[1]; [1]Dept. of Integrative Physiol. and Neurosci., Washington State Univ. Vancouver, Vancouver, WA; [2]Dept. of Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Addiction involves drug-induced neuroplasticity of the circuitry of motivated behavior. Emerging at the forefront of neuroplasticity regulation are specialized extracellular matrix structures that form perineuronal nets (PNNs) making them a promising target for the regulation of drug-induced neuroplasticity. Brain regions within the circuitry of motivated behavior with comparatively high PNN expression may provide neurobiological insight into maladaptive drug-induced neuroplasticity and subsequent drug seeking. Despite the emerging significance of PNNs in drug-induced neuroplasticity and the well-established role of the lateral hypothalamic area (LHA) in reward/reinforcement/motivation, little is known about how PNN-expressing neurons in the LHA control drug-seeking behavior. The goals of this set of experiments were: 1) to determine and characterize areas of high PNN expression within the LHA, and 2) whether PNN expression within the LHA is necessary for the rewarding and reinforcing effects of cocaine exposure, measured by the acquisition of conditioned place preference (CPP) and self-administration, respectively. A discrete region of the anterior dorsal LHA (LHAad) was found to exhibit robust PNN expression, while other remaining areas of the anterior LHA (LHAa) exhibited comparatively sparse PNN expression. We determined that approximately 90% of parvalbumin positive (PV+) neurons co-expressed the PNN marker *Wisteria floribunda* agglutinin (WFA), while 62% of WFA positive (WFA+) neurons co-expressed parvalbumin in the LHAad of drug naïve rats. Moreover, robust co-expression of the excitatory presynaptic marker, vesicular glutamate transporter 2 (VGLUT2), was found with WFA in the LHAad, suggestive of significant glutamatergic input within this brain region. Compellingly, PNN removal via chrondroitinase ABC (Ch-ABC) administration within but not outside the LHAad prior to conditioning abolished acquisition of cocaine-induced conditioned place preference and it attenuated cocaine self-administration, highlighting the importance and specificity of PNN removal within this subregion of the LHA in the rewarding and reinforcing effects of cocaine. Removal of PNNs within the LHAad did not affect total locomotor activity, high-fat food intake, or sucrose intake in a separate group of cocaine naïve animals. In summary, these data indicate that PNN expression in the LHAad: 1) is predominantly co-localized with parvalbumin, 2) receives robust glutamatergic inputs, 3) is necessary for acquisition of both cocaine-induced CPP and self-administration, and 4) is not necessary for normal locomotor or ingestive behavior.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Perineuronal nets protect parvalbumin neurons from the effects of cocaine-induced oxidative stress in the rat prefrontal cortex

Authors: *M. SLAKER, K. REYES, B. A. SORG;
Washington State Univ. Vancouver, Vancouver, WA

Abstract: Perineuronal nets (PNNs) are aggregations of extracellular matrix molecules that form a perimeter around the soma and proximal dendrites of primarily parvalbumin (PV) interneurons in the medial prefrontal cortex (mPFC). These cells are fast-spiking and are susceptible to damage via oxidative stress. Cocaine induces oxidative stress in the brain, including in the mPFC. Markers of oxidative stress increase following both acute and chronic exposure to cocaine. This increase can be reversed by administration of an antioxidant, either N-acetylcysteine (NAC) or Tempol. Additionally, PNNs protect their underlying cells from the harmful effects of oxidative stress. Data from our laboratory demonstrate that following acute exposure to cocaine, PNN staining intensity decreases, and following chronic exposure to cocaine, PNN staining intensity increases. We hypothesize that these dynamic changes in PNNs lead to protection of their underlying PV cells from cocaine-induced oxidative stress. We tested this hypothesis by examining levels of the oxidative stress marker, 8-oxo-dG, in PV-containing cells with and without a PNN following pretreatment with an antioxidant, NAC, and acute exposure to cocaine. Exposure to acute cocaine increases the level of oxidative stress in all PV cells, regardless of the presence of a PNN. Pretreatment with NAC prior to acute cocaine exposure prevents this increase in oxidative stress, but only in PV cells surrounded by PNNs. This prevention is associated with an increase in PNN staining intensity. In PV cells lacking a PNN, NAC pretreatment or cocaine exposure alone increases oxidative stress levels and NAC pretreatment combined with cocaine exposure exacerbates this increase. These findings demonstrate that PV cells respond differently to cocaine-induced oxidative stress that depends on the presence of a PNN. This acute response to cocaine may be beneficial in the short term for protecting PV cells from oxidative stress, but in turn may prime the persistent and chronic nature of drug addiction.

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Poster

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Title: Optogenetic manipulation of Parvalbumin interneurons in the central amygdala (CeA) modulates the negative affective states and the expression of corticotropin-releasing hormone within morphine withdrawal

Authors: *L. WANG[1,2], J. M. SHEN[2], F. F. WANG[2], L. MA[2];
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Abstract: The central nucleus of amygdala (CeA) is a crucial component of the neuronal circuitry mediating aversively emotion. Its role in the negative affective states during drug withdrawal includes changes in opioidergic, GABAergic, and CRF neurotransmission, *etc*. However, the modulation of the neurobiological interconnectivity in CeA, and the effects in the negative reinforcement of drug dependents are poorly understood. Here, we found chronic morphine withdrawal influenced the firing rate of CeA Parvalbumin (PV) $^{+}$ interneurons, indicating the changed activity of CeA PV^{+} interneurons during the chronic morphine withdrawal. Optogenetic inhibition of the activity of CeA PV^{+} interneurons changed the morphine withdrawal-induced negative affective states, such as the aversive (assessed by CPA), anxiety (assessed by EPM), and anhedonic-like (assessed by SPT) behaviors. Direct activation of CeA PV^{+} interneurons was sufficient to trigger those negative affective-like behaviors. We found the CRH^{+} interneurons are present in the CeA, and had the connections with the PV^{+} interneurons. Optogenetic manipulating the CeA PV^{+} interneurons during the morphine withdrawal significantly influenced the *CRH* mRNA level in the CeA. These results indicate that the activation of PV^{+} interneurons during morphine withdrawal was crucial for the induction of the negative emotion and the upregulation of *CRH* mRNA level in the CeA.

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Poster

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Title: Optogenetics reveals that dopamine signaling in the rostral-caudal NAc shell differentially inhibits/facilitates cocaine-induced natural reward devaluation and negative affect in a preclinical model

Authors: *S. W. HURLEY, E. A. WEST, R. M. CARELLI;
Psychology and Neurosci., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: In cocaine addicts, continued drug use is driven, in part, by the desire to eliminate negative affective states (e.g., dysphoria, anxiety, irritability) that typically emerge during both drug withdrawal and drug craving. Further, addicts also experience a devaluation of natural rewards (jobs, friends, family) that pale in comparison to the drug. Our lab developed a preclinical model that assesses these phenomena. In this model, rats receive daily 45 intraoral infusions of a saccharin solution (3.5 s/inf; ~1 inf/min), followed by cocaine self-administration (2 hrs). Rats initially exhibit appetitive responses (e.g., lateral tongue protrusions) but shift to primarily aversive responses (e.g., gapes) as they learn the taste-drug association. Further, rapid dopamine signaling in the nucleus accumbens shell (NAcSh) tracks this shift in affect and natural reward devaluation (Wheeler et al., 2011). Here, we examined if dopamine release in the NAcSh is causally linked to the emergence of negative affect and devaluation of the sweet using optogenetics. Tyrosine hydroxylase Cre (TH::Cre$^{+/-}$, n=10) and non-transgenic (TH::Cre$^{-/-}$, n=10) rats received bilateral injections of Cre-dependent channel rhodopsin in the VTA. Eight weeks later, rats were implanted with optical fibers directed at the NAcSh. Following 1 week recovery, rats underwent training (7 daily sessions) where they received 45 intraoral infusions of a saccharin solution that preceded an i.p. injection of cocaine (20 mg). On days 2-7, rats received optical stimulation of the NAcSh during the onset of each saccharin infusion. The objective was to prevent the shift (decrease) in dopamine signaling that occurs with multiple taste-drug pairings, and determine if this blocks the emergence of aversive taste reactivity. Preliminary data show that non-transgenic rats exhibited a significant increase in the percent of trials with aversive taste reactivity (Day 1, 2.9 ± 0.6 vs. Day 7, 20.3 ± 2.6, $p<0.05$). In contrast, transgenic rats with fiber placements in the rostral NAcSh failed to display a significant increase in aversive responses (Day 1, 2.2 ± 2.2 vs. Day 8, 8.1 ± 4.7). Remarkably, transgenic rats with fibers in the caudal NAcSh showed a robust *increase* in aversive taste reactivity beyond that of non-transgenic rats (Day 1, 5.3 ± 2.7 vs. Day 7, 57.3 ± 9.6, $p<0.05$). Together, these preliminary data

suggest that NAcSh dopamine differentially modulates cocaine-induced negative affect depending on its actions across the rostral-caudal shell; rostral NAcSh promotes appetitive behaviors while the caudal NAcSh appears to mediate aversive responses in our model.

Disclosures: **S.W. Hurley:** None. **E.A. West:** None. **R.M. Carelli:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Longitudinal changes in brain metabolic activity after escalation of cocaine self-administration

Authors: *C. NICOLAS[1,2], C. TAUBER[3], F.-X. LEPELLETIER[3], S. CHALON[3], P. BELUJON[2], L. GALINEAU[3], M. SOLINAS[2];
[1]Behavioral Neurosci. Res. Br., Natl. Inst. On Drug Abuse-Irp, Baltimore, MD; [2]Univ. of Poitiers, LNEC, INSERM, U1084, Poitiers, France; [3]UMR INSERM U930, Univ. François Rabelais de Tours, Tours, France

Abstract: Brain imaging has been shown to be a powerful technique to identify pathological brain functioning in drug addicts compared to naive individuals, with some of these neuroadaptations persisting long after discontinuation of drug use. In animal models of addiction, only two studies have used brain imaging to investigate drug-induced changes in brain functioning and they have focused on early withdrawal after chronic cocaine intake. Therefore, here we conducted a longitudinal study to determine metabolic changes in specific brain regions occurring after short and long periods of cocaine withdrawal. For this, after 7 days of cocaine self-administration training (2h/day), rats were given either short-access (ShA,1h/day) or long-access (LgA,6h/day) to cocaine self-administration for 20 days. A group that did not undergo self-administration served as control. After that, rats underwent forced abstinence, including two microPET scans with 2-deoxy-2-(^{18}F)fluoro-d-glucose (^{18}FDG) after 7 and 28 days of abstinence to assess modifications in brain activity. We found that voluntary intake of cocaine produces changes in brain metabolic activity that depends on the level of cocaine self-administration and on the duration of abstinence. Some neurometabolic changes were similar in ShA and LgA regardless of the withdrawal stage including decreased activity in the cingulate cortex and specific modifications in selective parts of the dorsal striatum and may reflect a general

consequence of voluntary cocaine intake. On the other hand, a number of changes in metabolic activity were specific to the LgA group and therefore might be the consequences of excessive and escalated cocaine intake. In particular, LgA rats showed a decrease in the activity of the infralimbic cortex and the nucleus accumbens after 7 days of withdrawal whereas they displayed a decrease in the activity of the insular cortex and an increase in the activity of the amygdala, the dorsal hippocampus and the substantia nigra after 28 days of withdrawal. Our results provide new insights into the short and long-term cerebral metabolic modifications related to cocaine withdrawal after chronic controlled and addicted cocaine intakes.

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Poster

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Support: RO1DA038613

Title: Mitochondrial fission in nucleus accumbens projection neurons subtype promotes cocaine behavioral plasticity

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Abstract: Altered brain energy homeostasis is a key adaptation occurring in the cocaine-addicted brain. However, the underlying mechanisms that govern these homeostatic adaptations are not clearly defined. One such mechanism, which is a fundamental component of energy homeostasis and has not been addressed in cocaine abuse are mitochondrial dynamics; which can include mitochondrial biogenesis, fission, and fusion. To provide insight into this we assess mitochondrial dynamics in the two nucleus accumbens (NAc) projection medium spiny neuron

(MSN) subtypes, those enriched in dopamine D1 vs. D2 receptors in mice that self-administer cocaine. Using a Cre inducible adeno-associated virus (AAV)-double inverted floxed open reading frame (DIO)-mito-dsRed combined with D1-Cre and D2-Cre mouse lines we label mitochondria in MSN subtypes. Cocaine self-administration caused an increase in the frequency of smaller mitochondria in D1-MSN dendrites, implicating enhanced mitochondrial fission in D1-MSNs. Consistent with our data we observe an increase in mRNA of Dynamin-related protein 1 (Drp1), a mediator of outer mitochondrial membrane fission, in NAc of rodents that self-administer cocaine and in postmortem NAc of cocaine dependent individuals. Using the RiboTag approach, we observe an upregulation of ribosome-associated mRNA of Drp1in D1-MSNs and a decrease in D2-MSNs after repeated cocaine. Additionally, the activated form of Drp1 protein is increased in NAc of mice after repeated cocaine injections. We next used a small molecule inhibitor of mitochondrial fission, Mdivi-1, which blocks the activated form of Drp1. Mdivi-1 treatment blocked seeking after cocaine conditioned place preference and the expression of cocaine locomotor sensitization. Finally, we generated Cre-inducible Drp1-constitutively active (CA) and Drp1-wild-type (WT) AAVs. Overexpression of Drp1-CA in D1-MSNs increased cocaine taking during acquisition to self-administration and seeking behavior after abstinence. Our findings demonstrate a novel role for altered mitochondrial fission in NAc in cocaine abuse and implicate that blockade of mitochondrial fission has potential therapeutic treatment for cocaine addiction.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Cocaine effects on dopamine neurons in mice are reduced by both deletion of GIRK2 channels specifically in dopamine neurons and with cocaine self-administration experience

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Abstract: Dopaminergic neurons are key mediators of substance abuse and addiction-related behaviors. In the midbrain, somatodendritic dopamine release activates inhibitory postsynaptic currents through D2 autoreceptors to increase G-protein gated inward rectifying potassium subtype 2 (GIRK2) conductance in dopamine neurons. Cocaine is a highly addictive stimulant that alters the mesolimbic dopamine pathway; however the role of the GIRK2 channel in dopamine neuron adaptations during cocaine abuse is unclear. These experiments used a knockout mouse line ($GIRK2_{DA}KO$) where GIRK2 channels were selectively deleted in neurons expressing the dopamine transporter (DAT). Male $GIRK2_{DA}KO$ mice and their wild-type littermates ($GIRK2_{DA}WT$) were trained to nose-poke for IV infusions of cocaine (0.5 mg/kg/infusion) during daily 2 h sessions until responding was stable. Electrophysiological recordings were taken from $GIRK2_{DA}KO$ and $GIRK2_{DA}WT$ mice under 3 different conditions: naive-controls, one day following cocaine self-administration, and after extended abstinence from cocaine. Cell-attached electrophysiological recordings were taken from brain slices containing midbrain dopamine neurons to measure adaptations in dopamine neuron activity in response to bath perfusion of cocaine. Results indicated a downward shift in the cocaine concentration-response curve in $GIRK2_{DA}KO$ mice compared to $GIRK2_{DA}WT$ mice. This effect was mediated by D2 autoreceptors, determined by preincubation of sulpiride (200 nM, D2 antagonist). Interestingly, immediately following cocaine-self administration, dopamine neurons exhibited decreased sensitivity to cocaine both in $GIRK2_{DA}KO$ and $GIRK2_{DA}WT$ mice. This effect was transient, since after a period of abstinence, dopamine neurons from mice of both genotypes returned to naive-like levels of cocaine sensitivity. These results indicate that the GIRK2 channels in dopamine neurons could play an important role in neuroadaptations that take place with cocaine abuse.

Disclosures: A.M. Hager: None. **S. Dominguez Lopez:** None. **K. Wickman:** None. **M.J. Beckstead:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Selective ablation of GIRK channels in dopamine neurons alters behavioral effects of cocaine in mice

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Abstract: *In vivo* cocaine exposure triggers the suppression of inhibitory G protein signaling in dopamine (DA) neurons of the ventral tegmental area (VTA). G protein-gated inwardly rectifying K^+ (GIRK/Kir3) channels are key effectors of inhibitory G protein signaling, and mediate the inhibitory effect of $GABA_B$ receptor ($GABA_BR$) and D_2 DA receptor (D_2R) activation in VTA DA neurons. Here, we examined the effect of the DA neuron-specific loss of GIRK channels on D_2R-dependent regulation of VTA DA neuron excitability, and cocaine-induced, locomotor and reward-related behaviors. Selective ablation of *Girk2* in DA neurons did not alter the baseline excitability of VTA DA neurons, but significantly reduced the magnitude of D_2R-dependent inhibitory currents and blunted the impact of D_2R activation on spontaneous activity and neuronal excitability. Mice lacking GIRK channels in DA neurons exhibited an enhanced motor-stimulatory effect of acute cocaine and altered sensitization, as well as increased intravenous self-administration of cocaine. However, these mice showed unaltered cocaine-induced conditioned place preference. Collectively, these data suggest that the GIRK channel in VTA DA neurons suppresses the locomotor stimulatory effect of cocaine and plays a role in the reinforcing effects of cocaine in the operant-based self-administration procedure but not the Pavlovian-based conditioned place preference procedure.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Signaling kinetics of stimulated dopamine release in the nucleus accumbens core and shell are differentially altered following abstinence from cocaine self-administration in behaving rats

Authors: *M. SADDORIS;
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Abstract: Repeated experience with drugs of abuse can induce persistent changes in neural signaling properties even after weeks of abstinence from the drug-taking episodes. We and others have shown that these alterations are associated with impairments in motivated and goal-directed behavior, including deficits in Pavlovian-to-instrumental transfer, conditioned approach, and second-order conditioning. At the neural level, these changes are correlated with alterations in the appropriate phasic DA signals in the nucleus accumbens (NAc), and these impairments showed distinct changes between the NAc core and shell. However, in these tasks, impaired signaling was investigated relative to task stimuli during the performance of a motivated task. Thus, impairments may be due to either an inability to appropriately signal the relevance of the stimuli (indicating a general motivational or learning deficit), or instead to fundamental alterations in the DA release and reuptake kinetics that occur as neuroadaptations following drug experience. To specifically test the latter hypothesis, we trained rats to self-administer cocaine intravenously (~1mg/kg) in daily 2hr sessions for 14d. Controls self-administered water to a foodcup and yoked i.v. vehicle infusions. Following at least 30d of enforced abstinence from self-administration and at least 1wk prior to recordings, rats were implanted with guide cannulas over the NAc core (n=7 Cocaine, n=17 Controls) or shell (n=6 Cocaine, n=12 Controls) and a bipolar electrical stimulating probe into the ventral tegmental area (VTA). On the day of recording, an acutely-placed carbon fiber electrode was lowered into the region of interest in awake and behaving rats. Electrical stimulation of the VTA was performed at a variety of frequencies (12-60 Hz) and pulse number (1-24) with a 4ms pulsewidth; rapid DA release and reuptake kinetics were measured via fast scan cyclic voltammetry. In Controls, we confirmed previous findings demonstrating a slower release rate to peak and slower reuptake rate following peak (Vmax) in the shell compared to the core. However, cocaine experienced rats showed significantly altered signaling kinetics. Shell DA kinetics were insensitive to changes in applied stimulation rates, while core DA kinetics slowed and came to resemble normal NAc shell

kinetics. Indeed, the distribution of peak [DA] in the core of Cocaine subjects was indistinguishable from Control shell recordings. These findings demonstrate persistent and region-specific changes in DA synaptic properties following cocaine self-administration which may contribute to ongoing deficits in motivated learning well after drug abstinence.

Disclosures: M. Saddoris: None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Role of endocannabinoid and dopamine signaling in cocaine-induced synaptic AMPAR depotentiation in the nucleus accumbens

Authors: *A. E. INGEBRETSON[1], M. C. HEARING[2], M. ESGUERRA[2], E. D. HUFFINGTON[2], M. J. THOMAS[2];
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Abstract: The nucleus accumbens (NAc) is a key mesocorticolimbic structure within which drugs of abuse exert their rewarding and reinforcing effects. Composed principally of medium spiny neurons (MSNs), the NAc receives converging glutamatergic input from cortical and limbic afferents that drive MSN activity and synaptic plasticity. In rodent models of addiction, changes in glutamatergic synaptic strength at NAc MSNs play a central role in drug-seeking behavior. Previous studies from our lab have shown that repeated exposure to cocaine potentiates AMPA receptor (AMPAR)-mediated signaling in the NAc shell during a period of abstinence (10-14 days) from drug exposure. This increase in synaptic strength is reversed (or, "depotentiated") by a single re-exposure to cocaine or bout of stress, suggesting that this switch in NAc synaptic excitability might act as a trigger for relapse, promoting renewal of drug-seeking behavior. However, the cellular mechanisms of this plasticity have not been well characterized. In the current study, using an *ex vivo* cocaine challenge model of synaptic

AMPAR depotentiation, we examined the contributions of endocannabinoid, dopamine and glutamate signaling. In the NAc, activation of postsynaptic group I metabotropic glutamate receptors (mGluRs) is known to promote synaptic depression through increased trafficking of AMPARs and release of endocannabinoids, which bind pre-synaptic CB1 receptors and decrease the probability of glutamate release. Consistent with this, application of the mGluR5 antagonist MTEP blocked the cocaine-induced reduction in mEPSC amplitude but not frequency, while the CB1 antagonist SR141716A blocked the reduction in both mEPSC amplitude and frequency. In addition, application of the non-selective dopamine antagonist flupenthixol blocked the depotentiation of mEPSC amplitude and frequency, confirming that dopamine receptor activation is a necessary step for the reduction in AMPAR synaptic transmission. Future studies will examine the role for D1 vs. D2 dopamine receptors and the relationship between dopamine and endocannabinoid signaling in cocaine-induced depotentiation in the NAc. Our findings demonstrate that re-exposure to cocaine co-opts multiple signaling pathways in the NAc shell to induce alterations in synaptic strength, identifying mechanisms that may be targeted for potential pharmacotherapies.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

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Title: NMDAR dependent intracellular responses associated with cocaine conditioned place preference behavior

Authors: S. NYGARD[1], A. KLAMBATSEN[2], B. BALOUCH[2], *V. L. QUINONES-JENAB[2], S. JENAB[2];
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Abstract: The aim of this study was to investigate the intracellular responses associated with the acquisition and expression of cocaine-context associations. ERK (extracellular regulated kinase), CREB (cAMP responsive element binding protein), FosB and deltaFosB proteins were of particular interest due to their involvement in cocaine reward and in synaptic plasticity underlying learning and memory. We used the conditioned place preference (CPP) paradigm,

which employs a Pavlovian conditioning procedure to establish an association between a drug-paired environment and the drug's rewarding effects, to study the role of these signaling pathways in cocaine-context associations. N-methyl-D-aspartate receptor (NMDAR) antagonism prior to cocaine administration during conditioning blocked the acquisition of cocaine CPP and reduced Nucleus Accumbens (NAc) phosphorylated-ERK (pERK) and phosphorylated CREB (pCREB) levels following the CPP test (drug-free). We also show that cocaine-induced increases in FosB and deltaFosB levels are dependent on NMDARs in the Caudate Putamen (CPu), but not in the NAc.These results will aid in the advancement of general knowledge about the molecular formation and retrieval of cocaine-associated memories that can be used in the future when designing treatments for cocaine addiction that target both prevention and relapse.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Title: Presynaptic adenosine A_{2A} receptor inhibition impacts behavioral sensitization to repeated cocaine but not repeated methamphetamine

Authors: *N. HAYNES, R. K. BACHTELL;
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Abstract: Repeated psychostimulant administration produces perturbations in dopamine and glutamate neurotransmission that contribute to enhanced psychostimulant-induced locomotion. The synaptic localization of adenosine A_{2A}Rs in the striatum can influence both dopamine and glutamate neurotransmission differentially and have different effects on behavior. A_{2A}Rs localized on presynaptic neurons that oppose direct pathway medium spiny neurons enhance glutamate neurotransmission, while postsynaptic A_{2A}Rs localized on indirect pathway neurons reduce dopamine signaling. These studies sought to elucidate differential effects of these subpopulations of A_{2A}R on psychostimulant-induced locomotor sensitization. Male Sprague-Dawley rats received 7 daily treatments of saline, cocaine, or methamphetamine to induce sensitization. Locomotor tests were conducted on days 1, 7, and 14 (following 7 days abstinence). Prior to the locomotor test on day 14, rats received a pretreatment of either

presynaptic $A_{2A}R$ antagonist (SCH 442416), postsynaptic $A_{2A}R$ antagonist (KW 6002), or vehicle prior to either a cocaine or methamphetamine challenge. Presynaptic $A_{2A}R$ blockade had no effect on either acute cocaine- or methamphetamine-induced locomotion and did not alter locomotion when administered alone. Interestingly, presynaptic $A_{2A}R$ blockade reversed behavioral sensitization to cocaine but not methamphetamine. Postsynaptic $A_{2A}R$ antagonism increased locomotion when administered alone but did not alter acute or sensitized cocaine- and methamphetamine-induced locomotion. These findings suggest that postsynaptic $A_{2A}R$ antagonism facilitates locomotion possibly via disinhibiting dopamine D_2 receptor activity on indirect pathway neurons, although this is insufficient to alter cocaine or methamphetamine-induced locomotion. Presynaptic blockade, on the other hand, reversed cocaine-induced sensitization, possibly by reducing glutamate neurotransmission onto direct pathway neurons. This effect was selective for cocaine sensitization as presynaptic $A_{2A}R$ antagonism had no effect on methamphetamine sensitization suggesting that reducing presynaptic glutamate transmission does not influence methamphetamine sensitization.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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KDA040004A

Title: Evaluating of the role of nucleus accumbens nitric oxide and somatostatin release in cocaine seeking

Authors: ***M. D. SCOFIELD**[1,2], J. A. HEINSBROEK[2], C. GARCIA-KELLER[2], A. W. SMITH[3], C. D. GIPSON[4], P. W. KALIVAS[2];
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Abstract: The gaseous transmitter nitric oxide (NO) is produced in the nucleus accumbens core (NAcore) by a subpopulation of GABAergic interneurons that express neuronal nitric oxide synthase (nNOS) as well as Somatostatin (SOM). NO plays a critical role in the nitrosylation and

activation of matrix metalloproteinases (MMPs) required for dendritic spine head (d_h) expansion on medium spiny neurons (MSNs), linked to cue-induced cocaine seeking. We have shown that microinfusion of the mGluR5 agonist CHPG into the NAcore promoted NO release, as well as cocaine seeking in the absence of conditioned cues. Interestingly, the induction of cocaine seeking produced by CHPG was nNOS-dependent. In order to isolate NAcore nNOS interneurons, we employed Cre-dependent expression of Gq-coupled DREADD receptors in NOS1-Cre transgenic mice. Similar to CHPG, activation of Gq-DREADD in NAcore nNOS neurons enhanced cocaine seeking in the absence of conditioned cues. We are currently examining the effects of inhibiting nNOS interneurons in the NAcore during cued reinstatement with Gi-coupled DREADD receptors and with other viral strategies designed to selectively destroy NAcore nNOS interneurons. Given that the nNOS neurons also release SOM, we evaluated the role of SOM in initiating cocaine seeking as described above with CHPG and activation of Gq-DREADD. Our data demonstrates that infusion of SOM, at doses reported to enhance locomotion, did not precipitate cocaine seeking. However, we observed a significant decrease in reinstatement-associated MMP activity and cued cocaine-seeking following microinfusion of cyclosomatostatin, a non-specific SOM receptor antagonist. We are currently performing experiments designed to directly compare the effects of nNOS inhibition or SOM receptor blockade in inhibiting Gq-DREADD-mediated cocaine seeking in NOS1-Cre transgenic mice. These data will provide further insight into the roles NO and SOM, two signaling molecules released from a class of GABAergic interneurons critically involved in the neurobiological underpinnings of cocaine seeking.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Title: Cocaine inhibits alpha6-containing nicotinic acetylcholine receptor-mediated currents

Authors: *D. CHEN[1,2], Q. SU[2,3], J. NEISEWANDER[3], J. WU[1];
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Abstract: Aim: Alpha6-containing nicotinic acetylcholine receptors (Alpha6*-nAChRs) are abundantly expressed in the midbrain dopaminergic system, which is thought to play an

important role in drug addiction and animal motivation behaviors. Emerging evidence suggests that nAChR in the ventral tegmental area (VTA) is an important target to mediate nicotinic effect, but is also involved in mediating other addictive drugs. Cocaine, a commonly abused drug, is well known for its inhibitory effect on dopamine transporter, but little is known about its effects on Alpha6*-nAChRs. In this study, we aimed to explore the acute effects of cocaine on α6*-nAChR function heterologously expressed in human SH-EP1 cell clone. We also examined the effects of cocaine on natural α6*-nAChR-mediated modulations in GABA release onto VTA neurons.**Methods:** Patch-clamp whole-cell recording techniques were used in this study. Functional α6*-nAChRs expressed in SH-EP1 cells were used for patch recordings, and Alpha6*-nAChR-mediated whole-cell currents were measured. Natural Alpha6*-nAChRs are located in GABAergic presynaptic boutons in acutely-dissociated mouse VTA DA neurons. Perforated whole-cell recordings were performed to measure miniature inhibitory postsynaptic currents (mIPSCs). **Results:**In transfected Alpha6-nAChRs in SH-EP1 cells, acute exposure of cocaine inhibited nicotine-induced inward current in a concentration-dependent manner with an IC50 of 34.5±2.3 μM, Hill coefficient 1.3±0.1. In the presence of 30 μM cocaine, the nicotine concentration response curve moved to right without change the IC50 value, suggesting a non-competitive inhibition. In mechanically- dissociated VTA DA neurons, 10 μM cocaine significantly reduced nicotine (1 μM)-induced enhancement of mIPSCs frequency. **Conclusion:**This study provides first evidence that cocaine directly inhibits both heterologously and naturally expressed Alpha6*-nAChR function, suggesting that VTA α6*-nAChRs may be involved in the process of cocaine reward and dependence.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Dysregulation of serotonergic function in orbitofrontal cortex during cocaine withdrawal

Authors: *A. M. WRIGHT, A. ZAPATA, A. F. HOFFMAN, C. R. LUPICA;
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Abstract: The orbitofrontal cortex (OFC) is a brain region key to flexible, goal-directed behavior. This flexibility is required for changing behavioral strategies and other higher-order

behavioral tasks. Loss of OFC function is classically linked to deficits in impulsivity, which can contribute to a multitude of human neuropsychiatric disorders including impulsive-aggression disorders and drug addiction. As the OFC receives dense serotonergic (5-HT) innervation and appears to be involved in remediation of psychiatric illnesses by drugs targeting the 5-HT system, it is hypothesized that 5-HT is integral to OFC function. However, the cellular mechanisms underlying serotonergic function in the OFC are poorly understood. Using whole-cell electrophysiological recordings in brain slices from naïve rats, we find that 5-HT_{1A} receptors generate hyperpolarizing outward currents in layer-V OFC pyramidal neurons, and that 5-HT_{2A} receptors increase glutamate release onto these cells and generate depolarizing inward currents. Additional experiments show that 5-HT signaling at these receptors is greatly attenuated during extended withdrawal (40 days) from cocaine self-administration (CSA) or yoked-administration (CYA). In situ hybridization at this extended withdrawal time point revealed a significant elevation in 5-HT_{2A} receptor mRNA, and no change in 5-HT_{1A} receptor mRNA in OFC from both CSA and CYA animals. We hypothesize that this dysregulation of 5-HT signaling leads to strong disruptions of OFC network activity, which may contribute to the impaired decision-making and craving associated with cocaine addiction.

Disclosures: A.M. Wright: None. **A. Zapata:** None. **A.F. Hoffman:** None. **C.R. Lupica:** None.

Poster

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DA031916

Title: The 5-HT1B serotonin receptor potentiates methylphenidate-induced gene regulation in the striatum

Authors: D. ALTER, J. A. BEVERLEY, *H. STEINER;
Chicago Med. School/RFUMS, North Chicago, IL

Abstract: The psychostimulant methylphenidate (Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer and party drug in the healthy. Methylphenidate, like cocaine, acts by blocking the reuptake of dopamine. However, unlike

cocaine, methylphenidate does not affect serotonin. Serotonin contributes to addiction-related gene regulation by cocaine. Thus, the lack of a serotonin effect may explain methylphenidate's more moderate gene regulation effects and addiction liability. Our previous studies show that enhancing serotonin action by adding a selective serotonin reuptake inhibitor (SSRI), fluoxetine, to methylphenidate indeed potentiates methylphenidate-induced gene regulation in the striatum. The 5-HT1B serotonin receptor subtype mediates serotonin effects in cocaine-induced gene regulation. Here, we investigated whether stimulation of the 5-HT1B receptor modifies methylphenidate-induced gene regulation. We assessed the effects of a 5-HT1B receptor-selective agonist, CP94253 (3-10 mg/kg), on methylphenidate-induced expression of the immediate-early genes Zif268, c-Fos and Homer1a, by in situ hybridization histochemistry. Our results show that CP94253 potentiated acute induction of Zif268 and c-Fos by methylphenidate (5 mg/kg) in a dose-dependent manner, thus mimicking the effects of fluoxetine. This potentiation was most pronounced in the lateral (sensorimotor) striatum on middle and caudal striatal levels. In contrast, these doses of the 5-HT1B agonist did not impact methylphenidate-induced Homer1a expression. These results indicate that stimulation of the 5-HT1B receptor is sufficient to facilitate Zif268 and c-Fos, but not Homer1a, induction by methylphenidate. Methylphenidate plus SSRI concomitant therapies are indicated in ADHD/depression comorbidity and other disorders, and co-exposure also occurs with cognitive enhancer use by patients on SSRIs. As SSRIs potentiate addiction-related gene regulation by methylphenidate, SSRIs may enhance the addiction liability of methylphenidate. Our results suggest that the 5-HT1B receptor contributes to this SSRI-induced potentiation of gene regulation. The 5-HT1B receptor may thus serve as a pharmacological target to prevent these SSRI-induced effects.

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Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA031900

Title: Dopamine neurotransmission in the medial dorsal striatum is associated with vulnerability to cocaine addiction.

Authors: *J. K. SHAW, R. A. ESPAÑA;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Cocaine addiction is a chronic, relapsing brain disease characterized by heavy use of, aberrant motivation for, and an inability to maintain abstinence from the drug. Considerable evidence suggests that the mesolimbic dopamine (DA) system plays a pivotal role in the development and maintenance of cocaine use and abuse, however the extent to which this pathway differs between those prone and resistant to cocaine use disorders remains unclear. To determine the relative contributions of DA release and uptake to vulnerability to use cocaine, we measured baseline DA release and uptake dynamics in the medial dorsal striatum of anesthetized rats using fast scan cyclic voltammetry prior to any behavioral testing. Following recovery, rats were provided access to cocaine-associated levers and the time to acquire, consumption of, and motivation for cocaine were measured using fixed ratio-1 (FR1), progressive ratio (PR), and within-subject threshold schedules of reinforcement. Preliminary results suggest an inverted-U relationship between DA release in the medial dorsal striatum and responding for cocaine under the FR1 schedule, and a strong linear relationship between the rate of DA uptake and motivation for cocaine under both the PR and threshold schedules—implicating the DA transporter in the risk for developing cocaine use disorders. These data suggest that inherent variability in the mesolimbic DA system may underlie behavioral components of cocaine self-administration. The current findings may aid in further development of targeted prevention efforts and therapies for the treatment of cocaine addiction.

Disclosures: **J.K. Shaw:** None. **R.A. España:** None.

Poster

349. Cellular and Circuit Mechanisms of Cocaine Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 349.27/EEE11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA033373

NIDA Grant DA033796

Title: Combining multiple schedules of reinforcement with glutamate biosensors to examine the effects of cocaine and food on prelimbic glutamatergic signaling

Authors: *S. R. BATTEN[1], G. A. GERHARDT[2], J. S. BECKMANN[1];
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Abstract: Drug-specific reward and associated effects on neural signaling are often studied between subjects, where one group self-administers drug and a separate group self-administers a

natural reinforcer. However, exposure to drugs of abuse can cause long-term neural adaptations that can affect how an organism responds to drug reward, natural reward, and their reward-associated stimuli. Thus, to isolate drug-specific effects it is important to use models that expose the same organism to all of the aforementioned. Multiple schedules provide a means of dissociating the rewarding effects of a drug from the rewarding effects of food along with their cues, within a single animal. Herein, we used glutamate biosensors implanted into the prelimbic cortex of freely-moving animals to assess glutamatergic and behavioral changes in rats performing under a cocaine-food multiple schedule. Our results show that there is a slight increase in the amplitude of glutamate release when animals press the cocaine lever compared to when they press the food lever. Further, glutamate release is greater when a cocaine infusion is earned compared to a food pellet. These data suggest that combining glutamate biosensors with multiple schedules provides a practical means for assessing differential glutamatergic signaling associated with cocaine and food. These data also suggest that prelimbic glutamate release is greater for cocaine lever presses and infusions compared to food lever presses and earning food.

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Poster

350. Cocaine: Brain Circuitry I

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Program#/Poster#: 350.01/EEE12

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA036582

Title: Resolving the contribution of midline thalamic nuclei efferents during reinstatement of drug-seeking using $G_{i/o}$-coupled DREADDs

Authors: ***A. M. WUNSCH**[1,2], L. M. YAGER[1], C. LE[1], E. A. DONCKELS[1], J. F. NEUMAIER[2,3,4], S. M. FERGUSON[1,2,4];
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Abstract: Addiction is a debilitating neuropsychiatric illness that produces profound alterations in neuronal function within the corticomesolimbic circuit. Despite advances in understanding the brain circuitry associated with addiction, rates of relapse remain incredibly high. Alterations in glutamatergic signaling within the nucleus accumbens (NAc) may be particularly important in

regulating relapse. Recent evidence indicates that NAc glutamatergic inputs originating in pre frontal cortex (PFC), amygdala (AMG), and hippocampus may regulate distinct aspects of addiction. Although projection neurons originating in midline thalamic nuclei (MTN) provide the second largest source of glutamate into the NAc, their role in relapse behavior has not yet been clearly identified. In order to define the role of MTN in the reinstatement of cocaine-seeking, we expressed inhibitory, $G_{i/o}$-coupled DREADDs (hM_4Di) in MTN. We found that reducing activity of MTN (ie. paraventricular, intermediodorsal, central medial, and mediodorsal nuclei) attenuated both cue-induced and drug-primed reinstatement of cocaine-seeking. Because MTN project to NAc, PFC, and BLA, all of which differentially regulate reinstatement, it is difficult to identify the exact contribution of MTN output neurons to each of these regions in reinstatement of drug-seeking. First, we confirmed MTN projections by assessing hM_4Di expression in these downstream structures. Indeed, we observed hM_4Di in axons within NAc, PFC, and BLA supporting the idea that our hM_4Di manipulation in all MTN neurons may have broad downstream effects. Next we will utilize a combinatorial viral and chemogenetic approach to assess the role of specific MTN efferents in reinstatement of cocaine-seeking. Rats will receive bilateral injections of retrogradely transported canine adenovirus expressing cre-recombinase (cre) into NAc, PFC, or AMG, and an adeno-associated virus expressing cre-dependent, hM_4Di into MTN. Because hM_4Di expression will be restricted to specific MTN efferents, we can selectively examine the role of $G_{i/o}$-signaling cascades in thalamostriatal, thalamocortical, and thalamoamygdalar neurons during cue-induced or drug-primed reinstatement of cocaine-seeking. By manipulating physiologically relevant signaling cascades within specific thalamic efferents, this work has the potential to better define the circuitry associated with relapse and lead to more targeted treatments in addiction.

Disclosures: **A.M. Wunsch:** None. **L.M. Yager:** None. **C. Le:** None. **E.A. Donckels:** None. **J.F. Neumaier:** None. **S.M. Ferguson:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758 to JRM

Title: Role of a CRF-mediated dopaminergic projection from the ventral tegmental area to the prelimbic cortex in stress-induced cocaine seeking

Authors: *E. VAN NEWENHIZEN[1], O. VRANJKOVIC[1], J. M. BLACKTOP[1], T. M. KLOEHN[1], G. S. STINNETT[2], C. H. GERNDT[1], K. KETCHESIN[2], M. E. NORDNESS[1], C. R. MUELLER[1], J. R. MCREYNOLDS[1], E. M. DONCHECK[1], D. A. BAKER[1], A. F. SEASHOLTZ[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Biol. Chem., Univ. of Michigan, Ann Arbor, MI

Abstract: The ability of stress to trigger cocaine seeking in humans and rodents is variable and is determined, in part, by the amount and pattern of prior drug use. This study examined the role of a corticotropin releasing factor- (CRF-) regulated dopaminergic projection from the ventral tegmental area (VTA) to the prelimbic cortex in shock-induced cocaine seeking and its recruitment under self-administration conditions that establish relapse vulnerability. Rats with a history of daily long-access (LgA; 14 x 6 hrs/day) but not short-access (ShA; 14 x 2 hrs/day) self-administration showed robust shock- and intra-VTA CRF-induced cocaine seeking. This was associated with a heightened shock-induced prelimbic cortex Fos response and activation of VTA neurons that project to the prelimbic cortex, as defined by Fos co-labeling with the retrograde tracer, cholera toxin B. Both shock-induced reinstatement and the prelimbic cortex Fos response were prevented by bilateral intra-VTA injections of the CRFR1 receptor antagonist, antalarmin. Pharmacological disconnection of the CRF-regulated dopaminergic projection to the prelimbic cortex by injection of antalarmin into the VTA in one hemisphere and the D1R antagonist SCH23390 into the prelimbic cortex of the contralateral hemisphere prevented shock-induced cocaine seeking, while antagonist administration within the same hemisphere or disconnection of the VTA projection to infralimbic cortex was without effect. Efforts to confirm the requirement for this VTA-prelimbic cortex pathway for stress-induced cocaine seeking using Designer Receptor Exclusively Activated by Designer Drugs- (DREADD) based approaches are ongoing. LgA, but not ShA, cocaine self-administration resulted in increased CRFR1 mRNA levels in the VTA as measured using *in situ* hybridization. Altogether, these findings suggest that excessive cocaine use establishes susceptibility to stress-induced relapse by recruiting CRF regulation of a key stressor-responsive mesocortical dopaminergic pathway.

Disclosures: E. Van Newenhizen: None. **O. Vranjkovic:** None. **J.M. Blacktop:** None. **T.M. Kloehn:** None. **G.S. Stinnett:** None. **C.H. Gerndt:** None. **K. Ketchesin:** None. **M.E. Nordness:** None. **C.R. Mueller:** None. **J.R. McReynolds:** None. **E.M. Doncheck:** None. **D.A. Baker:** None. **A.F. Seasholtz:** None. **J.R. Mantsch:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA038663 to Mantsch and Hillard

Title: Glucocorticoid-endocannabinoid interactions in the prelimbic cortex mediate stress-potentiated reinstatement of cocaine seeking through increased activation of the cortico-accumbens pathway

Authors: *J. R. MCREYNOLDS[1], E. M. DONCHECK[1], O. VRANJKOVIC[1], E. N. GRAF[1], X. LIU[2], T. STOLLENWERK[2], P. J. GOTTSHALL[1], Q.-S. LIU[2], C. J. HILLARD[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Pharmacol. & Toxicology and Neurosci. Res. Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Stress is a powerful trigger for relapse but also potentiates responses to other triggers for drug use. We have shown that under self-administration conditions where stress (electric footshock; EFS) alone does not directly reinstate cocaine seeking it can potentiate reinstatement when paired with low dose cocaine. This effect of EFS is corticosterone (CORT)-dependent and is mimicked by systemic or intra-prelimbic cortex (PL) CORT administration indicating that CORT is necessary and sufficient for stress-potentiated reinstatement and that the PL is a critical site of CORT action. CORT likely potentiates reinstatement through interactions with the endocannabinoid (eCB) system as stress increases eCB production in the PL in a glucocorticoid-dependent manner. Here we investigated CORT-endocannabinoid interactions in the PL and the resulting effect on cortico-accumbens pathway activation, a pathway critical for reinstatement. Male SD rats self-administered cocaine (14 x 2 hrs/day) and then underwent extinction training followed by reinstatement tests. We have shown that systemic or intra-PL cannabinoid receptor 1 (CB1R) antagonism blocks stress- and CORT-potentiated reinstatement. We have extended these findings to identify 2-AG as a main contributor in the eCB-dependent effect as upregulation of 2-AG with intra-PL administration of a MAGL inhibitor was sufficient to potentiate cocaine-induced reinstatement. Furthermore, inhibition of 2-AG production with intra-PL administration of a DAGL inhibitor prior to reinstatement tests blocks CORT-potentiated reinstatement. Taken together, this suggests that eCB signaling, more specifically 2-AG, in the PL is necessary and sufficient for CORT-potentiated reinstatement. CB1Rs are located on GABAergic interneurons in the PL so CORT effects may be the result of eCB-mediated inhibition of GABA. Indeed, bath application of CORT to PL slices attenuated inhibitory neurotransmission in a CB1R-dependent manner. Activation of the cortico-accumbens pathway following CORT-potentiated reinstatement is currently being investigated utilizing a retrograde tracer and double label immunohistochemical approach and determination of c-fos co-expression. Furthermore, the effects of CORT bath application on the cortico-accumbens pathway is currently being investigated. These findings support the hypothesis that CORT acts in the PL, through eCB-mediated, and more specifically 2-AG, inhibition of GABA, to potentiate reinstatement of cocaine seeking through increased activation of the cortico-accumbens pathway.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758 to JRM

NIDA Grant DA038663 to JRM and CH

Title: Neurocircuitry and cannabinoid receptor 1 involvement in cocaine-taking and cocaine-seeking behavior following chronic electric footshock stress-induced escalation of self-administration in rats

Authors: *C. P. WOLF[1], J. R. MCREYNOLDS[1], D. M. STARCK[1], C. J. HILLARD[2], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Pharmacol. & Toxicology and Neurosci. Res. Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: There is a strong interaction between stress and drug use, including a high co-morbidity between addiction and stress-associated psychological disorders. Rats given short access to cocaine for daily self-administration (SA) show stable drug intake, but if a stressor, electric footshock stress (EFS), is delivered daily at the time of SA, a glucocorticoid-dependent escalation of cocaine intake emerges. We hypothesize that stress-induced escalation of SA results from neuroplastic changes that persist after stress ends and involves neurobiological mediators that connect stress and reward systems in the brain, likely in afferent projections to the nucleus accumbens (NAc) shell, an area critical for the rewarding effects of cocaine. One candidate is the endocannabinoid system (eCB), as eCBs are implicated in cocaine-related behaviors and are regulated by chronic stress. We hypothesize that repeated stress at the time of SA induces a persistent increase in eCB signaling that results in stress-induced escalation of cocaine use and increased susceptibility to reinstatement. Male SD rats were trained to self-administer cocaine in 4 x 30 min SA sessions separated by 5-min drug-free periods, during which some rats received EFS in the SA chamber over a 14-day period. EFS administration resulted in escalation of cocaine intake and this effect persisted in the absence of EFS. To test the involvement of eCBs, the CB1R antagonist AM251 (1 mg/kg) was given prior to the SA session.

AM251 significantly attenuated cocaine intake only in rats with a history of EFS at the time of SA. We are currently examining CB1R binding following stress-induced escalation of cocaine intake and the activation of afferent projections to the NAc shell utilizing a retrograde tracer and an immunohistochemical approach examining co-expression of the tracer and c-fos following stress-induced escalation of cocaine intake. To assess changes in reinstatement, cocaine responding was extinguished and rats were tested for reinstatement by administration of a priming injection of cocaine (2.5, 5, or 10 mg/kg) prior to being placed in the SA box. Rats who received EFS during SA had augmented reinstatement at all doses of cocaine. To test the involvement of eCBs, AM251 (1 mg/kg) was given prior to high-dose cocaine (10 mg/kg) and, as with SA, significantly attenuated cocaine-primed reinstatement only in rats with a history of EFS at the time of SA. These data suggest that stress-induced neuroplastic changes occur, likely in the eCB system, in brain regions that influence expression of escalated cocaine intake and augmented cocaine-primed reinstatement and these changes may be glucocorticoid-dependent.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758

Title: Proestrus-level 17β-estradiol potentiates the reinstatement of cocaine seeking

Authors: *M. C. DEBAKER[1], E. M. DONCHECK[1], J. J. TUSCHER[2], L. A. URBANIK[1], L. M. BARRON[1], L. J. SCHUH[1], G. T. LIDDIARD[1], E. E. HERDEMAN[1], K. M. FRICK[2], J. R. MANTSCH[1];
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Abstract: Although it is generally accepted that peak physiological levels of the ovarian hormone estrogen confer enhanced relapse vulnerability in female cocaine addicts, the underlying mechanisms are not yet well understood. To investigate this, we sought to determine whether physiologically-relevant levels of the primary estrogen 17β-estradiol (E2), like the stress hormone corticosterone, can promote reinstatement of cocaine seeking in response to a "subthreshold" dose of cocaine that is otherwise insufficient for reinstatement. To identify

circulating E2 levels across the cycle, daily vaginal lavage and intravenous catheter blood samples were taken from sexually mature female Sprague Dawley rats (90 days old/280g minimum at study onset for all experiments). Estrous phases were confirmed using the Papanicoloau staining procedure, and corresponding blood samples assayed for E2 levels via ELISA. To identify E2 doses which recapitulate peak E2 levels, surgically ovariectomized (OVX) females received 1hr systemic E2 pretreatments (0, 10, 50, or 100 ug/kg, i.p.) and resultant blood levels were collected. The 10 and 50 ug/kg doses were determined to recapitulate proestrus-range E2 levels, and were subsequently tested for effects on the reinstatement of cocaine-seeking behavior. Female rats were implanted surgically with intravenous catheters and underwent short access (2 hour) cocaine self-administration (0.5mg/kg/0.2mL i.v. infusion) for 14 days prior to extinction training. To avoid effects on self-administration and extinction and isolate effects on reinstatement and related responses, rats did not undergo OVX until after reaching extinction criterion (<15 lever presses/2-hour session for 2 consecutive days). After allowing 7 days to recover and ensuring that responding still met extinction criterion, a counterbalanced design was used to administer the following tests to each rat: E2 (10 or 50 ug/kg, i.p; 1hr pretreatment) + saline, vehicle + saline, E2 + cocaine (0.625, 1.25, or 2.5 mg/kg, i.p.), vehicle + cocaine. We determined that, under these conditions, both 0.625mg/kg and 1.25mg/kg cocaine were subthreshold doses for reinstatement, while 2.5mg/kg cocaine was a supra-threshold dose. Although pretreatment with the proestrus-like 10 or 50 ug/kg E2 had no effect on 0.625mg/kg cocaine, potentiated reinstatement was seen with both E2 doses + 1.25mg/kg cocaine. These results indicate that we have developed a model to study how estrogen may set the stage for relapse in female cocaine addicts.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758

NIH Grant DA038663

Title: Localization and mechanisms underlying 17β-estradiol-potentiated reinstatement of cocaine-seeking behavior in female rats

Authors: *E. M. DONCHECK[1], J. J. TUSCHER[2], L. A. URBANIK[1], M. C. DEBAKER[1], L. M. BARRON[1], K. M. FRICK[2], Q.-S. LIU[3], C. J. HILLARD[3], J. R. MANTSCH[1];
[1]Biomed. Sci., Marquette Univ., Milwaukee, WI; [2]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [3]Pharmacol. & Toxicology, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Although peak physiological levels of the ovarian hormone estrogen correspond to enhanced relapse vulnerability in female cocaine addicts, the underlying mechanisms are not yet well understood. We recently developed a new preclinical self-administration model to study this phenomenon, in which sexually mature ovariectomized female rats given proestrus-levels of the primary estrogen 17β-estradiol (E2; 10 ug/kg, i.p., 1hr pretreatment) exhibit potentiated reinstatement of cocaine-seeking behavior in response to an ordinarily subthreshold dose of cocaine (1.25 mg/kg). Using this model, we find the potentiating effects of E2 to depend on activation of the cannabinoid-type 1 receptor (CB1R), as pretreatment with the CB1R antagonist AM-251 suppresses the E2-potentiation of reinstatement in a dose-dependent manner (1 & 3 mg/kg, i.p; 30min pretreatment). Additionally, we find that E2 can act directly within the prelimbic prefrontal cortex (PrL), a key node within the motivation circuitry, as intra-PrL application of E2 (5 ug/0.3 uL, bilateral; 15min pretreatment) reproduces the potentiation effect. Ongoing experiments aim to determine whether the systemic CB1R dependence in E2-potentiated reinstatement can be localized to the PrL. Concurrently with these behavioral studies, whole-cell voltage clamp recordings were made in slices from intact female PrL layer V pyramidal neurons to determine the effects of E2 on PrL neurotransmission. These recordings revealed that E2 (100 nM, 10min application) enhances the frequency of spontaneous miniature excitatory postsynaptic currents, indicating that E2 can act within the PrL to enhance presynaptic glutamate release. No effects of E2 on inhibitory postsynaptic current frequency or amplitude were observed. Further investigations into the mechanism underlying E2-mediated enhanced glutamatergic neurotransmission within the PrL are currently underway. These results indicate that E2-enhanced reinstatement vulnerability in female rats involves the endocannabinoid system, and that E2 may act through enhancement of excitatory synaptic transmission in the PrL. Investigations are underway to determine the degree to which these mechanisms may be intertwined.

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Poster

350. Cocaine: Brain Circuitry I

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Support: NIDA R01-DA-038599 (SBF)

NIDA Training Grant T32DA007821 (BNK)

University of Michigan Department of Psychiatry (SBF)

Title: Transient inactivation of the paraventricular nucleus of the thalamus differentially affects cue-induced reinstatement in sign-trackers and goal-trackers

Authors: *B. N. KUHN[1,2], M. S. KLUMPNER[3], S. FLAGEL[3];
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Abstract: Relapse remains the biggest problem in the treatment of addiction, with rates as high as 90%. Cues associated with the drug-taking experience can turn into powerful motivators that result in drug-seeking behaviors via Pavlovian-learning processes. However, there is individual variation in the extent to which a cue can attain such motivational value and only when it is attributed with incentive salience does it gain inordinate control over behavior. We use an animal model that allows us to study individual variation in the propensity to attribute incentive salience to reward-paired cues. In this model, sign-trackers (STs) are those rats that attribute incentive salience to a reward-predicting cue, and will approach and manipulate the cue upon its presentation; whereas goal-trackers (GTs) assign only predictive value to the cue and go to the location of reward delivery upon cue presentation. Relative to GTs, STs are also more impulsive, have higher cocaine break-point and are more susceptible to cue-induced reinstatement of drug-seeking behavior. The paraventricular nucleus of the thalamus (PVT) is a brain region previously implicated in sign- and goal-tracking and has recently been shown to mediate drug-seeking behavior in various cocaine relapse models. The current study examined how individual variation in the motivational value of a drug cue was impacted by inactivation of the PVT during a test for cue-induced reinstatement of cocaine-seeking behavior. After being characterized as STs and GTs, rats underwent 2 weeks of cocaine self-administration followed by a 2-week abstinence period. Rats then underwent extinction and subsequently received an infusion of either baclofen/muscimol (B/M; GABA agonists used to inactivate brain regions) or saline into the PVT prior to a test for cue-induced reinstatement. During the test, responses into the "active" port resulted in presentation of the discrete cue that had been associated with drug delivery. In agreement with previous results, saline-treated STs exhibited greater cue-induced reinstatement than saline-treated GTs. However, PVT inactivation decreased drug-seeking behavior in STs, rendering these rats indistinguishable from the saline-treated GTs. Conversely, in GTs, PVT inactivation increased drug-seeking behavior to levels of saline-treated STs. These findings support a role for the PVT in mediating cue-induced drug-seeking behavior, and demonstrate a role for this nucleus in mediating individual variation in the motivational value of a drug cue.

Disclosures: **B.N. Kuhn:** None. **M.S. Klumpner:** None. **S. Flagel:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA015222

5T32 MH14276

Title: Molecular and region specific effects of garcinol on cocaine-associated memory reconsolidation.

Authors: *M. S. MONSEY[1], D. M. GERHARD[1,3], R. S. DUMAN[1,2], J. R. TAYLOR[1,2,3]; [1]Mol. Psychiatry, [2]Interdepartmental Neurosci. Program, Yale Univ. Sch. of Med., New Haven, CT; [3]Psychology, Yale Univ., New Haven, CT

Abstract: Sustained abstinence from cocaine use is often compromised by exposure to environmental stimuli that have been strongly associated with drug taking. Such cues trigger memories of the drugs effects, leading to craving and potential relapse. Our work has demonstrated that manipulating cocaine-cue memories by interfering with the reconsolidation process is a potential therapeutic tool by which to prolong abstinence. We have previously shown that the naturally occurring histone acetyltransferase (HAT) inhibitor, garcinol, can impair the reconsolidation of cocaine-cue memories in a manner that is specific to reactivated memories only, temporally constrained, and long-lasting. Here, we examined molecular and region specific effects of garcinol in impairing cocaine-cue memory reconsolidation. Rats underwent 12 d of cocaine self-administration training where active lever presses resulted in an i.v. infusion of cocaine that was paired with a cue. Next rats underwent lever extinction for 8 d followed by cue reactivation and a reinstatement test 24 hr later. In our first experiment we examined whether systemic garcinol alters levels of histone H3 acetylation (AcH3) in the lateral nucleus of the amygdala (LA): a region involved in appetitive memory reconsolidation. Using immunofluorescence we found that memory retrieval enhanced levels of AcH3 in the LA and that garcinol administration reversed this retrieval-induced increase. Next, to examine whether garcinol's ability to impair reconsolidation is due to actions as a HAT inhibitor, we performed a rescue experiment. Systemic injections of garcinol (or vehicle) were given 30 min after retrieval followed by the histone deacetylase (HDAC) inhibitor, TSA (or vehicle) 45 min after retrieval. Results indicated that the garcinol/vehicle-injected group once again showed a significant reconsolidation impairment compared to the vehicle/vehicle group, while, as predicted the HAT inhibitor/HDAC inhibitor combination of garcinol/TSA, was able to rescue this impairment. Interestingly, the vehicle/TSA group showed an enhancement in cocaine-cue memory reinstatement compared to controls. Finally, we asked whether garcinol's effects were due to

local molecular alterations in the LA. We found that intra-LA garcinol infusion 1 hr after reactivation significantly impaired reconsolidation. Further testing revealed that intra-LA garcinol impaired reconsolidation only following memory retrieval (i.e., no reactivation controls). These data provide evidence for region specific effects of garcinol to impair cocaine-cue memory reconsolidation and for mechanisms of HAT inhibition in garcinol's mnemonic actions.

Disclosures: M.S. Monsey: None. **D.M. Gerhard:** None. **R.S. Duman:** None. **J.R. Taylor:** None.

Poster

350. Cocaine: Brain Circuitry I

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Support: NIH Grant DA034684

NIH Grant T32GM108540

Title: Blocking D1 receptors in the agranular insular cortex reduces cued and cocaine-prime reinstatement in rats

Authors: *C. V. COSME, A. L. GUTMAN, R. T. LALUMIERE;
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Abstract: Recent work from our laboratory indicates that inactivating the dorsal agranular insular cortex (AId) attenuates cued reinstatement but has no effect on cocaine-prime reinstatement in a cocaine self-administration paradigm. Moreover, we found that AId inactivation has no effect on food seeking, indicating a specific role for the AId in cocaine-seeking behavior, as opposed to general reward seeking. Considering that the AId receives a significant dopaminergic innervation from the ventral tegmental area, it is likely that dopaminergic signaling within the AId is involved in the reinstatement of cocaine seeking. Additionally, it has been shown that blocking D1 receptors in the AId reduces the motivating properties of cocaine during self-administration. However, activity at D1 receptors within the AId has not been examined during reinstatement to cocaine seeking. To address this question we investigated how dopamine within the AId influences cocaine seeking by blocking D1 receptors during a variety of reinstatement tests.
Male Sprague-Dawley rats underwent surgery for implantation of bilateral guide cannulae aimed

at the AId and implantation of an intravenous jugular catheter. After undergoing cocaine self-administration for at least 12 days (2 h daily), rats underwent extinction training prior to reinstatement testing. Reinstatement tests consisted of cue-induced, cocaine-prime, and cue-induced + cocaine-prime (cue +cocaine) reinstatement. Rats received either the D1 receptor antagonist SCH 23390 or its vehicle control immediately prior to reinstatement testing. Blocking D1 receptors within the AId reduced cued and cocaine-prime reinstatement. Although previous studies have found that intra-prefrontal cortex D2 receptor blockade has no effect on cocaine seeking, we are currently examining the effects of intra-IL D2 receptor blockade during reinstatement to cocaine seeking to determine whether activity at these receptors is involved in the cocaine-seeking behaviors.
The current findings suggest that dopamine within the AId is driving the previous effects observed with general AId inactivation. Surprisingly, although inactivating the AId does not alter cocaine-prime reinstatement, blocking activity at D1 receptors reduces this type of reinstatement. These findings are particularly interesting given that D1 receptor blockade within other regions of the prefrontal cortex has produced similar effects, in which dopamine manipulation alters cocaine seeking despite findings showing general inactivation of the same structure has no effect.

Disclosures: C.V. Cosme: None. **A.L. Gutman:** None. **R.T. LaLumiere:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA034684

Title: The infralimbic and prelimbic cortices contribute to the inhibitory control of cocaine-seeking behavior during a discriminative stimulus task in rats

Authors: *A. L. GUTMAN[1], V. A. EWALD[2], C. V. COSME[1], W. R. WORTH[1], R. T. LALUMIERE[1];
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Abstract: The infralimbic and prelimbic (IL and PL, respectively) regions of the medial prefrontal cortex regulate the control of drug-seeking behavior. However, their roles in cocaine seeking in a discriminative stimulus (DS)-based self-administration task are unclear. To address this issue, we examined the roles of the IL and PL in a cocaine self-administration task in which,

on a trial-by-trial basis, a DS+ indicated that a lever press would produce a cocaine infusion, whereas a distinct DS- indicated that a lever press would produce nothing. Bilateral cannulae were targeted at the IL and PL, and during separate 2-hour self-administration sessions, these regions were inactivated with the $GABA_{B/A}$ receptor agonists baclofen and muscimol. Inactivation of either the IL or PL decreased performance accuracy and disinhibited behavioral responding during the DS- presentation. This was accompanied by a decrease in cocaine infusions obtained, a finding confirmed in a subsequent experiment using a standard FR1 cocaine self-administration paradigm. We repeated the DS study using a food reward and found that inactivation of each region decreased performance accuracy but had no effect on the total number of food pellets earned. Additional experiments with the cocaine DS task found that IL, but not PL, dopamine receptor blockade with fluphenazine reduced performance accuracy and disinhibited behavioral responding on DS- trials, whereas IL or PL AMPA receptor blockade with CNQX had no effect on performance accuracy. These findings suggest that, in a DS-based self-administration task in which rats must actively decide whether to engage in lever pressing (DS+) or withhold lever pressing (DS-) on a trial-by-trial basis, both the IL and PL contribute to the inhibitory control of cocaine-seeking behavior.

Disclosures: A.L. Gutman: None. **V.A. Ewald:** None. **C.V. Cosme:** None. **W.R. Worth:** None. **R.T. LaLumiere:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA034684 (RTL).

Title: Activation of the infralimbic cortex using a stable step-function opsin attenuates cocaine seeking during reinstatement after extinction training

Authors: *V. A. MULLER EWALD, W. R. WORTH, R. T. LALUMIERE;
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Abstract: Prior work has implicated the infralimbic cortex (IL) in the consolidation of extinction learning as well as active suppression of cocaine-seeking behavior. Although optogenetically stimulating the IL to reduce cocaine seeking would be a beneficial approach for future experiments, optical activation of the IL via channelrhodopsin would also present significant difficulties as the patterns of stimulation (e.g. firing frequency) for optimal excitation are not

known and may simply disrupt, rather than enhance, normal IL activity. Therefore, to potentiate endogenous activity, rather than directly control neuronal firing, we used a Stable Step-function Opsin (SSFO) in order to provide a general enhancement of IL activity during the reinstatement of cocaine seeking. Male Sprague-Dawley rats underwent surgery for intravenous catheter implantation and implantation of bilateral fiber optic probes aimed at the IL. The rats then underwent cocaine self-administration for at least 10 consecutive d, in which active lever presses produced an intravenous infusion of cocaine and light and tone cues. Upon completion of self-administration, rats underwent 21 d of withdrawal, following which animals were returned to the operant chambers to begin testing. On the first day after withdrawal, rats underwent a cue-induced drug seeking test. Stimulation of the IL produced no effects on this cue-driven cocaine seeking. Similarly, IL activation had no effect on cocaine prime-induced seeking. Following these initial tests, rats then underwent a minimum of 8 d of extinction training during which active lever presses had no consequences in order to extinguish their lever pressing behavior. After this extinction, rats underwent cue and cocaine-prime reinstatement in a manner similar to what they had done before the extinction training. In this case, IL activation reduced cocaine seeking for both types of reinstatement. These results suggest the utility of an SSFO-based approach for activating a structure without trying to drive specific patterns of activity. Moreover, they also suggest that the ability of the IL to reduce cocaine seeking depends, at least in part, on having undergone extinction training.

Disclosures: **V.A. Muller Ewald:** None. **W.R. Worth:** None. **R.T. LaLumiere:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: MH-97988

Title: Cocaine self-administration alters endogenous pituitary adenylate cyclase activating peptide (PACAP) levels in the bed nucleus of the stria terminalis (BNST)

Authors: *O. MILES, E. A. THRAILKILL, A. K. LINDEN, V. MAY, M. E. BOUTON, S. E. HAMMACK;
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Abstract: We have previously shown that the activation of pituitary adenylate cyclase activating peptide (PACAP) systems in the bed nucleus of the stria terminalis (BNST) mediates both

consequences of stressor exposure and also mediates stress-induced reinstatement of cocaine-seeking behavior. Hence, intra-BNST PACAP 38 infusions reinstate previously extinguished cocaine seeking, and BNST infusions of the PAC1/VPAC2 antagonist, PACAP6-38, block the reinstatement of cocaine-seeking behavior normally associated with stressor exposure. In order to determine whether cocaine self-administration alters BNST PACAP systems, we assessed BNST PACAP transcript levels at two time points during a cocaine self-administration procedure. All rats self-administered cocaine (3mg/ml; 0.5mg/kg/infusion, i.v.) for 1hr daily for either 1 or 10 days, and were sacrificed 1hr after their final drug-taking session. Brains were quickly sectioned and dorsal BNST was dissected with a brain punch set. Total RNA was isolated, reverse transcribed, and underwent real-time Taqman qPCR amplification. Cycle threshold (Ct) data were normalized to the ribosomal protein (18s) reference gene and fold change relative to surgical control animals was determined. Rats allowed 10d access to cocaine for 1hr daily showed a significant decrease in PACAP expression in the BNST compared to controls. No changes in PACAP expression were found with rats allowed 1d access to the drug, and PAC1 expression was not altered at either timepoint. Overall, these data suggest that endogenous BNST PACAP transcript levels are significantly altered in response to acute cocaine self-administration. Future studies will determine BNST PACAP levels during withdrawal phases and aim to characterize the role of PACAP in stress-induced relapse.

Disclosures: O. Miles: None. **E.A. Thrailkill:** None. **A.K. Linden:** None. **V. May:** None. **M.E. Bouton:** None. **S.E. Hammack:** None.

Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R00 DA031767

Title: Orexin/hypocretin and dynorphin innervation within bed nucleus of stria terminalis: neuroanatomy and behavioral pharmacology in models of mood and addiction

Authors: *S. J. SIMMONS[1,2], L. MO[2], F. H. TRAN[2], T. A. GENTILE[2], J. W. MUSCHAMP[2]; [2]Ctr. for Substance Abuse Res., [1]Temple University, Sch. of Med., Philadelphia, PA

Abstract: Hypothalamic neurons that contain excitatory orexin/hypocretin peptides project to numerous structures implicated in affect, motivation, and reward processing. Interestingly, ~95% of orexinergic somata co-transmit the inhibitory ligand of the kappa opioid receptor (KOR)

dynorphin, and KOR-mediated transmission has been implicated in stress, anxiety, and negative affect. While these peptides may typically function in a coordinated manner (e.g., co-released dynorphin providing negative feedback), pathological conditions such as cocaine addiction may cause aberrant transmission in one or both peptidergic systems. The bed nucleus of stria terminalis (BNST) is a heterogeneous structure composed in part of neurons producing corticotropin-releasing hormone (CRH), which itself has been implicated in mental disorders precipitated by stress and characterized by negative affect. Independent lines of work have revealed extensive fiber innervation and dense receptor expression from both orexin and dynorphin transmitter systems within BNST. To date, however, limited functional evidence has examined roles of orexin-dynorphin transmission within BNST, and anatomical topography has not been comprehensively mapped. The present studies were designed to map projections of hypothalamic afferents within BNST and to examine functional roles in models of mood and addiction. Accordingly, adult male rats underwent unilateral cannulation surgery targeting BNST subregions. All rats received three 100-nL injections of flurophore-conjugated latex microspheres, separated each by 24-hours, for retrograde neuronal tracing. Immunofluorescent analyses were used to identify putative orexinergic afferents projecting to histologically-verified BNST target structures. Behavioral assays were used to characterize effects of direct infusion of peptides orexin-A (hypocretin-1) and dynorphin-A (1-17) on negatively-valenced 22- and positively-valenced 50-kHz ultrasonic vocalizations (USVs). A separate cohort of rats underwent jugular vein catheterization surgery for intravenous drug delivery, and effects of intra-BNST orexin-dynorphin pharmacological manipulations on cocaine-evoked affective changes were investigated by recording and analyzing USVs. Results extend our understanding of hypothalamic topography as it relates to peptidergic innervation of extended amygdala nuclei. Further, results elaborate on functional involvement of orexin-dynorphin transmission within BNST in governing affective changes associated with cocaine use that ultimately promote the escalation to addiction.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA-033370

Title: Serotonin 1B receptors in the Bed Nucleus of the Stria Terminalis contribute to the negative/anxiogenic effects of cocaine

Authors: *A. KLEIN, S. AKHAVAN, M. BRITO, D. FLANAGAN, K. LEE, A. S. PATIL, E. M. PURVIS, A. WEI, L. ZHOU, A. ETTENBERG;
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Abstract: In addition to its initial rewarding effects, cocaine produces a significant "crash" characterized by a state of dysphoria and anxiety. Any full account of a subject's motivation to seek cocaine must therefore consider the relative balance between these positive and negative effects of the drug. While the neurobiology of the reinforcing aspects of cocaine has been well established, less is known about the systems responsible for its negative effects. Cocaine alters many neurotransmitter systems, including serotonin (5-HT), which has been linked to states of anxiety and depression in both human and animal subjects. The Bed Nucleus of the Stria Terminalis (BNST) has similarly been implicated in the modulation of negative affective states and receives a strong serotonergic projection from the dorsal raphé nucleus. The present study aimed to test the hypothesis that serotonergic signaling in the BNST contributes to the anxiogenic effects of cocaine. A runway self-administration paradigm in which animals traverse a straight alley to enter a goal box for an infusion of IV cocaine (1.0mg/kg) on each of 16 single daily trials. The dual positive and negative effects of cocaine were reflected in the animals' development of a unique approach/avoidance conflict about entering the drug-paired environment as it learns the dual effects of the drug. Pretreatment with bilateral intra-BNST infusions of the selective 5-HT_{1B} auto-receptor agonist CP94,253 (0.0µg, 0.25µg, 0.5µg, or 1.0µg/side in 0.5/µl) reduced the frequency of these approach-avoidance "retreat" behaviors while leaving the positive incentive properties of the drug intact (i.e., start latencies were unchanged). This effect of the autoreceptor agonist was then reversed by co-treatment with the selective 5-HT_{1B} antagonist, NAS-181. Together these data suggest a role for 5-HT signaling within the BNST in mediating the aversive effects of cocaine.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA-033370

Title: Effects of dopamine receptor modulation in the lateral habenula on operant responding for IV cocaine

Authors: *K. SHELTON[1], E. M. PURVIS[2], A. GUILLEN[2], T. DO[2], A. ETTENBERG[2];
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Abstract: Human users of cocaine report that the initial euphoric "high" produced by the drug is soon displaced by an aversive "crash" characterized by agitation, anxiety and anhedonia. Our laboratory has previously reported that self-administered cocaine in rats produces behavioral effects consistent with the human report - that is, an initial experience of reward followed by a period of anxiety and anhedonia. While the positive motivating effects of cocaine have long been thought to require an intact mesolimbic dopamine (DA) system, the neural mechanisms that give rise to the negative effects of the drug remain less clearly defined. Recent literature points to the lateral habenula (LHb) as a site for the encoding of aversive or anxiogenic events and has also been shown to "gate" the activity of the DA reward system by inhibiting the activity of DA cells within the VTA. In previous work we have shown that antagonism of LHb-DA receptors attenuated the negative/anxiogenic effects of cocaine in a runway model of drug self-administration. Here we examined the effects of LHb-DA receptor modulation in an operant lever-press model of cocaine self-administration. Male rats were fitted with bilateral cannulae aimed at the LHb and then trained to lever-press for IV cocaine (0.25 mg/kg/infusion/response). Once responding had stabilized, subjects received intra-LHb infusions of the dopamine D1/D2 antagonist, cis-flupenthixol (0, 7.5, 15 µg/side) or the selective D2 agonist, sumanirole (0, 5, 10 µg/side) prior to self-administration. Application of both high and low doses of the antagonist significantly decreased the initial "loading dose" of cocaine that animals self-administer at the outset of each trial, while infusions of the high dose of the D2 agonist sumanirole had the opposite effect. These results are consistent with the view that DA antagonism within the LHb enhances the net reward (less drug is presumably required to reach each subject's "optimal" affective state) while D2 receptor activation required more cocaine. These results add to the growing body of research suggesting a role for the LHb in modulating the affective response to self-administered cocaine. This work was supported by NIDA grant DA-033370 awarded to AE.

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Poster

350. Cocaine: Brain Circuitry I

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Topic: G.08. Drugs of Abuse and Addiction

Title: The involvement of D2 receptor in basolateral amygdala in the companions-exerted decreasing effects on cocaine conditioning

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Abstract: Lately, we report that the presence of three cocaine-free or -treated companions reliably decrease the magnitude of cocaine-induced conditioned place preference (CPP). This study is undertaken to study the anatomical substrates involving in such companions-exerted decreasing effects on cocaine CPP. Ibotenic acid (5 μg/side) infusion was performed in bilateral basolateral amygdala, dorsal hippocampus and dorsolateral stratum to cause irreversible excitotoxic lesions in these brain regions. Moreover, microinfusion of selective dopamine receptor antagonists is used to study the role of dopamine receptor in mediating such effects. We find that basolateral amygdalar, dorsal hippocampal or dorsolateral stratal lesion do not affect the magnitude of cocaine-induced CPP. Interestingly, basolateral amygdalar, not dorsal hippocampal or dorsolateral stratum, lesion is found to abolish the companion-exerted decreasing effects on cocaine CPP. Moreover, microinfusions of a D2 receptor antagonist (Raclopride, 0.5 μg/0.2 μl in each side) abolish the companion-exerted decreasing effect on the CPP. These results, taken together, suggest that the presence of companions during conditionings may diminish the cocaine CPP, at least in part, by activating D2 receptor in basolateral amygdala.

Disclosures: W. Tzeng: None. **L. Yu:** None.

Poster

351. Cocaine: Cell Signaling

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R01 DA33641

Title: AKAP150 in the nucleus accumbens shell promotes cocaine reinstatement by facilitating PKA phosphorylation of GluA1 AMPA receptors

Authors: *L. A. GUERCIO[1], M. E. WIMMER[2], H. D. SCHMIDT[3], C. PIERCE[2];
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Abstract: Cocaine abuse is a major public health concern, with more than 2 million current users in the United States alone. One of the major obstacles in treating cocaine addiction is the discouragingly high rate of relapse after detoxification. Drug craving and relapse to cocaine seeking are modeled in rodents using the reinstatement paradigm. Previous work has shown that cyclic AMP (cAMP) production and Protein Kinase A (PKA) activation in the nucleus accumbens play a critical role in self-administration and reinstatement. A-Kinase Anchoring Proteins (AKAPs) are proteins that bind PKA and localize them to ion channels and receptors, notably AMPA receptors (AMPARs), at the synapse to mediate synaptic plasticity. We have shown that intra-accumbal infusions of St-Ht31, a cell-permeable peptide that disrupts PKA binding to all AKAP isoforms, attenuated the reinstatement of cocaine-seeking, but not sucrose-seeking behavior. Furthermore, cocaine reinstatement is associated with increased expression of a specific AKAP isoform, AKAP150, in the PSD of the nucleus accumbens. Here, we show that PKA binding to AKAP150 selectively in the accumbens shell is required for cocaine reinstatement. Initially, rats were trained to press a lever for cocaine (0.254 mg/59 µL, i.v.) using a fixed-ratio 5 (FR5) schedule of reinforcement. After 21 days of cocaine self-administration, responding was extinguished by substituting saline for cocaine. Following extinction, we infused a herpes simplex virus containing a dominant negative form of AKAP150 with a mutation in the PKA binding domain (AKAP150ΔPKA) directly into the accumbens shell and assessed cocaine priming-induced reinstatement of drug seeking. Our findings show that PKA binding to AKAP150 is required for cocaine, but not sucrose reinstatement. Additionally, we also show that AKAP150 promotes cocaine reinstatement by facilitating PKA phosphorylation of GluA1-containing AMPARs. Finally, we used electrophysiological approaches to determine the role of AKAP150 in mediating AMPA currents and rectification index after cocaine reinstatement. Collectively, these results suggest a role for AKAP150-mediated localization of PKA in the nucleus accumbens shell during the reinstatement of cocaine-seeking behavior in rats.

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Poster

351. Cocaine: Cell Signaling

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Title: Neuronal-enriched rna-binding protein hud and microrna mir-495 oppositely regulate cocaine-induced addiction-related gene expression and place preference behavior

Authors: *R. J. OLIVER, JR[1], R. M. BASTLE[2], J. L. BRIGMAN[1], A. M. ALLAN[1], J. L. NEISEWANDER[2], N. I. PERRONE-BIZZOZERO[1];
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Abstract: Post-transcriptional regulation (PTR) plays an important role in nervous system development and function, but very little is known about its role in substance use disorders. Both RNA-binding proteins (RBPs) and microRNAs provide PTR of gene expression. For instance, HuD is a neuronal-enriched RBP that binds to and stabilizes mRNAs containing AU-rich instability elements (AREs) in the 3'UTR. microRNAs similarly target specific regions in the 3'UTR, but instead lead to translational repression or degradation of the target mRNA. We found that HuD and miR-495 target similar sequences in a set of mRNAs which have previously been implicated in addiction (addiction-related genes; ARGs), including *Camk2a*, *Bdnf*, *Mef2c*, and *Arc*. Additionally, HuD itself is considered to be an ARG that is listed in the Knowledgebase of Addiction-Related Genes database (http://karg.cbi.pku.edu.cn), further suggesting it may play a role in this disorder. Finally, many of these shared targets have been shown to play a role in drug conditioned place preference (CPP). Since HuD and miR-495 have contrasting effects on mRNA stability, regulation of HuD or miR-495 could oppositely alter target ARG mRNAs, leading to changes in associated CPP behavior. To test this, we initially measured expression of miR-495, HuD, and its targets after the acquisition of cocaine CPP (15 mg/kg, i.p) in male C57BL/6 mice. miR-495 was significantly decreased within the nucleus accumbens (NAc) and dorsomedial striatum (Dms), but not the dorsolateral striatum (Dls). Conversely, HuD mRNA and protein were significantly increased in the same regional pattern. Additionally, CaMKIIα and BDNF mRNA and protein were increased in a similar fashion. To isolate the contribution of HuD in CPP, we used mice overexpressing HuD (HuD_{OE}) in the striatum. We found that HuD_{OE} mice exhibited increased cocaine CPP compared to wild-type littermates, suggesting HuD increases

cocaine reward. Additionally, NAc isolated from these animals showed significantly increased Bdnf and CaMKIIα protein. Next, we used lentiviral vectors to test the effect of NAc miR-495 overexpression on cocaine CPP in Sprague-Dawley rats. While both the overexpression and control groups acquired CPP to a similar degree, NAc miR-495 overexpression facilitated extinction of CPP compared to control animals, suggesting miR-495 may regulate motivation for cocaine during abstinence. These effects were associated with decreases in NAc *Bdnf* and *Arc* mRNA expression. Collectively, these findings suggest that miR-495 and HuD may competitively regulate ARG expression and cocaine abuse-related behavior.

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Poster

351. Cocaine: Cell Signaling

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T32 DA07287 (CW & EAW)

F31 DA038922 (CW)

Title: Discovery of selective serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) positive allosteric modulators as potential pharmacotherapy for cocaine use disorder

Authors: *J. ZHOU[1,2], E. A. WOLD[1,2], C. WILD[1,2], C. MCALLISTER[2], Y. DING[1], N. C. ANASTASIO[2], R. G. FOX[2], S. J. STUTZ[2], M. A. WHITE[3], H. CHEN[1], K. A. CUNNINGHAM[1,2];
[1]Dept. of Pharmacol. and Toxicology, [2]Ctr. for Addiction Res., [3]Sealy Ctr. for Structural Biol. & Mol. Biophysics, Univ. of Texas Med. Br., Galveston, TX

Abstract: Allosteric modulation of G protein-coupled receptors (GPCRs) is a unique strategy in drug discovery that may yield novel drug candidates for previously elusive targets. The serotonergic system utilizes 14 functionally distinct serotonin receptors that share topologically

similar orthosteric sites, lending great importance to achieving selective sub-type modulation. Recent evidence suggests that decreased signaling capacity at the serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) underlies regulatory processes implicated in various mental health disorders, including cocaine use disorder. Intriguingly, decreased 5-HT_{2C}R signaling has been shown to play an important role in stimulating relapse-related phenotypes in a preclinical animal model of cocaine addiction, and rescuing this dysfunction may provide clinical utility to promote and maintain abstinence. We hypothesize that rational design of novel 5-HT_{2C}R positive allosteric modulators (PAMs) will serve as an attractive strategy to rescue pathologically decreased 5-HT_{2C}R signaling and suppress relapse-related behavioral phenotypes. To this end, a series of 4-alkylpiperidine-2-carboxamides were designed and synthesized. Our efforts have resulted in five compounds demonstrating a >20% increase to enhance 5-HT-evoked Ca_i^{2+} release in h5-HT_{2C}R-CHO cells, no significant effect on 5-HT-evoked Ca_i^{2+} release in h5-HT_{2A}R-CHO, and no intrinsic activity on either 5-HT_{2C}R or 5-HT_{2A}R in absence of 5-HT, suggesting selective 5-HT_{2C}R positive allosteric modulation. Lead PAM CYD-1-79 displayed favorable *in vivo* PK and was counterscreened to determine no interaction at all additional 5-HT receptor orthosteric sites. Excitingly, behavioral studies in rat, using CYD-1-79, demonstrated suppression of impulsive action in a dose-dependent manner by 60% at the highest dose (2 mg/kg), in a one-choice serial reaction time (1-CSRT) task. Additionally CYD-1-79 significantly suppressed context-induced and cue-reinforced cocaine seeking (1 mg/kg). Taken together, positive allosteric modulation of the 5-HT_{2C}R represents a novel approach towards the discovery of neurotherapeutics for cocaine use disorder and our recently characterized selective PAMs may provide valuable tools to elucidate neuropathological serotonergic function. Finally, further medicinal chemistry optimization and preclinical development will yield promising therapeutic candidates with a potential for a first-in-class neurotherapeutic.

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Poster

351. Cocaine: Cell Signaling

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DA08259 (TAM)

HL096571 (TAM)

DA016735 (MJG)

Title: $Ca_v1.2$ expression in dopamine D1-receptor containing neurons is required for extinction of cocaine-associated behaviors

Authors: *C. E. BURGDORF[1], K. C. SCHIERBERL[1], A. S. LEE[1], S. BROOKSHIRE[2], T. A. VAN KEMPEN[1], V. MUDRAGEL[1], T. A. MILNER[1], M. J. GLASS[1], R. L. HUGANIR[3], A. M. RAJADHYAKSHA[1];
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Abstract: Addiction to cocaine, which affects millions of individuals worldwide, is characterized by a high rate of relapse, typically in response to drug-associated cues or contexts, despite extended drug-free periods. Drug exposure induces activity-dependent synaptic remodeling in forebrain regions receiving dopaminergic inputs, including the hippocampus (HPC), allowing for rapid learning of predictive contexts and cues associated with the reward. However, to date, little progress has been made in understanding the molecular mechanisms underlying extinction of cocaine related memories. Recent studies suggest that extinction of cocaine-related behavioral adaptations are dependent on tightly regulated synaptic changes in the AMPA receptor subunit GluA1. Our studies demonstrate that the L-type calcium channel $Ca_v1.2$ and activation of downstream CaMKII signaling pathways regulate phosphorylation of GluA1 at Ser 831 and aid in extinction of cocaine seeking behaviors. Using the cocaine conditioned place preference (CPP) behavioral paradigm, we find that extinction of cocaine CPP in wildtype mice increases $Ca_v1.2$ protein levels, as well as total and phosphorylated CaMKIIα, and GluA1 phosphorylation at Ser 831, a CaMKIIα site, in postsynaptic densities (PSD) of HPC. Using viral vector-mediated, site-specific knockdown, we demonstrate that $Ca_v1.2$ in the dorsal HPC is essential for cocaine CPP extinction. Using conditional knockout mice, we find that loss of $Ca_v1.2$ specifically in dopamine D1R-containing neurons ($D1^{Cre}$, $Ca_v1.2^{fl/fl}$ mice) attenuates extinction of cocaine CPP. D1Cre, $Ca_v1.2$ KO mice have lower levels of CaMKIIα at the PSD and lower levels of S831 phospho-GluA1, while also showing a decrease in regulators of gene transcription (CaMKIIγ, phosphorylated CREB, and phosphorylated NFATc3) in the nucleus of HPC neurons. Extinction of cocaine CPP was dependent on S831 GluA1 phosphorylation, as S831A phosphomutant mice were unable to extinguish cocaine CPP and had correspondingly lower levels of GluA1 protein in dendritic spines of pyramidal cells in the CA1, as determined by immunoelectron microscopy. Experiments examining the implications of downstream changes on gene transcription, protein expression and synaptic plasticity are currently ongoing. In summary, we have identified a role of $Ca_v1.2$ channels in D1R-containing neurons of the hippocampus in extinction of cocaine CPP via regulation of GluA1 phosphorylation and

trafficking. By further investigating the synaptic plasticity of the HPC pathway underlying cocaine extinction, this work can identify novel drug targets to decrease relapse rates in cocaine addicts.

Disclosures: **C.E. Burgdorf:** None. **K.C. Schierberl:** None. **A.S. Lee:** None. **S. Brookshire:** None. **T.A. Van Kempen:** None. **V. Mudragel:** None. **T.A. Milner:** None. **M.J. Glass:** None. **R.L. Huganir:** None. **A.M. Rajadhyaksha:** None.

Poster

351. Cocaine: Cell Signaling

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Support: R21 DA038048

MH 014276

R25 GM104552

Title: Ventral tegmental area L-type calcium channels mediate cue-induced cocaine seeking and dopamine release during early withdrawal.

Authors: *E. J. NUNES[1], S. M. HUGHLEY[1], K. M. SMALL[1], A. M. RAJADHYAKSHA[2], N. A. ADDY[1];
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Abstract: Exposure to drug associated cues facilitates drug-seeking behavior and promotes relapse in humans. In rodent models of addiction, the presentation of drug paired cues also induces burst firing in ventral tegmental area (VTA) DA neurons, increase phasic DA release in the nucleus accumbens core (NAc) and increases drug-seeking behavior. Nicotinic and muscarinic acetylcholine receptors in the VTA are critical regulators of burst firing of DA neurons, phasic DA release in NAc and cue-induced cocaine seeking. L-type calcium channels (LTCCs) are also expressed on VTA DA neurons and have also been shown to regulate burst firing, but their role in cue-induced cocaine-seeking following self-administration and withdrawal is unknown. First, we trained a cohort of male Sprague Dawley rats on 10 days of intravenous cocaine self-administration (0.5 mg/kg/infusion on a FR1 schedule), where active lever response resulted in intravenous cocaine delivery and the presentation of a compound cue (tone + light), while inactive lever responses had no programmed consequence. Following 10 days of cocaine abstinence, with no exposure to cocaine or the cues, rats were tested for cue-

induced cocaine-seeking. We found that blockade of VTA LTCCs, with isradipine (74 or 223 pg/side), dose-dependently reduced cue-induced cocaine-seeking. In contrast, VTA isradipine administration (223 pg/side) did not alter cue-induced sucrose-seeking. Furthermore, enhancing VTA acetylcholine tone with an acetylcholinesterase inhibitor, physostigmine (2 μg/side), increased cue-induced cocaine-seeking - an effect that was reversed by co-administration of isradipine (223 pg/side). Next, we used fast scan cyclic voltammetry (FSCV) to determine if VTA LTCCs mediate NAc phasic DA release before or after cocaine self-administration and withdrawal. In cocaine naïve rats, VTA infusion of isradipine (223 pg) did not alter VTA evoked phasic DA release in the NAc, compared to vehicle infusion. Surprisingly, in rats that had undergone cocaine self-administration and withdrawal, VTA infusion of isradipine (223 pg) increased phasic DA release in the NAc. Together, these results suggest a plasticity-dependent ability of VTA LTCCs to modulate cue-induced drug seeking and phasic DA release during early cocaine withdrawal. In ongoing experiments, we are testing the hypothesis that these DA effects are specific to cocaine, and not observed during sucrose withdrawal. Future experiments will also examine the role of VTA LTCCs in mediating phasic DA release during cue-induced cocaine and sucrose seeking in awake, behaving rats.

Disclosures: E.J. Nunes: None. **S.M. Hughley:** None. **K.M. Small:** None. **A.M. Rajadhyaksha:** None. **N.A. Addy:** None.

Poster

351. Cocaine: Cell Signaling

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Austrian Sience Fund FWF F44020

Title: $Ca_v1.3$ activation within the VTA regulates addictive and depressive-like behaviors

Authors: *A. MARTINEZ-RIVERA[1,2], J. HAO[1], T. F. TROPEA[1], J. STRIESSNIG[3,4], N. A. ADDY[5,6], A. M. RAJADHYAKSHA[1,2];
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Austria; [4]Ctr. for Mol. Biosci., Univ. of Innsbruck, Innsbruck, Austria; [5]Dept. of Psychiatry and Dept. of Cell. and Mol. Physiol., Yale Sch. of Med., New Haven, CT; [6]Interdepartmental Neurosci. Program, Yale Grad. Sch. of Arts and Sci., New Haven, CT

Abstract: Overlapping neurocircuitry and common genetic risk factors have been suggested to underlie the high co-morbidity in drug abuse and mood disorders. However, the neuropathological mechanisms remain unknown, particularly those resulting from genetic risk variants. Recent findings provide evidence that human mutations in the *CACNA1D* gene, that codes for the $Ca_v1.3$ subunit of L-type Ca^{2+} channels (LTCCs) can confer risk for the development of neuropsychiatric disorders. In particular, *CACNA1D* has been linked to bipolar disorder that is highly co-morbid with substance abuse disorders. The ventral tegmental area (VTA) plays a key role in rewarding processes, however it also modulates aversive outcomes such as depressive behavior. As $Ca_v1.3$ channels are highly abundant in the VTA, we investigated the role of $Ca_v1.3$ within the VTA in the modulation of addictive and depressive-like behaviors. In the present study we used $Ca_v1.2$ dihydropyridine (DHP) insensitive mutant mice that harbor a single mutation in the $Ca_v1.2$ DHP-binding site, thus, allowing us to selectively manipulate $Ca_v1.3$ pharmacologically. Our results revealed that VTA $Ca_v1.3$ activation with BayK 8644 enhances the development of cocaine conditioned place preference (CPP) and potentiates cocaine-induced locomotor activity. VTA $Ca_v1.3$ stimulation also resulted in depressive-like behavior, as revealed by increased immobility in the forced swim test and decreased sucrose preference in the sucrose preference test. Using DREADDs in C57BL/6J mice, we have begun to examine the VTA/$Ca_v1.3$ circuitry involved in cocaine and depressive-like behaviors. We targeted the laterodorsal tegmental nucleus (LDTg), a projection to the VTA that has been shown to mediate reward behavior. In addition, the LDTg comprises one of the major cholinergic inputs to the VTA, and we have recently demonstrated the importance of VTA-cholinergic activity in the regulation of depressive like behaviors. Preliminary results find that inhibiting the LDTg with hM4Di-DREADDs blocks the acquisition of cocaine CPP, however inhibiting LDTg did not have an effect on depressive-like behaviors. Studies are currently ongoing to examine the LDTg-VTA pathway in cocaine and depressive behaviors. Taken together, these findings demonstrate a role for VTA $Ca_v1.3$ channels in driving both, addictive and depressive-like behaviors, and a possible contribution of the LDTg inputs to VTA in driving $Ca_v1.3$-mediated cocaine behaviors.

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Poster

351. Cocaine: Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

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R01 DA033479

P50 DA15369

Title: Prelimbic cortical firing is decreased during cocaine self-administration in rats

Authors: *T. S. DENNIS, T. C. JHOU, J. F. MCGINTY;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous work from our laboratory has identified a down-regulation of activity-related phospho-proteins (p-GluN2A/B, pERK and pCREB) in the prelimbic (PL) cortex during early withdrawal from cocaine self-administration (SA) that is crucial for subsequent relapse. Reversing these changes with a single infusion of brain-derived neurotrophic factor into the PL cortex immediately after the last cocaine SA session suppresses subsequent cue- and cocaine-induced drug-seeking in a TrkB- and ERK-dependent manner, and normalizes cocaine-induced dysregulation of glutamate levels in the nucleus accumbens that are associated with relapse. Given the therapeutic potential of intervening during early withdrawal to prevent relapse, the current study investigated neuronal activity in the PL cortex during SA and early withdrawal through the use of *in vivo* single-unit electrophysiological recordings in awake, behaving rats. Male Sprague Dawley rats were implanted with an intra-jugular catheter and a drivable 16-wire electrode bundle into the PL cortex and allowed to recover for 5 days. Rats were habituated to the electrophysiological rig for 2 days and then trained to self-administer cocaine on an FR1 schedule, with electrophysiological recordings taken on days 1-2 and days 11-14. Within each recording session, neural activity was logged continuously during a 20 min baseline, a 2 hr cocaine SA period, and 2.5 hr after cocaine SA. Our data show that SA of cocaine on the last day suppresses the majority (54%) of recorded neurons in the PL cortex when compared to the 20 min baseline. In contrast, only 13% of neurons were suppressed on the first day of cocaine SA. Interestingly, after the levers are withdrawn and cocaine access is removed, neuronal activity rebounds within 40 min. This effect was not observed to the same extent in rats that received yoked cocaine throughout the entire protocol (30% of neurons were decreased on the first day and 31% of neurons were decreased on the last day). Preliminary data from additional controls suggest that cocaine is both necessary and sufficient for this effect, i.e. no decrease in firing occurs when levers and cues are made available without cocaine, while non-contingent cocaine

in the absence of levers and cues still decreases firing. The alterations in electrophysiological activity observed may act as an indicator of maladaptive plasticity induced by continued cocaine SA. Future studies will focus on pharmacologically reversing the observed electrophysiological signature and dissecting neuronal subtypes and PL cortical projections involved in this phenomenon.

Disclosures: **T.S. Dennis:** None. **T.C. Jhou:** None. **J.F. McGinty:** None.

Poster

351. Cocaine: Cell Signaling

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NIDA T32 DA007288

NIDA P50 DA015369

Title: SRC family kinase inhibition prevents the suppressive effect of BDNF on cocaine-seeking and BDNF induced phosphorylation of ERK, GluN2A, and GluN2B

Authors: *S. M. BARRY, J. F. MCGINTY;
Neurosci. Inst., Med. Univ. of South Carolina, Charleston, SC

Abstract: Relapse to drug seeking remains a major obstacle in the treatment of cocaine addiction in human addicts. Animal models of relapse have demonstrated that neuroadaptations in reward circuits following cocaine self-administration underlie reinstatement to drug seeking. Specifically, dysregulation of the pathway from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) is implicated in reinstatement. Brain-derived neurotrophic factor (BDNF) is synthesized in PFC pyramidal neurons and anterogradely transported to the NAc where it is the primary source of BDNF. Our lab has shown that a single BDNF infusion into the prelimbic cortex following a final cocaine self-administration session results in attenuation of reinstatement to cocaine-seeking. Inhibiting BDNF's receptor, TrkB, ERK/MAP Kinase activation, or NMDA receptors blocks this attenuating effect. These results imply that the interaction between glutamate-mediated synaptic activity and TrkB signaling is imperative to BDNF's suppressive effect on drug-seeking. Src family kinases (SFKs) are involved in both NMDA-mediated activation of TrkB and TrkB-mediated tyrosine phosphorylation of NMDA receptors. Thus, we hypothesized that infusion of the SFK inhibitor, PP2, into the prelimbic cortex prior to a BDNF

infusion immediately after the end of the last cocaine self-administration session will block BDNF's suppression of reinstatement of cocaine-seeking in rats with a cocaine self-administration history. PP2, but not the negative control, PP3, blocked BDNF's suppressive effect on context-induced relapse after one week of abstinence and cue-induced reinstatement after extinction. Because cocaine induces a dephosphorylation of Y1325-GluN2A and Y1472-GluN2B receptors and BDNF reverses this action (Go et al 2016), PP2 is likely blocking this reversal. Accordingly, we saw that PP2 blocked BDNF mediated induction of phospho-GluN2A/B, and phospho-ERK. All analyses were performed on the automated immunoblotting system WES^{TM} (Protein Simple). Additionally, p-Src and p-Fyn levels will be presented to determine if PP2's blocking action occurs from dysregulation of TrkB-mediated SFK activation and subsequent NMDA receptor activation.

Disclosures: S.M. Barry: None. **J.F. McGinty:** None.

Poster

351. Cocaine: Cell Signaling

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F31 DA041021

R01 DA033479

P50 DA015369

Title: Intra-prelimbic inhibition of striatal-enriched tyrosine phosphatase prevents relapse to cocaine-seeking in rats

Authors: *B. M. SIEMSEN[1], S. M. BARRY[1], P. L. LOMBROSO[2], J. F. MCGINTY[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Child Study Ctr., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Relapse following presentation of drug-conditioned contexts and cues is a major clinical obstacle in the treatment of substance use disorder. It has been hypothesized that a major contributor to relapse in human populations is decreased cerebral metabolism in the prefrontal cortex during abstinence (i.e. hypofrontality). In rats trained to self-administer (SA) cocaine, the phosphorylation of extracellular signal-regulated kinase 1/2 (pERK1/2), cAMP response-element

binding protein (pCREB), and Y1472-GluN2B- and Y1325-GluN2A-containing NMDA receptors is diminished in the prelimbic (PrL) cortex two hours after 12-14 short access cocaine SA sessions (Whitfield et al., 2011; Go et al., 2016), signifying cocaine SA-induced neurotransmission deficits in the PrL cortex. Moreover, these dephosphorylation events are associated with increased activity of STriatal-Enriched tyrosine Phosphatase (STEP), and pERK1/2 and pGluN2B are known substrates of STEP (Sun et al., 2013). A single infusion of brain-derived neurotrophic factor (BDNF) immediately following the final SA session suppresses context-induced relapse following abstinence and cue- and cocaine prime-induced reinstatement following extinction (Berglind et al., 2007) by reversing the dephosphorylation of pERK1/2, pCREB, and pGluN2A/B in the PrL cortex during early withdrawal from cocaine SA (Whitfield et al., 2011; Go et al., 2016). Thus, we hypothesized that an intra-PrL microinfusion of the selective STEP inhibitor, TC-2153, immediately after the final SA session would suppress context-induced relapse after abstinence as well as cue and cocaine prime-induced reinstatement following extinction by preventing the cocaine-induced decrease in phospho-proteins in the PrL cortex during early withdrawal. A dose-response study indicated that a single intra-PrL cortical infusion of 1 μM TC-2153 increased pERK1/2 in naïve rats two hours after infusion. An intra-PrL cortex infusion of 1 μM TC-2153 immediately following the final SA session suppressed context-induced relapse after 6 days of abstinence as well as cue-, but not cocaine prime, induced reinstatement following extinction. Additionally, preliminary evidence (n=3/group) indicates that an intra-PrL infusion of TC-2153 immediately after the final SA session may prevent the cocaine-induced decrease in pERK1/2 in the PrL cortex during early withdrawal. A complete analysis of pERK1/2 and additional analyses of pGluN-containing receptors is ongoing. Future experiments will investigate whether TC-2153 microinfusions at the same timepoint have an effect on operant responding to cues associated with a natural reward (i.e. sucrose).

Disclosures: B.M. Siemsen: None. **S.M. Barry:** None. **P.L. Lombroso:** None. **J.F. McGinty:** None.

Poster

351. Cocaine: Cell Signaling

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Support: National Institute on Drug Abuse (NIDA) Grant R00 DA033372

Brain & Behavior Research Foundation NARSAD award

Title: Effect of adolescent isolation on drug responsivity: Alterations in C-fos activation and paired pulse facilitation.

Authors: *A. FOSNOCHT[1], A. U. DEUTSCHMANN[1], A. S. ELLIS[1], L. BRIAND[2]; [2]Psychology & Neurosci., [1]Temple Univ., Philadelphia, PA

Abstract: Adolescence is a critical period for psychological and physiological development. Stress during this period of increased plasticity leaves adolescents vulnerable to engage in risky behavior, including substance abuse. However, the mechanisms underlying this increased vulnerability are largely unknown. Therefore, we utilized two adolescent stress paradigms to examine the relationship between adolescent stress and addictive phenotypes in adulthood. We found that social isolation and chronic unpredictable stress in adolescence increased motivation for cocaine and cocaine-seeking behavior in adulthood. As alterations in synaptic plasticity within the nucleus accumbens are thought to underlie these addictive phenotypes we examined the impact of adolescent stress on this brain region. We found that social isolation during adolescence led to a decrease in paired pulse facilitation within the nucleus accumbens, while not affecting long-term depression. This suggests an increase in excitatory drive onto nucleus accumbens MSNs, however further physiological measurements are being collected to confirm this. Additional studies are underway to determine how adolescent stress affects neural and synaptic activity in response to cocaine. Taken together, these studies provide insight into how stress during adolescence changes the nucleus accumbens and may lead to alterations in addictive vulnerability. *This work was supported by National Institute on Drug Abuse (NIDA) Grant R00 DA033372 (L.A.B.) and a Brain & Behavior Research Foundation NARSAD award (L.A.B.).*

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Poster

351. Cocaine: Cell Signaling

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Support: NIDA Grant R00 DA033372

Brain & Behavior Research Foundation NARSAD award

Title: Cocaine addiction increases vulnerability to stress: role of AMPAR trafficking

Authors: *A. S. ELLIS, A. Q. FOSNOCHT, K. E. LUCERNE, L. A. BRIAND;
Psychology&Neuroscience, Temple Univ., Philadelphia, PA

Abstract: Cocaine addiction is characterized by extremely high rates of relapse and these are, in part, mediated by life stress. Much research has focused on the ability of early life stress to increase vulnerability to addiction, however less is known about the mechanisms by which drug use can alter stress reactivity. It is known that both cocaine taking and stress can alter glutamatergic transmission and AMPA receptor trafficking. Therefore, the current studies examined the role of AMPA receptor trafficking in the response to stress following cocaine exposure. To do this we utilized a transgenic mouse with a point mutation preventing PKC-dependent phosphorylation of GluA2 subunits. As this phosphorylation event leads to activity-dependent internalization of AMPA receptors containing the GluA2 subunit, this mouse model exhibits blunted AMPA receptor trafficking. In the first study, we examined the stress response to a forced swim exposure in mice that had either experimenter administered or self-administered cocaine. We found that disrupting AMPA trafficking led to an enhanced response to forced swim stress in mice with either experimenter or self-administered cocaine experience compared to saline controls. Next, we examined whether this increased stress response would influence the ability of stress to initiate relapse. We found that a sub-threshold stressor was sufficient to initiate reinstatement of cocaine seeking and cocaine conditioned reward in mice with disrupted AMPAR trafficking, but did not initiate relapse in wildtype controls. Additional studies are underway to determine whether disrupting AMPA receptor trafficking alters the proportion of mice that are vulnerable to social defeat. Taken together, these studies suggest a role for glutamate trafficking in the ability of cocaine to mediate future responses to stress.

Disclosures: **A.S. Ellis:** None. **A.Q. Fosnocht:** None. **K.E. Lucerne:** None. **L.A. Briand:** None.

Poster

351. Cocaine: Cell Signaling

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Support: NIDA Grant DA033372

Brain & Behavior Research Foundation NARSAD

Title: Intraaccumbal administration of zeta inhibitory peptide (ZIP) blocks cocaine reinstatement and restores accumbal LTD.

Authors: *L. A. BRIAND, A. U. DEUTSCHMANN, J. D. LENZ;
Psychology, Temple Univ., Philadelphia, PA

Abstract: During abstinence, memories associated with drug-taking persist and the inability to eliminate these drug memories is thought to underlie addiction. Eliminating these drug-paired memories could provide an opportunity for therapeutic intervention. Converging evidence suggest that zeta inhibitory peptide (ZIP) eliminates memories for experience-dependent behaviors, included conditioned drug associations. However, it is not known whether the elimination of these memories alters drug relapse. The current study examined the effect of ZIP administration in the nucleus accumbens on cocaine-primed reinstatement of cocaine seeking, a rodent model of relapse. We demonstrate that intraaccumbal ZIP blocks cocaine-primed reinstatement when administered 24-hours or 1 week prior to testing. Interestingly, ZIP infusion has no effect on the reinstatement of food seeking. ZIP is a synthetic compound designed bind the constitutively active form of atypical PKC, PKMζ, a protein implicated in learning and memory. However, recent evidence from PKMζ knockout mice suggests that ZIP may function through alternative mechanisms. In support of this, we found that ZIP was able to block reinstatement in PKMζ knockout mice. One possible mechanism underlying addictive phenotypes is the ability of cocaine to block further plasticity. We hypothesized that ZIP may be working to reverse this anaplasticity. While ZIP has no effect on accumbal LTD in slices from naïve or yoked saline mice, it is able to restore LTD in animals following cocaine self-administration and withdrawal. Additional experiments are underway to determine whether ZIP is able to reverse other forms of cocaine-induced synaptic plasticity.

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Poster

351. Cocaine: Cell Signaling

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Title: The effects of cocaine self-administration and extinction on NMDA receptor-mediated currents.

Authors: *M. T. SEPULVEDA-ORENGO, K. L. HEALEY, K. J. REISSNER;
Dept. Psychology and Neurosci., Univ. of North Carolina at Chapel Hill Dept. of Psychology, Chapel Hill, NC

Abstract: We have recently reported that extinction from cocaine self-administration in male rats is associated with a decreased in surface area and volume of astrocytes in the nucleus accumbens (NAc) core, as well as a decreased in colocalization of astrocytes with synapses (Scofield et al, 2016). These observations raise the hypothesis that a barrier in volume transmission may impair the ability of astrocytes to modulate synaptic transmission. One salient means by which astrocytes modulate synaptic transmission is by generation of D-serine, an important NMDA receptor co-agonist. It has previously been reported that non-contingent cocaine administration leads to decreased D-serine levels in the NAc suggesting a cocaine-induced impairment in NMDA receptor-mediated plasticity (Curcio, 2013). In order to test the hypothesis that basal NMDA receptor function is impaired following operant cocaine self-administration, we employed whole cell patch-clamp electrophysiology of NAc core medium spiny neurons (MSNs) to measure properties of NMDA receptor-mediated currents following cocaine self-administration and 16-17 days of extinction training. Preliminary results indicate a decrease in stimulus-evoked current amplitude across increasing stimulation intensities, but no effect on the current-voltage relationship or decay kinetics. If this impaired function is mediated by decreased tone of D-serine, then administration of D-serine should restore responsiveness of NMDA receptor-mediated currents as well as NMDAR-mediated plasticity; these studies are ongoing. Previous studies have indicated an increase in NMDA receptor-containing silent synapses in the NAc during early (< 2 weeks) withdrawal from cocaine (for a review, see Huang et al 2015). We hypothesize that the emergence of silent synapses is followed by a later stage of matured yet hypo-functional synapses, characterized by decreased NMDA receptor function but increased synaptic strength.

Disclosures: **M.T. Sepulveda-Orengo:** None. **K.L. Healey:** None. **K.J. Reissner:** None.

Poster

351. Cocaine: Cell Signaling

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Title: Augmentation of D-serine reduces reinstatement to cocaine seeking.

Authors: ***K. L. HEALEY**, B. WU, M. SEPULVEDA-ORENGO, K. J. REISSNER;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Withdrawal from chronic cocaine use is characterized by cellular adaptations and structural remodeling within the brain's reward circuitry, which are believed to drive persistent drug seeking and relapse. We have recently reported that astrocytes in the nucleus accumbens (NAc) core make fewer synaptic connections following cocaine self-administration and extinction (Scofield et al., 2016), suggesting impairment in astroglial volume transmission. D-serine is an important NMDA receptor co-agonist released by neurons and astrocytes, which is derived from L-serine produced in astrocytes. Previous studies have found reduced NAc D-serine following non-contingent cocaine administration (Curcio, 2013). These findings suggest that withdrawal from cocaine is characterized by a deficit in NMDA receptor stimulation by D-serine, which may contribute to impaired synaptic function. In support of this, preliminary data suggest that NMDA receptors are hypoactive following cocaine self-administration and extinction (see Sepulveda-Orengo, SFN abstract 2016). Here, we tested the hypothesis that systemic augmentation of D-serine would attenuate reinstatement to cue- and drug- primed cocaine seeking. Male rats (Sprague Dawley) were trained to self-administer cocaine (2 hr/day) for 12 days, followed by 14 days of extinction and a final test of compound cue- plus drug-primed reinstatement behavior. Animals received three days of systemic administrations (on reinstatement test and two extinction days prior; intraperitoneal, *i.p.*) of either saline, D-serine, sodium benzoate (SB), an inhibitor of the D-serine metabolizing enzyme D-Amino Acid Oxidase (DAAO), or both. D-serine has been shown to facilitate extinction learning in multiple paradigms; however, administration prior to the last two days of extinction should uncouple effects from consolidation of extinction. We found that combined administration of D-serine and SB significantly reduced reinstatement of cocaine seeking compared to vehicle control animals. In contrast, administration of D-serine or SB alone was without effect. Ongoing studies are designed to elucidate the precise cellular mechanism(s) responsible for the effect of D-serine augmentation on reinstatement. We propose this mechanism may include NMDA receptor-triggered endocytosis of AMPA receptors, thereby normalizing synaptic strength in the NAc core.

Disclosures: **K.L. Healey:** None. **B. Wu:** None. **M. Sepulveda-Orengo:** None. **K.J. Reissner:** None.

Poster

351. Cocaine: Cell Signaling

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DFG, Reinhart-Koselleck Award, SP 383/5-1

DFG, SPP1226, SP 383/4-1

Title: The potential role of Ras in cocaine and nicotine self-administration in mice

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Abstract: Acute exposure to drugs of abuse activates a variety of intracellular pathways, and following repeated exposure, persistent changes in these pathways may contribute to drug dependence. The MAPK/ERK signaling cascade has been implicated in the short- and long-term effects of various drugs of abuse, including cocaine and nicotine, by activating downstream signaling and subsequent gene expression. Interestingly, the Ras family of small GTPases that serve as key upstream mediators of ERK phosphorylation, has not been extensively examined in drug-related behaviors. We examined cocaine and nicotine self-administration in two mouse lines in which Ras activity is altered. RasGRF2 KO mice lack one of the guanine nucleotide exchange factors (GEF) that controls the cycling of Ras isoforms between an inactive GDP-bound state and an active GTP-bound state, and synRAS mice have constitutive activity of H-Ras, the predominant Ras isoform in adult neurons. Male RasGRF2 KO and synRas mice and their corresponding wildtype (WT) littermates self-administered cocaine (0.5mg/kg/14µl infusion for 7d) or nicotine (0.03mg/kg/35µl infusion for 10d) under an FR2 schedule of reinforcement. RasGRF2 KO mice demonstrated increased cocaine self-administration relative to WTs, likely due to a decreased sensitivity to the rewarding properties of cocaine, and a consequent increase in drug intake to compensate for this reduced hedonic effect. This finding is likely due to inhibition of the Ras/Raf/MEK/ERK signaling pathway; in fact, we further show that a MEK inhibitor administered prior to daily cocaine SA sessions similarly increased cocaine intake. SynRas mice demonstrated the opposite effect, demonstrating a decrease in cocaine self-administration, likely by increasing the putative rewarding properties of cocaine. These data suggest that RasGRF2 mediates cocaine reinforcement, at least in part, via its activity on H-Ras, and implicates a role for H-Ras in cocaine-mediated behaviors. RasGRF2 KO mice also demonstrated increased nicotine self-administration relative to WTs. However, in contrast, synRas mice and their WT conspecifics demonstrated no difference in nicotine self-administration. These findings suggest that although RasGRF2 also appears to modulate nicotine reinforcement, nicotine reward may not involve the actions of H-Ras, implicating another downstream target or mechanism mediating nicotine reinforcement. For example, RasGRF2 is a dual Ras/Rac GEF, and cocaine and nicotine may differentially target these specific effectors. These findings demonstrate specificity for H-Ras in mediating drug reinforcement related to psychostimulants.

Disclosures: R.E. Bernardi: None. **A. Olevska:** None. **R. Heumann:** None. **E. Santos:** None. **R. Spanagel:** None.

Poster

351. Cocaine: Cell Signaling

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 351.16/GGG3

Topic: G.08. Drugs of Abuse and Addiction

Support: DA000266

Title: Cocaine induced neurotoxicity &It stimulant behavior is mediated by ulk1 dependent autophagy

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Abstract: Cocaine exerts its behavioral stimulant effects by facilitating synaptic actions of neurotransmitters such as dopamine and serotonin. It is also neurotoxic and broadly cytotoxic, leading to overdose deaths. We found that cocaine dose dependently activated autophagy in cortical neuronal cultures. Cocaine treatment also induced autophagy in different areas of the adult mice brain. Electron microscopic examination revealed induction of autophagy in brains of fetal mice whose pregnant mothers were treated with toxic doses of cocaine. Genetic and inhibitor screening indicated that apoptosis, necrosis and necroptosis fail to induce cocaine-mediated neurotoxicity, which is exclusively autophagy dependent. Autophagic actions of cocaine are mediated by the nitric oxide-glyceraldehyde-3-phosphate dehydrogenase-signaling pathway. Thus, depleting GAPDH via shRNA abolished cocaine-associated autophagy and neurotoxicity as did the drug CGP3466B, which prevents GAPDH nitrosylation. Cocaine induced behavioral abnormalities were evident with very low, non-toxic doses. Concentrations of cocaine as low as 1μM elicited autophagy. Genetic depletion of the autophagy-mediating protein ULK1 (ATG1) or inhibition of ULK1 with the specific inhibitor SBI-0206965 impaired cocaine induced autophagy.SBI-0206965 significantly diminished cocaine induced behavioral stimulant effects in mice. Thus, ULK1 dependent autophagy appears to be a major mediator of cocaine's behavioral actions.

Disclosures: P.P. Guha: None. **P. Guha:** None.

Poster

351. Cocaine: Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA00266

Title: Autophagy mediates cocaine-induced behavioral effects in mice

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Abstract: Cocaine abuse is the most prevalent stimulant use disorder in the U.S. Recently, we demonstrated that high concentrations of cocaine induce autophagy in neurons, which mediate its neurotoxic actions. Using biochemical and microscopic analysis, we now show that lower, recreationally relevant concentrations of cocaine rapidly (within 10 min) induce autophagy in primary cortical neurons, suggesting a role for autophagy in the behavioral actions of cocaine. Recreationally relevant concentrations of cocaine also elicit autophagy in mouse embryonic fibroblasts (MEFs). Conversely, molecular derivatives of cocaine that are not psychoactive, such as sodium benzoate and ecgonine, fail to induce autophagy. Cocaine injection (a low dose of 15 mg/kg) in mice also stimulates autophagy in the brain. Pharmacologic inhibition of autophagy blocks cocaine induced behavioral changes in mice. Cocaine fails to induce autophagy in nNOS knockout neurons in which both rapamycin and amino acid starvation-induced autophagy are preserved. On the other hand, in wild type neurons, cocaine, rapamycin and amino acid starvation elicit autophagy. It is widely accepted that cocaine binds the dopamine transporter (DAT) and inhibits its function. This leads to increased DA signaling, which mediates cocaine reward/stimulant effect. We find that DAT is rapidly depleted in the striatum following cocaine treatment of mice. Our data suggest that cocaine-induced DAT degradation is mediated by autophagy. These findings implicate autophagy in the regulation of cocaine-induced behavioral changes and reveal novel therapeutic targets for cocaine addiction.

Disclosures: M.M. Harraz: None. **P. Guha:** None. **P. Cortes:** None. **S.H. Snyder:** None.

Poster

351. Cocaine: Cell Signaling

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Title: Metabolomics changes within the brain mesolimbic dopamine system following cocaine self- administration

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Abstract: The American Psychiatric Association defines addiction as the uncontrollable need to take a drug. People who suffer from addiction prioritize seeking and taking the drug before their careers, family and responsibilities. About 1.5 million people of the 25 million illicit drugs users in the United States abuse cocaine. Today, there is no effective pharmacotherapy for cocaine addiction. In addition, little is known on how cocaine impacts specific metabolic pathways within the brain. Metabolomics approaches can provide new evidence to understand how cocaine regulates metabolic activity within the dopamine reward system. The present study aims to examine changes in metabolic pathways present within mesolimbic structures following cocaine intravenous self-administration in male Sprague Dawley rats. First, animals were implanted with intravenous catheters (n=20). Following recovery from surgery, rats learned to self-administer cocaine in a cue elicited experimental design on a fixed ratio (FR5) of lever responding. After reaching stable behavioral criteria on FR5, animals were euthanized and their brains were removed. Brain tissue samples were dissected from: (a) the nucleus accumbens (NAc), (b) the prefrontal cortex (PFC), (c) the hippocampus and (d) the cerebellum. Finally, samples were prepared for metabolomics analysis. Metabolomics analysis was done using Gas Chromatography/ Mass Spectrometry. Results show several metabolites within different brain regions, for example: 31 metabolites within the PFC, 17 in the hippocampus and 43 in the cerebellum. Bioinformatics analysis was done using Mataboanalyst.ca web based resource including Partial least squares Discriminant Analysis (PLSDA) and Metabolite Enrichment Analysis (MEA). PLSDA analysis revealed that cocaine self-administration elicited significant metabolic changes within different brain regions. The MEA identified that the amino acid metabolism, protein synthesis and glucose alanine cycle were affected within the cerebellum. In contrast, in the PFC significant changes were found in the citric cycle, insulin signaling, fatty

acid, glycolipid and amino acid metabolism. Our data indicates that cocaine exposure affects diverse metabolic pathways necessary for normal cognition, appropriate decision making and normal physiological function.

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Poster

351. Cocaine: Cell Signaling

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA014133

P01DA008227

Title: Dopaminergic dynamics underlying sex-specific reward processing

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Abstract: In cocaine addiction, females are more motivated to take drug, transition to addiction more quickly and relapse at higher rates than males. Here we define a sex-specific neural mechanism underlying enhanced cocaine reward in females. Using a combination of in vivo electrophysiology of ventral tegmental area (VTA) dopamine neurons and fast scan cyclic voltammetry in the nucleus accumbens (NAc), we identified an estrous-cycle dependent enhancement of dopaminergic function. This enhancement converged with an increased ability of cocaine to augment dopamine, which leads females in estrus to form stronger associations between the rewarding effects of cocaine and the cues that predict its availability, a measure of reward. This increased cocaine reward is mediated by a robust estrous cycle-dependent enhancement of cocaine's ability to bind directly to the dopamine transporter. Further, this enhancement of reward circuitry and cocaine effects directly on this system leads to potent and long-lasting associations between the rewarding effects of cocaine and the environmental cues that predict its availability. By integrating conditioned place preference with fiber photometry/in vivo calcium imaging, we defined the temporal dynamics that underlie these persistent associations and concomitant drug seeking. These dopaminergic responses to the environmental cues alone, in the absence of drug, occur even at later stages in the estrous cycle, and act to

enhance motivation, drug seeking and relapse. Together, we define a basic mechanism by which estrous-cycle dependent changes in dopamine transporter function and dopaminergic neurotransmission underlie susceptibility to cocaine addiction by promoting cue-reward associations following cocaine exposure. Supported by NIDA and NIMH

Disclosures: **E.S. Calipari:** None. **B. Juarez:** None. **C. Morel:** None. **D.M. Walker:** None. **E. Riberio:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None. **M. Han:** None. **E.J. Nestler:** None.

Poster

351. Cocaine: Cell Signaling

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Pennsylvania Department of Health

Title: Casein-kinase 2 activity may mediate camkiia-dependent effects on reconsolidation of a cocaine-associated cue memory

Authors: ***J. J. WEEKS**, M. T. RICH, V. NAGARAJAN, M. M. TORREGROSSA;
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Abstract: Drug addiction is a widespread public health issue, the resolution of which depends on treatment strategies that can produce long-term abstinence from drug use. However, the complex milieu of cues that come to be associated with the drug presents a persistent challenge, as these stimuli gain powerful incentive salience and can lead to robust motivation to seek the drug (craving) and relapse. Understanding of the processes by which these maladaptive memories are consolidated, retrieved, and, potentially, manipulated, may present a critical outlet in developing more effective and lasting treatment strategies for drug addiction. Previous research in our lab has implicated Ca2+/calmodulin-dependent protein kinase II (CaMKIIα) in the reconsolidation of a cocaine-associated memory, including phosphorylation on three threonine residues that have not previously been studied in the context of memory regulation. Bioinformatic databases suggest that these threonine residues (T334, 336, & 337) are substrates for casein kinase 2 (CK2). Therefore, the present experiments aimed to determine if CK2 is involved in CaMKIIα-mediated effects on reconsolidation of a cocaine-associated memory. Male, Sprague-Dawley rats were trained to self-administer cocaine paired with an audiovisual cue. After lever extinction,

rats had the cue memory reactivated by brief presentation in a novel context. After reactivation, rats were given vehicle or an inhibitor of CK2 activity 4,5,6,7-Tetrabromobenzotriazole (TBB), into the basolateral amygdala (BLA). A control group was exposed to TBB in the absence of memory reactivation (i.e., no cue presentation). We found that when cue memories were reactivated, treatment with TBB, but not vehicle, produced a significant reduction in reinstatement responding, while TBB did not reduce reinstatement in the no reactivation condition. Further experiments will aim to determine whether or not CK2 inhibition can reduce CaMKIIα activity *in vitro,* as hypothesized, by expressing CaMKIIα in HEK293T cells, treating cultured cells with varying doses of TBB, then assessing resulting CaMKIIα activity. The behavioral results of this study suggest that CK2, through its effects on CaMKIIα function, may play a critical role in drug-related memory processes, and thus serve as a target for future research and, ultimately, therapeutic applications.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Program#/Poster#: 352.01/GGG8

Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA025876

R01DA033459

R01DA038700

T32DA07288

Title: A corticothalamic inhibitory control pathway mediates smoking relapse vulnerability

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Abstract: *Rationale.* Nicotine addiction is associated with inhibitory control (IC) deficits and concomitant alterations in brain function/structure; however, relations between function, structure and smoking cessation remain poorly characterized. We examined relations between

neural indices of IC and smoking lapse/relapse in two independent studies of adult smokers. In Study-1, we examined baseline differences in grey matter volume (GMV) and IC-fMRI BOLD response as predictors of treatment outcomes over a 10-week clinical trial. In Study-2, we examined IC-fMRI BOLD response as predictors of time to smoke during a laboratory analog of relapse within a brief 3-hr. study visit. *Methods.* Study 1: Smokers (*N*=81) were MRI scanned prior to quitting, followed for 10-weeks and classified as Abstinent (*n* = 41) or Relapsed (7 consecutive days ≥ 1 cigarette/day; *n* = 40). Differences in IC-BOLD response, IC task-based functional connectivity (tbFC; α =.05) and GMV differences in IC networks were assessed (FWE_{voxel} <.05). Study 2: Sated smokers (*n*=26) were MRI scanned while performing the same IC task as in Study 1. Following scanning, subjects performed a Smoking Relapse analog Task (SRT) in the lab, where time to first cigarette and smoking topography was recorded. Relations between IC-BOLD response, tbFC and SRT outcomes were examined. *Results.* In study 1, we found that less gray matter volume and greater IC-task related BOLD response in right IFG, along with weaker task-based corticothalamic functional connectivity, were associated with smoking relapse. Similarly, in the separate laboratory study (Study 2), greater IFG BOLD response and decreased corticothalamic FC predicted smoking during the SRT. *Conclusions.* The current studies provide novel findings for the role of corticothalamic circuitry function—involved in stimulus-driven IC over prepotent behavioral responding—in smoking relapse vulnerability. These findings suggest that baseline IC behavior and neural function may provide a biomarker for early detection of smoking lapse/relapse vulnerability.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH 1R15DA034912

Title: Nucleus accumbens BDNF overexpression alters the behavioral response to nicotine

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Abstract: Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor involved in synaptic differentiation, growth, and maintenance. Increases in BDNF have been shown in substance abuse and decreases in BDNF have been shown in response to stress and major depressive disorder (MDD). We analyzed the effects of BDNF upregulation via lentivirus on Pavlovian conditioned approach (PCA), behavioral sensitization, and nicotine self-administration in rats. Lentiviral-mediated expression cassettes, with dual promoters to drive the BDNF gene and the reporter gene were constructed according to the manufacturer's instruction and surgically injected into the nucleus accumbens (Nac), the primary brain area that mediates drug reward and reinforcement. Rats were allowed to recover for three weeks before behavioral testing commenced. All rats were trained to associate the presentation of a lever and illumination of a stimulus light with delivery of 20% sucrose in a Pavlovian conditioned approach (PCA) task. Head entries into the receptacle where sucrose was delivered (goal tracking) and lever pressing (sign tracking) during the conditioned stimulus (CS) were measured to determine if BDNF over-expression (BDNF+) altered approach to the sign or goal location. Rats in the BDNF+ group made more goal directed behaviors during the CS than sham group. There were no differences in sign tracking and no differences in basal activity. This pattern suggests that BDNF over-expression may increase reward-related learning in a manner specific to goal tracking. Three days after completion of the PCA task, all animals were habituated to a locomotor arena followed by nicotine behavioral sensitization, and were administered nicotine (ip, 0.5 mg/kg free base) or saline every second day for seven days. Results revealed that the BDNF+ group demonstrated enhanced sensitivity to the hypoactive response to nicotine. At day 7, BDNF+ animals demonstrated enhanced behavioral sensitization to nicotine as compared to all other groups, and Sham NIC animals demonstrated sensitization compared to Sham SAL controls. Thus, it appears increasing NAc BDNF expression enhances the behavioral response to nicotine. Animals were then surgically implanted with a jugular catheter and commenced nicotine self-administration. Interestingly, BDNF+ rats demonstrated reduced nicotine self-administration and motivation to obtain nicotine. Global changes in BDNF expression could be a mediating variable in endophenotypes that are more or less susceptible to drug-taking and substance dependence.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Title: Does periadolescent nicotine-induced sensitization to cocaine require activation of microglia and expression of ΔFosB in the brain of the rat?

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Abstract: Nicotine use during adolescence is considered a 'gateway' that leads to the brain's sensitization to illicit substances in the future. Psychoactive drugs such as nicotine are known to induce the expression of the transcription factor ΔFosB that may facilitate this sensitization process. Previous studies demonstrated that a once daily, 10-day administration of nicotine that bracketed the onset of puberty in rats (PD 35-44) at an intraperitoneal (i.p.) dose of 0.4 mg/kg induced the expression of ΔFosB in important memory and reward areas of the rat brain. The increased expression persisted into adulthood (PD 80), especially in the nucleus accumbens (NAc) and dentate gyrus of the hippocampus (DG) (K Soderstrom et al. Psychopharm 191:891, 2007). Also, periadolescent nicotine exposure (PD 35-44) sensitized the rat to cocaine at adulthood as determined by a conditioned place preference (CPP) paradigm (B A McMillen et al. Eur J Pharm 509:161, 2005). Our goal here is to determine whether whether there is a link between ΔFosB induction and the activation of microglia. In this study, minocycline, a lipophilic tetracycline antibiotic was used to suppress the activation of microglia (Iba-1 immunohistology) *in vivo* by injection into periadolescent male Sprague Dawley rats prior to each nicotine administration. Immunohistology for ΔFosB was used to determine the effect of nicotine. As expected, 0.4 mg/kg i.p. of nicotine-bitartrate (dosed as free base) during PD 35-44 increased the density of ΔFosB in the DG from the vehicle control by 52.4% ($n=7-8$, $p<0.05$). A dose of 30 mg/kg i.p. minocycline 30 min. prior to each dose of nicotine increased the density of ΔFosB labeled nuclei in the DG by 18% over vehicle ($n=6-8$, $p>0.05$). In the NAc, the number of activated microglia in the nicotine-only group increased by 53% ($n=5$, $p<0.05$) when compared to the vehicle control, and by 82% ($n=5$, $p>0.05$) when compared to the minocycline-prior-to nicotine group. In the medial prefrontal cortex (mPFC), the number of activated microglia in the nicotine-only group increased from the vehicle control by 88% ($n=4-5$, $p<0.05$), and from the minocycline+nicotine group by 45% ($n=4-5$, $p>0.05$). The effect of minocycline-pretreatment to nicotine during PD 35-44 on behavioral sensitization to cocaine at PD 80 was determined with CPP. The difference in time in the cocaine-paired chamber between pre- and post-conditioning for the nicotine-only group was increased by 80% over vehicle. Minocycline injections prior to nicotine did not attenuate the time the rats spent in the cocaine-paired chamber. These data suggest that induction of ΔFosB may not be responsible for the nicotine-induced sensitization to cocaine.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA036691

AA013588

AA017072

Title: Dysregulation of ACh-GABA-CRF neurotransmission in the CeA contributes to nicotine self-administration in dependent rats

Authors: *M. KALLUPI[1], G. DE GUGLIELMO[2], P. SCHWEITZER[2], R. O. MESSING[3], O. GEORGE[2];
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Abstract: The mechanisms underlying the escalation of nicotine intake are largely unknown. Converging evidence suggest that recruitment of the corticotropin releasing factor (CRF) system is a key factor in the development of disorders associated with increased stress level, including addiction. Here, we studied the cellular mechanisms responsible for the recruitment of CRF neurons in the central amygdala (CeA) in nicotine dependent rats and the mechanisms mediating CRF effects. Using an electrophysiological approach in CeA slices, we recorded pharmacologically isolated spontaneous inhibitory postsynaptic currents (sIPSCs) in neurons from Wistar rats trained to self-administer nicotine in short (ShA 1h/day) or long (LgA, 21h/day) access. Exposure of CeA neurons to 1 μM nicotine increased sIPSC frequency in both groups. This effect was persistent in ShA neurons but appeared to desensitize in LgA neurons. The amplitude of sIPSCs was not affected by nicotine, pointing at a presynaptic effect of nicotine on GABA release. AAV-DIO-eYFP virus was injected in the CeA of CRF-cre rats in order to visualize CRF+ neurons. Animals were then implanted with nicotine minipumps (9 mg/kg/day) for 7 consecutive days to produce nicotine dependence and whole-cell recordings were performed 16 h into withdrawal. In naïve rats, nicotine increased sIPSC frequency in CRF- but not CRF+ neurons. In nicotine dependent and withdrawn rats, nicotine increased sIPSC frequency in CRF+ but not CRF- neurons. No difference in sIPSC amplitude and kinetic across all conditions was shown. A separate group of AAV-DIO-EYFP rats self-administrated ShA nicotine. CRF neurons were inhibited using a 532 nm laser for alternating periods of 10 min

ON/OFF. The results showed a decrease in nicotine intake during ON periods compared with OFF periods**.** To test if the nAChRs containing α7 and α6 subunits control nicotine S-A in the CeA, we infused the α7 antagonist (MLA) or the α6 antagonist (α-Con) in the CeA of non-dependent rats self-administering ShA nicotine. Both MLA and α-Con decreased nicotine intake, suggesting that activation of α7 and α6 nAChR subunits may contribute to nicotine self-administration in ShA rats. These results demonstrate that nicotine dependence is associated with decreased nicotine-induced GABA transmission onto CRF- neurons and increased GABA transmission onto CRF+ neurons in the CeA. Moreover, blockade of CRF1 receptors, *α7 and α6* nAChRs and optogenetic inhibition of CRF+ neurons decreased nicotine S-A. These results suggest that increased GABA transmission onto CRF+ neurons in CeA, possibly through activation of *α7 and α6* nAChRs may be a key mechanism responsible for nicotine S-A in rats.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Pearson Center for Alcoholism and Addiction Research

Title: Kappa opioid modulation of GABA transmission in the central amygdala is reversed upon chronic nicotine exposure

Authors: *P. SCHWEITZER[1], M. KALLUPI[1], G. F. KOOB[2], O. GEORGE[1];
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Abstract: Identification of the cellular mechanisms that control the negative affective aspects of nicotine abstinence is pivotal in the development of novel medications. No study to date has investigated the effects of nicotine self-administration and abstinence on kappa opioid receptor (KOR) regulation of neural activity. Here we sought to determine changes in the modulation of GABA transmission by the KOR system in the central nucleus of the amygdala (CeA) following chronic exposure to nicotine. Male Wistar rats (400-600g) were trained to self-administered

nicotine (21h/day) to establish dependence. CeA slices were prepared from three groups of animals: 1) naïve; 2) following 48 h of withdrawal from nicotine self-administration (WD); 3) immediately after completion of nicotine self-administration with continuous exposure of the slices to nicotine throughout preparation and recording ("nicotine on board", NOB). We then performed intracellular recordings in the whole-cell configuration in the medial subdivision of the CeA and studied pharmacologically isolated spontaneous inhibitory postsynaptic currents (sIPSCs). Basal GABAergic transmission was unchanged in WD or NOB neurons compared to naïve neurons. Superfusion of the selective KOR ligand U-69593 (500 nM) decreased sIPSC frequency (but not amplitude) in naïve CeA neurons to 73% of control. This effect was inverted in WD neurons where U-69593 increased sIPSC frequency to 115% of control. Such chronic-nicotine elicited KOR dysregulation, however, was absent in NOB neurons continuously bathed in nicotine where U69 decreased sIPSP frequency to 63% of control. The KOR antagonist norbinaltorphimine (norBNI; 200 nM) increased sIPSP frequency to 138% of control in naïve animals, indicative of tonic endogenous dynorphin activity at KOR. But norBNI decreased sIPSC frequency in withdrawn neurons to 78% of control, an inverted effect consistent with that observed with U69. Such inversion of the norBNI action was not seen in NOB neurons where norBNI increased GABA transmission to 132% of control. Our results show that chronic nicotine exposure reverses the cellular KOR response, an effect rescued by acute nicotine. Targeting the KOR system may represent a novel approach to reduce the negative emotional states of nicotine abstinence and reduce stress-induced relapse.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Title: Neurochemical profile of CNS neurons activated by menthol in GAD67-GFP knock in mice

Authors: *O. DEHKORDI[1], J. E. ROSE[4], A. JAYAM-TROUTH[1], R. M. MILLIS[5], K. F. MANAYE[2], M. I. DÁVILA-GARCÍA[3];
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Abstract: Menthol cigarette use is prevalent among African-American smokers and may contribute to health-related disparities in this population. However, the data regarding the pharmacological impact of menthol on nicotine addiction is limited. Menthol, a widely used cooling-anesthetic and flavoring agent regulates sensory transduction by activating TRPM8 channels located specifically in sensory neurons. In addition to its peripheral sensory impact, recent studies have demonstrated the presence of menthol in the CNS after in vivo exposure. This implies that menthol may impact nicotine addiction through central mechanisms. Menthol has been shown to upregulate nicotinic acetylcholine receptors (nAChRs), to stimulate $GABA_A$ receptors and to enhance GABAergic transmission in the spinal cord and hippocampal neurons. However, the neuroanatomical and neurochemical profile of CNS neurons targeted by peripheral and/or direct central effects of menthol is not known. Thus, in the present study using GAD67-GFP knock-in mice, we hypothesized that GABAergic neurons of the reward-addiction circuitry are one of the targets of menthol in the CNS. We tested this hypothesis by utilizing c-Fos immunohistochemical technique to identify the neuroanatomical location of menthol-induced c-Fos activated GAD67-GFP positive cells in the addiction circuitry. Menthol (200 mg/kg, I.P), produced c-Fos activation at multiple sites in the CNS including several structures previously shown to be activated by nicotine such as the periaqueductal gray, dorsal raphe, ventral tegmental area, hypothalamus, paraventricular thalamic nucleus, lateral habenular nucleus, hippocampus, piriform cortex, anterior olfactory nucleus and prefrontal cortex. However, with the exception of a sparse number of cells in the posterior hypothalamus, somatodendritic components of GAD67-GFP positive cells of the reward-addiction circuitry were not activated by menthol in the CNS. Finally, the overlap of CNS structures activated by nicotine and menthol implies that nAChRs may be one of the targets of menthol in the CNS.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Support: NIDA grant DA027840

Title: The dopamine D1 antagonist SCH23390 and the serotonin 5HT2C agonist lorcaserin potentiate chronic nicotine infusion induced reduction of nicotine self-administration in rats

Authors: D. DIPALMA[1], B. WILLETTE[1], C. WELLS[2], S. SLADE[2], B. J. HALL[2], A. H. REZVANI[2], *E. D. LEVIN[2];
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Abstract: A variety of neural systems are involved in tobacco addiction. In addition to nicotinic acetylcholine receptors, dopamine D_1 and serotonin $5HT_{2c}$ receptors among others, have been found to play key roles in nicotine self-administration. In prior work, we have found that D_1 antagonist treatment with SCH23390 and serotonin $5HT_{2c}$ agonist treatment with lorcaserin significantly reduced nicotine self-administration in rats. Since nicotine replacement for smokers trying to quit is only modestly effective, we hypothesized that chronic nicotine in combination with either D_1 antagonist treatment or $5HT_{2c}$ agonist treatment may provide improved efficacy. We tested these combinations in a rat model of nicotine self-administration (SA). Adult female Sprague-Dawley rats were trained for operant responding using food reinforcement and then fitted with jugular catheters and given access to nicotine infusions. After five sessions of nicotine self-administration osmotic pumps (Alzet 2ML4) delivering 0 or 2.5 mg/kg/day of nicotine for four weeks were implanted to reproduce chronic nicotine replacement therapy. After one week of nicotine delivery by the minipump, repeated s.c. injections of SCH23390 (0 or 0.02 mg/kg) or lorcaserin (0 or 0.6 mg/kg) were done prior to each nicotine SA session. After another week of nicotine SA, the rats were give a week of enforced abstinence during which they continued to receive drug treatment but did not have access to nicotine to self-administer. Then, the rats received one final week of treatment and resumed access to nicotine for self-administration. Finally, the rats continued nicotine SA for a week after the end of drug therapy. Both SCH23390 and nicotine replacement therapy were effective at reducing nicotine self-administration both before and after the enforced abstinence. The combination of nicotine and SCH23390 caused a greater reduction in nicotine SA, an effect which persisted even after the termination of the drug therapy. Lorcaserin also augmented the effect of chronic nicotine in reducing nicotine SA and resulted in a persistent reduction in nicotine SA. These studies demonstrate the potential utility of combination therapies targeting non-nicotinic receptors as treatment options for tobacco addiction.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: Virgina Tobacco Settlement Foundation

Title: Nicotine selectively remodels dendrites in the ventral and dorsolateral striatum

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Abstract: Nicotine is one of the most addictive substances known, targeting multiple memory systems, including the striatum. The striatum is functionally and anatomically heterogeneous. While the dorsal striatum interfaces corticolimbic systems required for decision-making the ventral striatum has been most classically associated with reinforcement learning. No study has measured and simultaneously compared, using a statistical model, the dendritic morphology of neurons located in dorsal and ventral striatum after chronic adolescent nicotine exposure. Further, the role of D1 receptors in nicotine-induced structural plasticity has been understudied, especially in the dorsal striatum. To address this gap, rats were injected (s.c.) on an intermittent schedule (every other day) with either a D1DR antagonist (SCH23390) or vehicle 20 minutes prior to either nicotine (0.5 mg/kg) or vehicle over 14 days (8 total injections) during an adolescent timeframe (PN 28-42). Following 21 drug-free days, brains were processed (Golgi stain) to visualize dendritic structure. Dendrites from medium spiny neurons (MSNs) located in the dorsomedial striatum (DMS), dorsolateral striatum (DLS) and nucleus accumbens shell region of the ventral striatum (VS) were digitally reconstructed in 3D and analyzed. Results revealed both an expansion and increased complexity of dendrites located in DLS and VS, but not DMS, in the nicotine compared to vehicle control groups. D1 receptor antagonism (SCH23390) abolished nicotine-induced dendritic elaboration in the VS and attenuated nicotine-induced dendritic complexity in the DLS. These findings support previous work suggesting a necessary role for activity at D1DRs in nicotine-induced dendrite remodeling in the VS and other data showing an anatomical gradient for dopaminergic innervation along the dorsoventral axis of the striatum. One model of addiction in the brain suggests a shift in neuronal processing, from DMS to DLS, underlies the progression from recreational to habitual drug use. The current experiments demonstrate a selective and long-lasting form of nicotine-induced dendrite remodeling in the DLS. Considering the role of the DLS in shaping action selection (habits),

these data support a model in which persisting neuroadaptions in the DLS are a general feature of a nicotine addiction phenotype.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Support: R01DA033396 (M.R.B)

T32DA007261-22 (S.K.N.)

DECODE(INSCOPIX)

Title: imaging CA1-hippocampal ensembles during nicotine-contextual associations

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Abstract: Nicotine, the main addictive component of tobacco products, causes compulsive drug-seeking and drug-taking behavior in users. Learned associations between environmental cues and the rewarding properties of nicotine are a major cause for relapse among abstinent nicotine users. The conditioned place preference (CPP) paradigm attempts to model this aspect of drug reward-associations and can be useful in examining the underlying neural circuitry involved in the formation of drug-associated memories. However, how the hippocampus and specifically excitatory neurons of the CA1 region contribute to the development of nicotine-associations is unknown. Here we combined *in vivo* calcium imaging with nicotine conditioned place preference behavior, to determine the role of CA1 neuronal ensembles in the acquisition and expression of nicotine contextual associations. By tracking neurons over the course of a five-day nicotine-conditioning procedure, we show that during nicotine place preference expression, CA1 neuronal activity increases upon entry into the nicotine-paired chamber. We also determined the necessity of the CA1 neurons for the acquisition and expression of nicotine place preference behavior

using chemogenetic (DREADD-based) approaches. We show that CA1 CaMKIIa neuronal ensembles are involved in encoding reward-context associations, but do not play a role in encoding discrete cues associated with reward as assessed in an operant Lickometer task. Taken together, our data provide unique evidence for a key role of the CA1 HIP region in nicotine-contextual associations and begin to dissect the circuitry mediating the development of drug-reward cue associations. Supported by R01DA033396 (M.R.B), and T32DA007261-22 (S.K.N.) from NIDA and DECODE (Inscopix).

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Support: NIH Grant R01DA033459, PI: Froeliger

NIH Grant T32DA7288-23 S1, PI: McGinty

Title: Neural mechanisms of emotion regulation in cigarette smokers

Authors: *S. BELL[1], C. EICHBERG[1], P. A. MCCONNELL[1], K. GRAY[2], F. J. MCCLERNON[5], B. FROELIGER[3,1,4,2];
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Abstract: Introduction: Smokers report smoking in the face of negative affect (NA) or stress-inducing situations and often report that smoking reduces NA. Furthermore, smokers report that two primary factors precipitating relapse during a quit attempt are increased NA and difficulties with cognitive control (e.g. attention, memory, filtering distraction). Proactive cognitive control over NA is pivotal to executing goal-directed processes in the face of negative emotional stimuli. Smokers also exhibit dysregulated reward processing and positive affect (PA), especially following a quit attempt. The neural mechanisms underlying nicotine addiction and associated deficits in proactive emotion regulation (ER) remain to be elucidated. The purpose of this study, therefore, is to utilize fMRI to examine the effects of smoking abstinence, as compared to satiety, on ER-BOLD response during positive and negative ER and relations between ER-BOLD and

smoking behavior.
Methods: Smokers (N= 26) were fMRI scanned while performing a well-validated ER Task on two occasions: once while smoking satiated, once while abstinent from smoking for 24 hours. During the ER Task, participants were presented with positive and negative emotional images and instructed to either reappraise or passively view them. Smokers performed a Smoking Relapse Analog Task (SRT) following scanning in which a monetary incentive was given incrementally for delaying smoking. Regression analyses were conducted between latency to smoke during the SRT and BOLD response during ER (reappraise minus view) within a prefrontal/subcortical ROI-mask based on a priori hypotheses (pFWE <.05: $p<0.005$, Monte Carlo cluster corrected K).
Results: While sated, greater negative ER-BOLD response in left dorsolateral prefrontal cortex (dlPFC; peak = -40 16 33) predicted smoking sooner on the SRT. A similar inverse relationship between ER-BOLD response in left lateral PFC (peak = -51 18 10) BOLD and latency to smoke during the SRT was observed, but during positive ER.
Conclusions: These results suggest that individual differences in ER in smokers may predict smoking behavior. Greater lateral PFC BOLD response leads to diminished capacity to resist smoking; however, current study findings suggest this relationship differs as a function of emotional valence and smoking state. Results will be discussed in the context of potential mediating variables (e.g. baseline affect, craving, dependence severity).

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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DFG Grant SFB 940/1

Title: Hippocampus goes depression: structural and functional correlates of negative mood states after smoking cessation

Authors: *M. N. SMOLKA, F. BÖHME, C. BURRASCH, N. B. KROEMER;
Technische Univ. Dresden, Dresden, Germany

Abstract: Objective: Affective symptoms often occur within the first days after smoking cessation and facilitate the risk to relapse. However, neural underpinnings of these cessation-related changes in mood are not well understood. **Methods**: We investigated nicotine-dependent smokers and non-dependent controls, who completed two magnetic resonance imaging (MRI) sessions within approximately one month. Abstinent smokers took part before and after successful cessation. Hierarchical linear modeling was used to investigate the effects of quitting on subcortical volumes. Additionally, we investigated cessation-related negative mood changes via self-reported depressive symptoms and a behavioral measure of motivational anhedonia. Moreover, we used functional MRI (fMRI) to assess whether quitting leads to increased activation in limbic regions as indication of heightened emotional reactivity. **Results:** We found greater post-cessational volume losses in the right hippocampus of smokers compared to controls. In addition, volume loss in the left hippocampus occurred in more dependent smokers. Further volume losses were observed in the left amygdala and right caudate in smokers after quitting. Critically, hippocampal volume loss was associated with motivational anhedonia. Analyses of fMRI data revealed significantly increased hippocampal response to unpleasant, but not to pleasant stimuli in smokers after cessation, which predicted relapse within the first 6 months after smoking cessation. **Conclusions:** Our results suggest that structural and functional changes in the hippocampal formation are associated with the occurrence of depressed mood after quitting. Changes in emotional reactivity might be caused by cessation-related elevated cholinergic levels and result in increased susceptibility to unpleasant events thereby increasing the risk for relapse.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: Ministry of Food and Drug Safety (14182MFD977), Korea

Title: Regulation of metabotropic glutamate receptor 5 phosphorylation by c-jun n-terminal kinase

Authors: ***S. SEO**[1], I. RYU[1], J. KIM[1], J. KIM[1], J. YANG[1], J. OH[2], E. CHOE[1];
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Abstract: Glutamate is crucial for regulating psychomotor behaviors in response to drug exposure. Activation of the Ca^{2+}-dependent protein kinases linked to glutamate receptors phosphorylates the receptor themselves, contributing to drug-induced reward. This study determined metabotropic glutamate receptor 5 (mGluR5) phosphorylation by c-Jun N-terminal kinase (JNK) both in vitro and in the dorsal striatum, and its role in IP_3 signaling linked to mGluR5 after repeated nicotine administration. The results demonstrated that JNK phosphorylates threonine residue at the position of 1055 in carboxyl terminus (CT) of mGluR5 in vitro. JNK binding to the amino acids sequence, $_{1060}$SYLIPKEIQLPT$_{1071}$, of mGluR5-CT is required for the phosphorylation in vitro. The interaction takes place in neurons of the dorsal striatum after repeated nicotine administration and is regulated by protein phosphorylation. Interfering of JNK binding to the sequence in mGluR5-CT results in a decrease in IP_3 production which is elevated by repeated nicotine administration. These findings suggest that mGluR5 phosphorylation by JNK plays an important role in nicotine-induced reward.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA

NIH Grant T32 MH018399

Title: Double jeopardy: Obese smokers show hypoactivation in inhibitory control brain regions compared to normal weight counterparts during smoking cue exposure

Authors: ***A. V. ELY**[1], K. JAGANNATHAN[2], N. HAGER[2], H. PATER[2], T. R. FRANKLIN[2];
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Abstract: A growing literature suggests that neural mechanisms of substance abuse and obesity may overlap. For example, smokers, versus nonsmokers, show reduced activation in reward-related brain regions in response to food stimuli. However, no study to date has examined the impact of high body weight on response to smoking stimuli. This study used arterial spin-labeled (ASL) perfusion fMRI, and highly evocative smoking stimuli, to examine neural activity to cigarette cues in an original experimental design that strongly minimizes contributions from pharmacological withdrawal. Thirteen normal-weight (NW) and 17 obese (OB) smokers, who were contemplating quitting completed smoking and nonsmoking cue fMRI scans. Data were analyzed in SPM8 within a MATLAB environment to compare group responses to smoking versus nonsmoking cues, controlling for age and sex. It was hypothesized that OB may be differentially sensitive to cigarette cues as compared to NW. Groups did not significantly differ on education, IQ, or smoking history. Whole-brain blood flow analyses showed that NW demonstrated significantly greater activation than OB bilaterally in the dorsolateral prefrontal cortex (Right $T=3.57$, $p<0.001$; Left $T=3.15$, $p=0.002$). NWs may thus be better able to recruit inhibitory control regions when evaluating cues while planning to abstain from smoking in the future. These findings are consistent with research pointing to overlapping neurobiological responding to drug and food reward, and suggest a mechanism whereby individuals prone to chronic overeating leading to obesity may have difficulty resisting rewards beyond food. Future research may contribute to a greater understanding of the interaction of weight and smoking and to the development of treatment strategies specific to those vulnerable to multiple cardiovascular risk factors.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Title: Substance P neurotransmission in the interpeduncular nucleus contributes to nicotine sensitization

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Abstract: The medial habenula (MHb) and interpeduncular nucleus (IPN) communicate directly via the fasciculus retroflexus (FR) primarily via acetylcholine (ACh), glutamate, and substance P

(SP) neurotransmission. The MHb-IPN pathway has been shown to modulate both the rewarding and aversive effects of nicotine, presumably by interacting with the mesolimbic reward pathway. While the role of ACh and glutamate in the MHb-IPN pathway has been extensively studied, there has been little investigation as to the role of SP signaling in any component of nicotine addiction.
Our preliminary data suggests SP transmission in the IPN, but not the MHb, is important for mediating both behavioral and neurochemical sensitization to nicotine. Administration of the selective neurokinin-1 (NK1) receptor antagonist, Ezlopitant (EZL) directly to the IPN significantly reduces sensitized locomotor responding and the sensitized release of accumbal dopamine in response to nicotine. When administered to the MHb, EZL has no effect on sensitized responses to nicotine. While the MHb projects exclusively to the IPN, severing the FR results in a near-complete loss of ACh and glutamate but only a partial loss of SP in the IPN (Artymyshyn & Murray, 1985) suggesting that the MHb is not the sole source of SP input to the IPN.
The goal of the present study was to identify the neurocircuitry underlying SP transmission in the IPN to elucidate potential mechanisms by which NK1 receptor blockade could be mediating sensitized responding to nicotine. Using retrograde tracing combined with immunohistochemistry, we will identify brain regions sending SP containing afferents to the IPN. Two candidates, the laterodorsal tegmental nucleus and triangular septum, project to the IPN, contain both ACh and SP cell bodies, and have been implicated in nicotine reward. With this data, we can better understand the role that SP in the MHb-IPN pathway is playing in mediating nicotine sensitized responses.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: ACADIA Pharmaceuticals Grant

UHCL Faculty Research Support Fund Grant

Title: Effects of nicotine withdrawal and Volinanserin on sleep quality in the rat

Authors: J. C. SHAHIN[1], J. J. BAUTISTA[1], J. J. IZYGON[1], D. M. NGHIEM[1], M. M. HENCEROTH[1], E. S. BURSTEIN[2], *C. P. WARD[1], D. H. MALIN[1];
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Abstract: Sleep disturbances have been reported in smoking cessation. The serotonin system and the 5HT2A receptor in particular appear to play a major role in nicotine dependence. Inactivating 5HT2ARs may increase slow-wave sleep and delta rhythm power. Therefore, we studied the effects of nicotine withdrawal on sleep quality in the rat, and how this was affected by the 5HT2A antagonist Volinanserin (MDL 100907). Eighteen male Sprague-Dawley rats were rendered nicotine-dependent by 7 days continuous subcutaneous infusion of nicotine bitartrate. They were injected i.p. with 1 mg/kg Volinanserin or injection vehicle alone prior to the succeeding normally sleep-dominated lights-on period and again before the normally active lights-off period. In vehicle-injected control rats, withdrawal from nicotine induced a near-significant increase in time awake, during the normally sleep-dominated 12 hour lights-on period. The Volinanserin-injected rats experienced significant decreases in the fragmentation of non-REM and REM sleep during the four hour window of peak withdrawal within the lights-on phase, compared with the control group. In the same time period, the low frequency, sleep-associated delta and theta rhythms were significantly reduced in the control group, while these were increased in the Volinanserin group. Conversely, the powers of the wake-associated higher frequency beta and gamma rhythms were significantly increased in the control, but not the volinanserin group. Overall, the control group experienced changes suggesting lower quality sleep; these changes were moderated by Volinanserin. In the subsequent 12 hour dark period, in which the rats are normally awake and active, both treatment groups spent proportionately less time awake compared to baseline. However, only the control group had significantly increased non-REM sleep compared to baseline, suggesting a rebound from poor quality sleep. This differed significantly from the Volinanserin group, which significantly reduced the power of its non-REM-associated delta waves, suggesting normal wakefulness during the active period. Similarly, only the control group experienced significant decreases in the power of its high frequency gamma band, indicating reduced intensive mental activity during the active period. This suggests a physiological basis for the cognitive and attentional deficits linked to nicotine withdrawal. In summary, the data suggests that nicotine withdrawal results in poor quality sleep, followed by an impaired waking period. Volinanserin effectively reversed these changes.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Support: ACADIA Pharmaceuticals Grant

UHCL Faculty Research Support Fund Grant

Title: Disruption of sleep patterns in rats during continuous nicotine infusion

Authors: *E. NEYHART, J. C. SHAHIN, J. J. BAUTISTA, J. J. IZYGON, D. M. NGHIEM, M. M. HENCEROTH, D. H. MALIN, C. P. WARD;
Psychology, Univ. of Houston-Clear Lake, Houston, TX

Abstract: Smoking has been shown to cause various disruptions in the sleep of humans. However, these effects have yet to be extensively modeled in rodents. In the present experiment, a well validated model of nicotine dependence was utilized to examine the effects of nicotine administration on sleep. Sprague Dawley rats (n=6) were implanted with EEG and EMG using standard procedures. Following recovery, polysomnography was recorded for a baseline day. Then, an osmotic mini-pump was implanted subcutaneously to administer nicotine bitartrate (9mg/kg/day) over 5 days. This infusion rate has been shown to produce blood levels of nicotine similar to those in heavy smokers. Sleep/Wake behavior during baseline and administration days 1, 3, and 5 were scored. The administration of nicotine resulted in an increase in wakefulness. During the normally sleep-dominated light cycle, there was a peak in wake on Day 1 ($p=.035$) that returned toward baseline by Day 5; however, during the normally wake-dominated dark cycle, there was a constant trend of increasing wake over the 5 days ($p=.001$). Non-REM sleep was decreased in comparison to baseline throughout the 5 days in both the light ($p<.001$) and dark cycle ($p<.001$). Nicotine resulted in an increase in REM sleep. During the light cycle there was a trend toward increasing REM over all 5 days ($p<.001$), while during the dark cycle, there was a peak on Day 1 ($p=.004$) and a return to baseline by Day 5. Average wake bout duration significantly increased on Day 1 ($p=.037$) of nicotine administration and then returned to baseline, but no changes were noted during the dark cycle. Average NREM bout duration decreased over the 5 days of nicotine administration in both light ($p=.002$) and dark cycles ($p=.043$). Average REM duration was not affected. Spectral analysis indicated that Gamma and Beta activity increased over baseline throughout nicotine administration. There were also significant changes in Delta, Theta, and Alpha activity that varied between the sleep/wake stages. Overall, Non-REM sleep was negatively impacted by nicotine in both time and average duration. Wake and REM were increased with nicotine administration, though the temporal pattern of change differed based on rats being in their active or inactive period. This suggests that although

certain sleep disruptions diminish as tolerance to nicotine develops, other patterns persist even after initial administration.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Support: P30 DA015663

Title: Nicotine administration and withdrawal alters sleep and EEG patterns in mice

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Abstract: Daytime sleepiness and difficulty sleeping are commonly reported adverse events experienced during nicotine withdrawal. Yet, the relationship between smoking cessation and sleep remains one of the most infrequently studied. Published reports in the human literature are contradictory, and there is a lack of published data using animal models. The purpose of the current study was to examine the relationship between nicotine administration, withdrawal, and sleep in a rodent model of physical dependence and spontaneous withdrawal. In the first experiment, 7 subjects were implanted with EEG and EMG recording devices using standard procedures. After a recovery/acclimation period, data was recorded continuously for a 4-week period. Mice had ad libitum access to food and a drinking water solution containing .2% saccharin. Baseline sleep and wake data was scored for two consecutive 24 periods, and subsequently averaged. Immediately following baseline, all subjects began receiving 200μg/ml of nicotine for a period of 2 weeks. 5 days of the nicotine period were scored and averaged. After 2 weeks, withdrawal was induced by excluding the nicotine from the drinking water. The first 2 days of withdrawal were scored and averaged; day 5 was also scored. Sleep patterns were analyzed by: (1) calculating the total amount of time spent in each state (Wake, NREM, REM); (2) determining the number of transitions; and (3) assessing the average bout duration. Data were assessed in 24 and 12 hour bins over each 24 hour period. In a second experiment, delta power (0-4hz) was analyzed, in 2-hour bins, in 4 nicotine receiving mice using the previously

mentioned protocol. Nicotine consumption deceased total sleep time. The largest effect was observed in the lights off phase where animals saw a 30% reduction in sleep. The trend during early withdrawal was a modest increase in sleep during the lights off phase. Sleep bouts and transitions remained stable until day 5 of withdrawal. At this time, average bout duration decreased by 20%, whereas the number of transitions increased by 13%. Compared to baseline, during the administration period, delta power decreased during the lights off phase and increased during the lights on phase. During the withdrawal period, delta power increased during both phases. These data suggest an effect of nicotine administration and withdrawal on the sleep/wake cycle and homeostatic measures of sleep

Disclosures: **H.L. Mathews:** None. **L. Jimenez:** None. **S. Ahmad:** None. **J.A. Stitzel:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 352.18/GGG25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA032246

Title: Investigating the role of the alpha 7 nicotinic acetylcholine receptors in nicotine reward

Authors: *A. JACKSON, P. MULDOON, M. DAMAJ;
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Abstract: Tobacco use is associated with many health problems and financial costs. Current smoking cessation therapies are modestly successful; therefore, there is a dire need for new molecular targets. Nicotine, the main addictive component in tobacco, exerts its dependency effects via nicotinic acetylcholine receptors (nAChRs). These receptors exist in multiple subtypes and are located in the mesolimbic pathway, which plays a role in the rewarding effects of nicotine. Activation of the homomeric α7 nAChR subtype has been shown to reduce nicotine's rewarding properties in the mouse conditioned place preference (CPP) test and in the nicotine i.v. self-administration rat model. However, the mechanisms underlying the effects of α7 nAChR agonists in these models are largely unknown. A recent study implicated the nuclear receptor transcription factor peroxisome proliferator-activated receptor type-α (PPARα) as a possible downstream signaling target of the α7 nAChR activation in VTA dopamine cells. Therefore, the purpose of the present study was to investigate the possibility of the involvement of PPARα in nicotine reward using the mouse unbiased CPP test. Our results showed that the selective α7 nAChR agonist, PNU282987, dose-dependently attenuated nicotine preference in

male ICR adult mice after systemic administration. Remarkably, the attenuating effect of α7 nAChR activation on nicotine CPP was blocked by the PPARα antagonist, GW6471. Furthermore, we also found that the systemic administration of the PPARα exogenous agonist, WY-14643, reduced nicotine preference in a dose-dependent manner in the CPP test. These initial results suggest that attenuation of nicotine reward by the α7 nAChR activation is PPARα mediated. Further studies will be conducted to investigate the role of PPARα signaling system in nicotine reward.

Disclosures: A. Jackson: None. **P. Muldoon:** None. **M. Damaj:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

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Program#/Poster#: 352.19/GGG26

Topic: G.08. Drugs of Abuse and Addiction

Support: UHCL Faculty Support Fund grant

Acadia Pharmacuticals grant

Title: The 5HT2a antagonist volinanserin attenuates spontaneous nicotine withdrawal syndrome in the rat.

Authors: *D. H. MALIN[1], S. GADAM[2], D. J. MCGHIEY[2], C. L. AGUILAR[2], J. R. CAMPBELL[2], R. N. HUGHES[2], L. CASTILLO[3], P. GOYARZU[2], E. S. BURSTEIN[4], C. A. MADISON[2];
[1]Human Sciences,, Univ. of Houston Clear Lake, Houston, TX; [3]Biol., [2]Univ. of Houston-Clear Lake, Houston, TX; [4]Biosci., ACADIA Pharmaceuticals, Houston, TX

Abstract: There are several reasons to hypothesize that the 5HT2a serotonin receptor is involved in the nicotine withdrawal syndrome. Nicotine withdrawal decreases pre-pulse inhibition, a type of impairment reversible by 5HT2A antagonists. Stimulation of this receptor intensifies shaking resulting from nicotine withdrawal in the rat. Conversely, clozapine, a drug which, among other actions, potently inhibits 5HT2A receptors, attenuates several measures of nicotine withdrawal. Therefore it seemed plausible that the selective 5HT2a antagonist Volinanserin (also known as MDL100907) might reduce the symptoms of nicotine withdrawal syndrome in the rat.
The subjects were 18 male Sprague-Dawley rats. They were all continuously infused for seven days with 9 mg/kg/day nicotine bitartrate via subcutaneous Alzet 2ML1 osmotic minipump, implanted under isoflurane inhalation anesthesia. At the end of this period, the pumps were

removed under the same anesthesia. Twenty-one hours later, groups of six rats were injected intraperitoneally with either zero, 0.2 mg/kg or 1.0 mg/kg Volinanserin in 20% DMSO, 20% Tween 80, and 60% saline. An hour later, each rat was observed over twenty minutes under "blind" conditions for nicotine withdrawal signs, such as gasps, shakes and tremors, vacuous chewing, ptosis, seminal ejaculation and hind foot scratches.
The rats receiving the injection vehicle alone displayed 28.0 ± 3.9 withdrawal signs (M ± SEM), cumulated across all categories. Rats receiving 0.2 mg/kg Volinanserin exhibited 32.0 ± 4.1 signs, while those receiving 1.0 mg/kg had only 12.5 ± 2.8 signs. One-way ANOVA indicated a significant treatment effect, $p = .004$. Post-hoc tests (Tukey's HSD) indicated that the high dose Volinanserin group differed significantly from both the vehicle control group, $p = .022$, and the low dose group, $p = .005$. ANOVA of individual categories of withdrawal signs, found significant treatment effects on shakes and tremors, $p = .013$, and seminal ejaculations, $p = .036$. These results suggest a dose-dependent attenuation of nicotine withdrawal by Volinanserin, consistent with the hypothesis that activation of 5HT2a receptors contributes to nicotine withdrawal syndrome.

Disclosures: **D.H. Malin:** None. **S. Gadam:** None. **D.J. McGhiey:** None. **C.L. Aguilar:** None. **J.R. Campbell:** None. **R.N. Hughes:** None. **L. Castillo:** None. **P. Goyarzu:** None. **E.S. Burstein:** None. **C.A. Madison:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01DA024330

1S10RR025128

1S10RR029398

Title: Molecular histochemistry identifies peptidomic organization and reorganization along striatal projection units.

Authors: *A. HISHIMOTO[1,2], H. NOMARU[2], A. NISHI[2], K. YE[3], J. LIM[4], J. T. AGUILAN[4], E. NIEVES[4], G. KANG[2], R. H. ANGELETTI[4], N. HIROI[2,5,6];
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Analysis & Proteomics (LMAP), [5]Dominick P. Purpura Dept. of Neurosci., [6]Dept. of Genet., Albert Einstein Col. of Med., New York, NY

Abstract: Peptides, defined as polymers of between 2 and 100 amino acids, are abundantly expressed in the mammalian brain; some 850 peptides are known to exist in the mouse brain. Intracellular peptides are associated with the nucleus, lysosome, or membrane and are involved in many processes within and across cells; they are collectively termed neuropeptides. While studies have shown that some neuropeptides contribute to many brain functions underlying behaviors relevant to emotion, motivation, learning and memory, their functional roles in the brain are still poorly understood. Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS), also known as in situ mass spectrometry, quantitatively and comprehensively profiles regulation of known and unknown peptides and their cleaved products with excellent spatial resolution in mammalian tissues . We explored the technical capability of MALDI-IMS for characterization of peptidomic regulation by an addictive substance along two distinct projection systems in the mouse striatum. We used six male C57BL/6J mice for MALDI-IMS and two male C57BL/6J mice for tandem mass spectrometry (MS/MS) validation. The six mice for MALDI-IMS were divided into saline (N=3) and nicotine (0.2 mg.kg, i.p, N=3) treatment groups. Two drug-free mice were used for MS/MS validation of peptide identities. The spatial expression patterns of Substance P and Proenkephalin, marker neuropeptides of two distinct striatal projection neurons, were negatively correlated at baseline. We detected 768 mass/charge (m/z) peaks whose expression levels were mostly negatively and positively correlated with those of substance P and Proenkephalin-A(218-228), respectively, within the dorsal striatum. Following nicotine administration, there was a positive shift in correlation of m/z peak expression levels with Substance P and with Proenkephalin-A (218-228). Our analyses demonstrate the technical capacity of MALDI-IMS for comprehensive identification of peptidomic regulation patterns along histochemically distinguishable striatal projection pathways.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

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Program#/Poster#: 352.21/HHH2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH NS083578

Title: Chronic nicotine induces neuroadaptations in striatopallidal D2 pathway mediated by NR2B containing silent synapses

Authors: J. XIA, *J. A. BEELER;
Psychology, Queens Col. CUNY, Flushing, NY

Abstract: Nicotine addiction is a chronic neuropsychological disorder. Success rates for smoking cessation are very low and risk of relapse continues even after prolonged abstinence. The challenge of maintaining long-term abstinence is believed to arise from nicotine-reinforced learning that gives rise to cue-induced cravings. Why this learning is so resistant to extinction, however, is poorly understood and may arise from nicotine-induced neuroadaptations in mechanisms regulating corticostriatal synaptic plasticity. In present study, we used whole-cell patch clamp recording to investigate the effect of chronic nicotine (cNIC) treatment on synaptic plasticity in D2 receptor-expressing medium spiny neurons in striatopallidal indirect pathway in the dorsolateral striatum. Mice were administered nicotine in their drinking water for minimally three weeks starting at 60 days of age. Mice exposed to chronic nicotine exhibited long-term potentiation (LTP) in response to high-frequency stimulation (HFS) with a concomitant loss of presynaptic depression that underlies the long-term depression (LTD) typically observed with HFS. We observed a dissociation between the rescue of presynaptic depression and blocking postsynaptic potentiation: bath application of the D2R agonist quinpirole induced presynaptic depression (paired pulse facilitation) and reduced the observed potentiation without inducing depression in EPSCs. Similarly, the NMDA receptor antagonist APV blocked potentiation without rescuing presynaptic depression while quinpirole and APV together restored HFS-LTD. Chronic nicotine altered AMPA/NMDA ratios and induced enrichment in the NR2B subunit. Using a minimal stimulation assay, we detected a substantial increase in the prevalence of silent synapses in the mice exposed to chronic nicotine, suggesting the LTP we observe in response to HFS in the chronic nicotine treated mice arises from activation or unsilencing of silent synapses. These data indicate that chronic, intermittent nicotine exposure induces changes in AMPA and NMDA receptor regulation in striatopallidal MSNs resulting in altered corticostriatal synaptic plasticity. The increased prevalence of silent synapses is consistent with previous reports of similar increases in silent synapses in response to cocaine and been proposed to underlie the incubation of craving observed after extended abstinence.

Disclosures: J. Xia: None. **J.A. Beeler:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: MRC UK, G1000403

NC3Rs G1000053

NIHR PGfAR

RP-PG-0407-10398

Title: A conditioned place preference forward genetic screen in zebrafish identifies a novel locus affecting nicotine preference in fish and smoking behavior in humans.

Authors: *C. H. BRENNAN[1], A. J. BROCK[2], M. O. PARKER[4], V. KUAN[3], D. JOLLIFFE[3], A. SUDWARTS[2], A. R. MARTINEAU[3], R. T. WALTON[3];
[2]SBCS, [3]Barts and The London Sch. of Med. and Dent., [1]Queen Mary Univ. of London, London, United Kingdom; [4]Portsmouth Univ., Portsmouth, United Kingdom

Abstract: Tobacco smoking is the leading preventable cause of death worldwide and places a heavy social and financial burden on society. Human smoking behavior is strongly heritable but the molecular mechanisms underlying nicotine preference are largely unknown. In a novel approach using a nicotine-induced, conditioned place preference, behavioral genetic screen of ethylnitrosurea-mutagenized zebrafish we demonstrate that nicotine preference is heritable in fish as in humans and identify loss-of-function mutations at specific loci (called QM1, 2) leading to altered nicotine preference. Conservation of the phenotype in independent lines carrying similar putative loss-of function mutations confirmed causative roles for the genes in fish nicotine preference. One of the affected genes, QM2, is a ligand for a family of proteins which have an established role in axon guidance and cell migration in the developing central nervous system. We show that larvae heterozygous or homozygous for loss-of-function mutations in QM2 have altered dopaminergic circuitry. Further, heterozygous larvae show increased expression of the cholinergic receptor *chrna5*. Analysis of the *QM2* locus in humans identified genetic markers that predict level of cigarette consumption and likelihood of cessation, suggesting that evolutionarily conserved *QM2* pathways acting through CHRNA5 regulate tobacco dependence in humans. These findings suggest a role for QM2 signaling in mechanisms underlying tobacco dependence and indicate the cell biological processes involved. To our knowledge, this is the first report of a novel human functional polymorphism identified using a forward genetic screen of adult zebrafish to uncover loci affecting a complex human behavioral

trait. These findings underline the potential of a zebrafish model for understanding the pathophysiology of behaviors associated with human psychiatric disease.

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Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 352.23/HHH3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant R01-DA021274

Title: Examination of the neurochemical mechanisms that modulate sex differences in nicotine withdrawal.

Authors: *R. J. FLORES GARCIA[1], L. CARCOBA[2], K. URIBE[2], L. E. O'DELL[2];
[1]Psychology, [2]Univ. of Texas at El Paso, El Paso, TX

Abstract: Introduction: Women are more vulnerable to tobacco use than men and experience greater symptoms of withdrawal during abstinence from nicotine in tobacco products. However, the neurochemical mechanisms that mediate sex differences in nicotine withdrawal are not well understood. Current work in our laboratory is focused on understanding the underlying neural circuitry of withdrawal within the nucleus accumbens (NAcc), where dopamine levels are decreased during withdrawal from nicotine. Our mechanistic hypothesis is that females display larger decreases in dopamine levels in the NAcc that are modulated via a greater gamma-aminobutyric acid (GABA)-mediated inhibition of dopamine in this region. To address this hypothesis, we assessed NAcc levels of GABA during nicotine withdrawal in male and female rats. Rats also received yohimbine to compare sex differences in response to a pharmacological stressor. **Methods:** Rats were prepared with an osmotic pump containing a dose of nicotine (3.2 mg/kg; base) that produces equivalent nicotine levels in female and male rats. Fourteen days later, the rats were prepared with dialysis probes in the shell of the NAcc. The following day, samples were collected every 20 min for a 1-hour period following baseline and administration of the nicotine receptor antagonist mecamylamine (1.5 and 3.0 mg/kg, ip) to precipitate withdrawal. **Results:** During nicotine withdrawal, females displayed a significantly larger increase in GABA release than males. Similarly, females displayed a larger increase in GABA release in response to administration of the pharmacological stressor, yohimbine.
Discussion: Our results suggest that females display larger withdrawal-related increases in

GABA release than males. This provides evidence for a potential mechanism involving GABA mediated sex differences in nicotine withdrawal. Ongoing studies are also examining the contribution of ovarian hormones in modulating nicotine withdrawal in females. In addition, we are employing mass spectrometry methods assess the relationship between GABA and other neurotransmitters that may mediate sex differences in nicotine withdrawal.

Disclosures: R.J. Flores Garcia: None. **L. Carcoba:** None. **K. Uribe:** None. **L.E. O'Dell:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 352.24/HHH4

Topic: G.08. Drugs of Abuse and Addiction

Title: Light-enhanced startle sensitivity to acute nicotine withdrawal

Authors: *R. C. BARNET, A. C. HENNINGS;
Col. William & Mary, Williamsburg, VA

Abstract: Nicotine is highly addictive and negative effects of withdrawal, including anxiety, contribute to relapse. Light-enhanced startle is a validated animal model of anxiety that has been relatively unexplored in its effectiveness as a model of withdrawal-associated anxiety (Jonkman et al., 2008). Two experiments evaluated sensitivity of the light-enhanced startle paradigm to anxiety associated with nicotine withdrawal in rats, following intraperitoneal (I.P.) administration of nicotine. In Experiment 1, adult male and female rats received twice/daily I.P. exposure to nicotine (0.40 mg/kg) or saline for 14 days followed by light-enhanced startle behavioral testing 24 hr (withdrawal day 1), 72 hr (withdrawal day 3), or 120 hr (withdrawal day 5) following nicotine cessation. In Experiment 2, the nicotine exposure period was extended to 25 days and animals were again tested for behavioral anxiety in light-enhanced startle during acute withdrawal. Findings from Experiment 1 revealed significantly higher light-enhanced startle during the withdrawal period in nicotine pretreated rats compared to saline pretreated rats. By contrast, Experiment 2 demonstrated significantly lower light-enhanced startle in nicotine pretreated rats compared to saline controls. Variation in nicotinic acetylcholine receptor sensitivity and alterations in sensory processing of environmental stimuli that vary with drug exposure are discussed as possible explanations for observed results. The light-enhanced startle paradigm may be a useful tool for examining how anxiety varies during nicotine withdrawal.

Disclosures: R.C. Barnet: None. **A.C. Hennings:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

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Program#/Poster#: 352.25/HHH5

Topic: G.08. Drugs of Abuse and Addiction

Support: DA012844

DA026356

Title: HIV-1 proteins influence novelty-seeking behavior and alter region-specific transcriptional responses to chronic nicotine treatment in HIV-1Tg rats

Authors: *Z. YANG[1], T. NESIL[2], S. L. CHANG[3,4], M. D. LI[1,3];
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Abstract: Clinical studies suggest that HIV-1-infected patients are more likely to use or abuse addictive drugs than is the general population. We hypothesized that HIV-1 proteins impact novelty-seeking behavior and enhance the transcriptional response to nicotine of genes implicated in both novelty-seeking behavior and drug addiction. In this study, we used the HIV-1 transgenic (HIV1-Tg) rat as an animal model to test this hypothesis. We first assessed the effect of HIV-1 proteins on novelty-seeking behavior by comparing baseline activity differences between HIV-1Tg and F344 control rats in the open field test, which is used routinely to measure dopamine-driven novelty-seeking behavior. One day after behavioral testing, the HIV-1Tg and F344 rats began daily injections of either nicotine (0.4 mg/kg, base, *s.c.*) or saline for 27 days. At the end of treatment, the prefrontal cortex (PFC), nucleus accumbens (NAc), and ventral tegmental area (VTA) were collected for analysis of RNA expression of four neurotransmitter receptor families: dopamine, GABA, glutamate, and serotonin. The HIV-1Tg rats spent more time in the center of the arena and made more entries to the center than did F344 rats. By quantitative RT-PCR analysis, mRNA of *Drd3* and *Grm2* in the PFC and of *Drd5* and *Gabra6* in the VTA was significantly upregulated, and that of *Drd5* in the NAc was downregulated in HIV-1Tg rats compared with F344 rats. Further, more addiction-related genes were significantly modulated by nicotine in HIV-1Tg rats. Genes altered in nicotine-treated HIV-1Tg rats included *Drd5, Gab*ra*1*, *Gabra2*, *Gabra6,* and *Grm5* in the NAc, *Drd1a*, *Drd2*, *Drd3, Drd5*, *Gabra5*, and *Grm2* in the PFC, and *Drd5*, *Gabra6, Grm1*, *Grm2,* and *Grm5* in the VTA. In contrast, only the genes of *Gabra5* in the NAc, *Gabra3* and *Grm2* in the PFC, and *Gabra6*, *Grm*1, and *Grm2* in the VTA were changed by nicotine in F344 rats. In conclusion, our findings indicate that HIV-1 proteins not only affect novelty-seeking behavior but also modulate the expression of genes related to drug addiction and novelty-seeking behavior.

Disclosures: **Z. Yang:** None. **T. Nesil:** None. **S.L. Chang:** None. **M.D. Li:** None.

Poster

352. Nicotine: Neural Mechanisms of Addiction

Location: Halls B-H

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Program#/Poster#: 352.26/HHH6

Topic: G.08. Drugs of Abuse and Addiction

Title: Blunted nicotine-induced neural activity in the habenula-interpeduncular nucleus circuit in response to chronic nicotine history

Authors: *H. ZHANG[1], M. D. EHLERS[1,2];
[1]Neurosci. Res. Unit, Pfizer, Inc, Cambridge, MA; [2]Biogen, Cambridge, MA

Abstract: Nicotine is the major addictive substance in tobacco. Chronic nicotine exposure induces molecular and neurochemical changes in various brain areas, including the medial habenula (MHb) and its major target the interpeduncular nucleus (IPN). The Hb-IPN circuit elicits robust acute responses to nicotine. However, the effect of chronic nicotine exposure on neural activity in these areas and associated changes in neural dynamics are poorly understood. We characterized neural dynamics in the habenula and IPN in response to acute nicotine challenges, and investigated how chronic nicotine history altered such responses. In urethane-anesthetized rats, acute intravenous nicotine induced transient, dose-dependent activation of local field potentials (LFP), including reduction of the delta oscillations (0.8-2.3 Hz) and increased oscillations in the broad theta (2.5-6.0 Hz) range. A substantial portion of single neurons showed excitatory, dose-dependent responses to nicotine, while their dose-dependency and background activity varied across neurons even within individual animals. Interestingly, although rats with chronic nicotine exposure showed responses that were qualitatively similar to naïve animals, the induced LFP activation, including transient Hb-IPN coherence, was substantially blunted relative to control rats with no chronic nicotine history. Our results show that chronic nicotine exposure diminishes the response magnitude of the habenular circuitry to acute nicotine intake. Given the postulated role of Hb-IPN output as a brake system known to exert negative regulation on dopamine reward circuitry and known to promote aversive responses to nicotine, such compromised Hb-IPN output upon chronic nicotine exposure reveals the neural representation of an adapted brain more permissive to nicotine intake.

Disclosures: **H. Zhang:** A. Employment/Salary (full or part-time): Pfizer Inc. **M.D. Ehlers:** A. Employment/Salary (full or part-time): Pfizer, Inc.

Poster

352. Nicotine: Neural Mechanisms of Addiction

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Program#/Poster#: 352.27/HHH7

Topic: G.08. Drugs of Abuse and Addiction

Support: CIHR postdoctoral fellowship

Title: Oxytocin phenocopies the effects of dopamine receptor antagonism on nicotine motivation

Authors: *T. E. GRIEDER[1,2], M. YEE[1], O. GEORGE[2], D. VAN DER KOOY[1];
[1]Inst. Med. Sci., Univ. Toronto, Toronto, ON, Canada; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Drug abuse, specifically nicotine addiction, is a worldwide epidemic that has led to many deaths. Our previous research has suggested that both social factors and a specific pattern of dopaminergic signalling in the ventral tegmental area play an important role in the motivational responses to nicotine in mice. The neuropeptide oxytocin has been implicated in various social behaviours as well as dopaminergic modulation, thus we hypothesized that oxytocin administration may affect the motivational responses to acute nicotine and withdrawal from chronic nicotine. We used a place-conditioning paradigm to examine the effect of oxytocin pretreatment on the conditioned response to nicotine in groups of nondependent or nicotine-dependent and -withdrawn C57Bl/6J mice, and compared these nicotinic motivational effects with those elicited after dopamine receptor antagonism. Nondependent mice given a low (0.35 mg/kg) or high (1.75 mg/kg) dose of acute nicotine showed no conditioned response and an aversive response to the nicotine-paired environment, respectively. Pretreatment with either oxytocin or with a dopamine receptor antagonist made the low dose of nicotine rewarding and prevented the conditioned aversive response to the high dose of nicotine. Similarly, nicotine-dependent and -withdrawn mice showed a conditioned aversive response to the withdrawal-paired environment that was blocked by either oxytocin or dopamine receptor antagonist pretreatment. These results suggest that oxytocin administration and dopamine receptor antagonism have the same effects in blocking the aversive effects of nicotine, and that oxytocin may be acting serially in a neurobiological circuit to modulate the specific pattern of dopaminergic activity that mediates nicotine's aversive motivational effects.

Disclosures: **T.E. Grieder:** None. **M. Yee:** None. **O. George:** None. **D. van der Kooy:** None.

Poster

353. Human Cognition and Memory II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.01/HHH8

Topic: H.02. Human Cognition and Behavior

Support: FISM project n. 2011/R/8

Title: Brain reorganization following adaptive working load cognitive training in multiple sclerosis

Authors: *L. BONZANO[1], L. PEDULLÀ[1], M. PARDINI[1], A. TACCHINO[2], G. BRICHETTO[2], M. BOVE[1];
[1]Univ. Genoa, Genoa, Italy; [2]Multiple Sclerosis Italian Fndn., Genoa, Italy

Abstract: Cognitive impairment is common in patients with multiple sclerosis (PwMS), however the best cognitive rehabilitation features definition is still an open issue. Also, little is known about the effects of cognitive rehabilitation on the central nervous system. We recently evaluated the efficacy of adaptive vs. non-adaptive training based on working memory (WM) on cognitively impaired PwMS, by using an ad hoc developed application software for portable devices named Cognitive Training Kit (COGNI-TRAcK), able to administer a user-friendly and personalized treatment based on WM exercises. COGNI-TRAcK was well accepted by PwMS and effective in improving their cognitive functioning, suggesting that an adaptive working load (i.e., fine-tuned to the individual's needs) is crucial to allow a transfer effect also to non-trained cognitive domains and ensure a long-term positive effect. Here, we investigated the effects of adaptive cognitive rehabilitation on cognitive function and related brain activity patterns in PwMS. We included 18 PwMS presenting with cognitive impairments and 18 healthy controls (HC). PwMS underwent a 8-week home-based treatment through COGNI-TRAcK, with automatic adjustment of tasks difficulty to subject's performance. Before and after treatment, cognitive status was assessed using a gold standard neuropsychological evaluation. In the same sessions, brain activity was investigated by fMRI at 1.5 T during the Paced Visual Serial Addition Test (PVSAT). HC underwent the same fMRI procedure. Cognitive performance significantly improved in all PwMS as effect of treatment. During the PVSAT, HC activated the left cingulate gyrus, inferior parietal lobule, precuneus, precentral gyrus, and middle frontal gyrus, and the right cerebellum, consistently with previous works. In PwMS, at baseline PVSAT-related activations were found in the precuneus, inferior parietal lobule and cerebellum bilaterally, and in the right superior parietal lobule and insula. In addition, the left Brodmann area (BA) 39 was activated. The different patterns of activation can be interpreted as the consequence of compensatory mechanisms. After treatment, the PVSAT elicited a lateralized fronto-parietal network with cerebellar activation reduced with respect to the previous session

and more similar to HC. In particular, PwMS after treatment showed increased activation of the left orbitofrontal cortex than HC. Interestingly, this area has a key role in fatigue perception in PwMS. In conclusion, the adaptive working load cognitive training was able to reduce brain resource demand, altered by the disease.

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Poster

353. Human Cognition and Memory II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.02/HHH9

Topic: H.02. Human Cognition and Behavior

Title: Predicting variations in cognitive load: a multimodal approach

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Abstract: Using joint multimodal signal features may improve cognitive performance prediction. Continuous physiological status monitoring and real-time data processing techniques can be combined to identify variations in the human state. This information could be used to improve individual or group decisions. In this study, we hypothesized that one can predict various levels of cognitive load using joint, multimodal signal processing techniques. Seventeen subjects (8 female) completed auditory working memory (Digitspan) and visual working memory (ColorK) tasks during separate experimental sessions. The Digitspan task consisted of 120 trials with intermediate sentence distractors. Subjects listened to a string of digits, a sentence, waited for one second, recalled the sentence, and finally recalled the digits. Two, four, or eight digits were presented to vary task difficulty, and therefore cognitive load. 40 different sentences were presented at each difficulty at random. In contrast, during the ColorK task subjects had to remember the color and position of squares presented on the screen. Subjects then had to hold these in memory for eight seconds before recall. Similar to the Digitspan session, the difficulty of the ColorK task was varied by presenting 2, 4 or 8 colored squares to the subject. Non-invasive electroencephalography (EEG), galvanic skin response (GSR), pupillometry, and speech measures were recorded in each experiment. Each modality was preprocessed to remove

signal artifacts and environmental noise. An initial analysis estimated cognitive load using joint speech features via a novel machine learning method over spectrotemporal speech features. Receiver operation curve (ROC) values were calculated from system cross-validation results. We achieved a classifier ROC area of 0.60 for single-trial difficulty classification using cross-correlation features and cross-spectral power densities from the first three vocal formant tracts. These results support the hypothesis that there are underlying sensorimotor changes in speech vocal features that vary with levels of cognitive load. Future work will integrate additional modalities into this analysis, which we believe will improve the system's cognitive load detection ability. Continuous cognitive performance prediction may help to improve operational efficiency and safety. This material is based upon work supported by the US Army Natick Soldier Center under Air Force Contract No. FA8721-05-C-0002 and/or FA8702-15-D-0001. Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the US Army.

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Poster

353. Human Cognition and Memory II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.03/HHH10

Topic: H.02. Human Cognition and Behavior

Title: Structural plasticity in healthy elderly after working memory training. A randomized control-group trial.

Authors: ***N. HUDL**[1,2], J. WEICKER[1,3], A. VILLRINGER[1,4], A. THOENE-OTTO[3]; [1]Neurol., Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Max Net Aging Res. Sch., Rostock, Germany; [3]Clin. for Cognitive Neurol., Univ. of Leipzig, Leipzig, Germany; [4]Clin. for Cognitive Neurol., Uni, Leipzig, Germany

Abstract: There is an ongoing debate, whether working memory (WM) capacity, which declines with old age, can be improved by training. However, several studies have proven behavioral and functional activation changes in the brain in young and healthy elderly. To our knowledge, only one study investigated structural changes after a WM training so far.
We investigated structural brain plasticity of healthy elderly (aged 60-70 years) after a WM training over 4 Weeks (12 sessions) using voxel-based morphometry (VBM). Therefore, 40 healthy elderly participated in either an adaptive training (n = 20) or a visually identical placebo training for 12 weeks. Neuropsychological and neuroimaging assessments were conducted at

baseline, after the training and three months follow-up.
On a behavioral level, we found increased WM performance in a WM composite score (Weicker et al. in prep.) after the training. On the basis of the VBM-Analysis we found increased GM density in the left inferior frontal gyrus, left anterior cingulate gyrus, left rectal gyrus, as well as the right culmen, right anterior cingulate, right middle temporal gyrus, right cerebellar tonsil and right postcentral gyrus ($p<0.001$ uncorrected) after training when comparing the working memory group with the placebo training group.
These effects were not stable until follow-up, however, we found decreased GM density in the right inferior frontal gyrus and increased GM density in the left lentiform nucleus, right insula and right precentral gyrus ($p<0.001$ uncorrected) after 3 months.
To conclude, we found increased GM density in frontal-parietal regions of the brain, cerebellum and anterior cingulate. This is in line with previous learning studies, which found increased GM density after a training period, mainly in the frontal cortex, precuneus, hippocampus and parietal areas. Our study demonstrates that a training of WM is possible, leading to beneficial behavioral as well as gray matter changes in healthy elderly.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Suppression of brain response to a task-irrelevant visual stimulus emerges in a visual hemifield on which VSTM task was imposed

Authors: ***A. SAYAMA**, T. URAKAWA, A. KITAMI, H. AZETAKA, O. ARAKI;
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Abstract: To effectively achieve a behavioral task of visual short-term memory (VSTM), we should appropriately pay attention to visual objects and then hold its information for a short period under limited neural capacity. Previous studies using dual task designs have reported that behavioral performance of detection task was deteriorated when the other task irrelevant to VSTM was simultaneously taxed. A previous fMRI study reported that neural activity for the task-irrelevant objects in the high load VSTM task was attenuated over retinotopic visual areas (Konstantinou et al, 2012), suggesting suppression of visual processing at early visual areas for task-irrelevant visual objects. Since this previous study employed a VSTM task exclusively in the central visual field, it remains to be addressed whether such attenuated activity for the task-

irrelevant objects depends on a portion of visual field on which the VSTM task is taxed. Focussing on early ERP components (P1 and N1), the present study attempted to clarify this issue, confining the high load VSTM task to either left or right visual field. Eleven healthy subjects performed the change-detection task in which the subjects were required to report whether or not two successive images were identical in the cued hemifield. Each of the two images appeared for 200 ms and contained four colored rectangles both in the left and right visual fields. Delay period (a blank image) was inserted between the two images for 1 s. One of the rectangles in the cued hemifield changed in one feature (either color or orientation) from the first to the second images at half of all trials. Regardless of the feature changes, the task-irrelevant rectangles appeared around the fixation point during the delay period at half of all trials. Under this stimulation paradigm, we calculated peak-to-peak amplitude of P1 and N1 for the task-irrelevant rectangles and compared the amplitude contralateral to the cued hemifield with that ipsilateral to the cued hemifield. Results obtained showed that the peak-to-peak amplitude contralateral to the cued hemisfield was significantly attenuated compared to that ipsilateral to the cued hemifield ($p < 0.01$). Our findings suggest that the suppression of visual processing is dependent on a portion on which the VSTM task was loaded.

Disclosures: **A. Sayama:** None. **T. Urakawa:** None. **A. Kitami:** None. **H. Azetaka:** None. **O. Araki:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Hamburg state cluster of excellence (neurodapt!)

German National Academic Foundation

Title: Working memory performance in the elderly closely relates to alpha oscillations and is predicted by integrity of the parahippocampal cortex and white matter tracts

Authors: ***T. K. STEIGER**[1,2], N. A. HERWEG[2], M. M. MENZ[2], N. BUNZECK[1,2]; [1]Dept. of Psychology, Univ. of Luebeck, Luebeck, Germany; [2]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: The ability to maintain and process physically absent information for a short period of time tends to decrease across the life span, but the underlying neural mechanisms remain unclear.

To address this open question, 31 healthy elderly participants (55-79 years) performed a working memory (WM) task while electroencephalography (EEG) was being recorded. Additionally, individual whole brain anatomical scans were acquired using voxel-based morphometry (VBM) and diffusion tensor imaging (DTI). During the WM task, participants were briefly presented with a pattern of colored squares (2, 4 or 6 items) and indicated whether one of the colors had changed after a delay of 4-5 seconds. As expected, reaction times were slower and accuracy decreased with increasing WM load. At the neural level, this effect was reflected in power modulations of the alpha-band (8-14 Hz). Importantly, between participant variance in grey matter volume of the parahippocampus strongly predicted performance accuracy in the load-6, but to a lesser degree in the load-4 condition. In line with these observations, Tract-Based Spatial Statistics (TBSS) revealed a significant correlation between accuracy in load-6 and fractional anisotropy (FA) maps within wide spread white matter tracts. Together, our findings shed new light on WM performance in the elderly, suggesting a close link between structural declines of the parahippocampal cortex, white matter integrity and alpha oscillations.

Disclosures: **T.K. Steiger:** None. **N.A. Herweg:** None. **M.M. Menz:** None. **N. Bunzeck:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: McDonnell Foundation (5-29727)

NINDS (R01 NS065046),

NIMH (R01 MH099078)

Title: Learning working memory gating policies

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[1]Cognitive Linguistics and Psychological Sci., [2]Brown Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: Working memory (WM) resources are capacity limited and their use requires executive control. WM control has been extensively analyzed within the gating framework. This framework postulates the existence of *gates* that control the updating of information into WM (input gate), and its influence on behavior (output gate). On this view, performing a task requires

learning a *gating policy* that is tuned to the input and output gating demands of the task. Computational models have demonstrated how gating might be implemented by neural circuits involving the prefrontal cortex (PFC) and basal ganglia (BG), and how sophisticated gating policies may be acquired via dopamine-dependent reinforcement learning mechanisms. In recent work, we have shown that human subjects rapidly learn and transfer gating policies to novel task environments. Subjects were first exposed to a task that required non-selective input gating for successful performance. They were then presented with a new task where selective input gating was beneficial. Initial behavior in the new task suggested that subjects generalized a learned tendency for non-selective input gating to new stimuli. Here, we build on this work by investigating the nature of such learning and transfer within the context of the PFC-BG gating framework. As subjects learn a WM gating policy, its parameters must be tuned to the statistics of input gating task demands. We built a PFC-BG network model of the task and examined potential circuit-level mechanisms by which such demands may be accommodated during learning. One class of mechanisms produces a global change in input-gating thresholds supporting broad generalization, while also making WM more susceptible to task-irrelevant distractors. A second class of mechanisms relies on category-specific changes in input gating thresholds that make WM less susceptible to irrelevant distractors, but reduces the scope of generalization. Finally, we tested the global v/s category-specific threshold hypotheses empirically by including task-relevant v/s irrelevant distractors and showed that the learned policy makes subjects more sensitive to the former. We conclude that WM gating policy learning results, in part, from changes in category-specific input gating thresholds, which may provide a balance between task efficiency and generalization.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1358839

Title: Single neuron study of memory for audio-visual episodes in the human brain.

Authors: ***E. KRAUSE**[1], H. TANG[2], M. ISON[2], I. FRIED[3], G. KREIMAN[2];
[1]Neurobio., [2]Harvard Univ., Boston, MA; [3]UCLA, Los Angeles, CA

Abstract: Our ability to remember past events is a critical function for everyday life. However, we only remember a small fraction of the events that occur in our lives. Our brain selectively filters our experiences in order to form episodic memories. How the brain accomplishes this selective filtering to consolidate memories is poorly understood. Using TV episodes as a proxy for real-life memory formation, this study investigates the neural correlates of memorability. We examined single unit recordings from epilepsy patients implanted with intracranial depth electrodes. A total of 761 single and multi units were obtained from 17 sessions in 11 subjects. The majority of electrodes were implanted within the medial temporal lobe, cingulate, and superior temporal gyrus. The experimental paradigm consisted of an encoding period, in which patients viewed a 42 minute episode of the TV show "24", and a testing period, in which patients completed a two-alternative forced choice memory task. In this task patients were presented with either a shot from the episode they viewed (target trial) or a shot from a later unseen episode (foil trial), and they selected whether or not they remembered seeing the shot. A shot refers to a series of uninterrupted frames between cuts (typically 1-2 seconds). The testing period consisted of 100 target trials and 100 foil trials. In addition to the group of epilepsy patients, a group of 80 volunteers completed the same experimental paradigm, but with 1000 target trials. Volunteers were tested at time intervals between 15 minutes and 1 year after encoding. Memory performance declined with time, as indicated by a repeated measures ANOVA on performance with time as a factor ($F = 4.03$, $P = 0.001$). The increased number of testing trials allowed for an exhaustive sampling of all shots, approximately 40 trials per shot across volunteers. From the volunteer performance we defined the "memorability" of each shot as the fraction of correct trials. We found a subset of units with firing rates showing a significant correlation with memorability. Furthermore, firing rate was predictive of memorability. Using a support vector machine algorithm to decode performance on single trials with firing rates as features, we found robust decoding in which neural data provided additional information beyond semantic content in the movie. These results provide initial steps to understand the neural circuits underlying the formation of episodic memories.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Strategic Information and Communications R&D Promotion Program (SCOPE), Ministry of Internal Affairs and Communications, Japan

Title: Relationships between ongoing activity fluctuation in the medial temporal lobe and subsequent memory performance

Authors: *R. KEERATIVITTAYAYUT, R. AOKI, M. TAGHIZADEH SARABI, K. NAKAHARA;
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Abstract: An intriguing question about episodic memory is: why do we remember something while forget other things? Previous studies showed that greater activation in the medial temporal lobe memory system during memory encoding is a key factor predicting subsequent memory performance (the subsequent memory effect). Growing evidence suggests that low-frequency (0.01-0.1 Hz) fluctuation of ongoing blood oxygenation level-dependent signal (low-frequency fluctuation: LFF) can account for much of the variability in task-related activation, and possibly variability in behavioral performances. Thus, in the present study, we hypothesized that the LFF in the medial temporal lobe memory system may be a root of moment-to-moment variability in memory encoding performance. Twenty-five subjects participated in functional magnetic resonance imaging (fMRI) scans. During the fMRI scans, the participants were sequentially presented with 720 color photographs of objects, and were asked to make semantic judgments on each photograph (man-made or natural-made). Twenty minutes later, the participants underwent a surprise test outside the scanner, in which participants' recognition memory of the objects presented during the fMRI sessions was tested. Based on the performance of the recognition memory test, trials during the fMRI sessions were classified into high-confidence hit, low-confidence hit, or miss trials. We first identified brain regions that showed the subsequent memory effect by contrasting the activation related to high-confidence hit trials and miss trials. Consistent with previous studies, the premotor cortex, the posterior parietal cortex, the hippocampus (HC), the parahippocampal cortex (PHC), and the fusiform gyrus (FUS) showed the subsequently memory effect bilaterally. We then extracted trial-independent LFF from the HC, PHC, and FUS as regions of interest. Preliminary analysis indicated that the amplitude of LFF in the PHC was greater in high-confidence hit trials than in miss trials. Our results suggest that trial-independent fluctuation of ongoing activity in the PHC may account for subsequent memory performance.

Disclosures: **R. Keerativittayayut:** None. **R. Aoki:** None. **M. Taghizadeh Sarabi:** None. **K. Nakahara:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Discovery 500491

Title: Shifting the balance between pattern separation and completion: Recent memory retrieval increases people's subsequent ability to recall associations

Authors: *A. PATIL, F. MIAN, J. LEE, K. DUNCAN;
Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: How can the hippocampus support the conflicting processes of pattern separation and pattern completion? Theoretical models propose that, after detecting novelty or familiarity, the hippocampus is biased towards encoding or retrieval states, respectively, by shifts in cholinergic input. This neuromodulatory effect is slow-acting and may linger for a few seconds, suggesting that biases in memory behavior may also be extended in time. Consistent with this memory state model, we have previously shown that novelty elicits temporally-extended behavioral bias towards pattern separation (Duncan, Sadanand, & Davachi, 2012). Here, we investigated the complementary effect of familiarity on subsequent memory retrieval. Specifically, we contrasted preceding familiarity's influence on associative retrieval (pattern completion dependent) to item recognition (pattern completion independent) (Exp. 1: N=32). Participants studied words paired with trial-unique images of either objects or scenes. They then made memory decisions, which simultaneously assessed whether they thought the images were 'old' or 'new' (recognition memory) and if they could remember the associated word (associative retrieval). Critically, we manipulated whether each retrieval decision occurred after an unrelated novel or familiar image. We found that recently identifying an image as familiar rather than novel improved participants' subsequent ability to recall word associates ($t(31)=4.22$; $p<0.0005$). Importantly, this boost in associative retrieval was not driven by response priming or conceptual priming; the preceding image had always been studied in a separate block and contained distinct material (objects vs. scenes). Recent familiarity judgments did not, however, affect participants' item recognition performance ($t(31)=0.38$; $p=0.71$). This suggests that preceding familiarity judgments do not benefit memory retrieval as a whole, but selectively benefit pattern completion dependent retrieval. This is consistent with the mechanisms proposed by computational models. In Exp. 2 (N=48), we varied the inter-stimulus interval (ISI) between retrieval trials to assess the time course of familiarity's influence on subsequent memory retrieval. Results for the short ISI condition were similar to that of Exp. 1. For longer ISIs, there was a significant reduction in the effect of preceding familiarity judgments on associative retrieval ($F(47)=7.12$; $p=0.01$). This

decay in the influence of recent familiarity suggests that the boost in associative retrieval is time-dependent, consistent with the timescale of cholinergic modulation of the hippocampus.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: FWO-Flanders Odysseus II Award #G.OC44.13N to WHA

Title: Task representations in the dorsolateral prefrontal cortex

Authors: ***J. DERAEVE**, E. VASSENA, W. ALEXANDER;
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Abstract: In cognitive experiments, participants are often required to perform tasks where they have to apply simple rules, such as “if target is a square, press left”. In everyday life, however, behavior is more complex and may be governed by collections of rules - task sets - that need to be selectively applied in order to achieve a goal. While previous research has demonstrated the involvement of dorsolateral prefrontal cortex (dlPFC) in representation and maintenance of relevant task sets, the nature of this representation remains an open question. One possibility is that task sets are represented as the co-activation of multiple neurons, each of which codes for a single rule. An alternative possibility is that the activity of individual neurons encodes the conjunction of simple rules. In order to answer this question, subjects performed a delayed match-to-sample task while undergoing fMRI. On each trial, subjects were shown a cue indicating one of three possible task sets. Each task set was composed of two out of three possible rules: color/orientation, orientation/shape or shape/color. Subjects were then presented with a sample stimulus of which they had to memorize the cued task set dimensions. Following a delay, a target stimulus was presented and the subjects indicated how many cued dimensions of the target stimulus matched the sample stimulus. A control condition was also included in which subjects indicated whether the direction of an arrow (left/right) matched a cued direction. Because each task set had one rule in common with another task set and the other rule in common with the remaining task set, this allowed us to ascertain through multivariate decoding if neural representations are conjoined or co-activated. Under the co-activation account, voxels important in classifying between task set A and task set B should be those coding for the rules these task sets do not have in common. Since these are the rules that constitute the remaining

task set C, classifying task set A and B against C and control using these important voxels as input features should yield classification accuracies at chance. Under the conjunction account, important voxels code for a specific conjunction of rules regardless of overlaps between task sets, and classification of task sets A and B against C and control trials is possible. We find evidence for conjunctive representation coding in dlPFC: voxels identified as important in classifying between two task sets yield accuracies above chance when used to classify against the remaining task set and control conditions.

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Poster

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Mortimer Zuckerman Mind Brain Behavior Institute

Foundation Adelis

Title: Working memory capacity determines maximum chunk sizes.

Authors: ***M. V. TSODYKS**, Y. MI;
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Abstract: Chunking is a strategy of grouping several individual pieces of information into a meaningful whole. It helps information processing by breaking a long string of inputs into chunks that are easier to remember. The individual items that constitute a chunk are inter-associated through learning and they are retrieved as an integrated group. Our recent study found that chunking emerges spontaneously during free recall, with typical chunks containing either 3 or 4 words. Despite being a general phenomenon in cognition, the neural mechanisms underlying how chunks are formed and recalled remain largely unknown. A synaptic theory for working memory (WM) in the frontal cortex proposes that memory items are stored temporally in the facilitated neuronal synapses via short-term plasticity (STP) without recruiting persistent neuronal firing. Based on this theory, the WM capacity, i.e., the number of memory items that can be simultaneously retrieved without interference, is restricted by the dynamics of STP. Since chunking involves associating multiple items in an integrated unit, the memory capacity for chunking should equal the number of individual units the system can keep in WM. In this study,

we build up a computational model to validate this idea. The model consists of a number of excitatory neuronal clusters, each of them representing a memory item, and an inhibitory neuron pool, which orchestrates the firings of excitatory clusters, so that no interference between memory items occurs. All excitatory clusters receive a homogeneous background input representing the attentional level. By increasing the background input, several neuronal clusters are activated in rapid succession, and by Hebbian learning, these clusters increase their connections to form an integrated group, i.e., chunking is achieved. In such a way, the neural system can construct multiple chunks, but the maximum size of a chunk the neural system can retrieve is still restricted by the dynamics of STP.

Disclosures: **M.V. Tsodyks:** None. **Y. Mi:** None.

Poster

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P50-MH094263

R01-MH106512

Title: Recollection precision is supported by posterior-medial hippocampal networks: causal evidence from non-invasive brain stimulation

Authors: ***A. NILAKANTAN**, D. BRIDGE, E. GAGNON, J. VOSS;
Med. Social Sci., Northwestern Univ., Chicago, IL

Abstract: Recollection memory depends critically on the hippocampus. The functional significance of fMRI-defined distributed networks of the hippocampus, such as the posterior-medial cortical network, remains unclear. Here we used a noninvasive brain stimulation method that has previously been used to increase fMRI connectivity of the hippocampal posterior-cortical networks to test their role in recollection success and precision. Subject specific parietal stimulation locations were determined based on high resting-state fMRI connectivity with the body of hippocampus. In a counter-balanced order, participants (n=12) went through five consecutive days of repetitive TMS (20Hz) and five days of vertex stimulation (sham). EEG was

recorded during a spatial memory task, involving the recall of 96 object-locations, administered 24 hours before and 24 hours after each 5-day stimulation period. Effects of stimulation were assessed on recollection success, defined as trials with error less than a threshold determined by a two-process model of recollection versus guessing, and on precision, defined as the mean error for successful trials. Within successfully recollected trials, memory precision improved following targeted stimulation, relative to sham. Furthermore, stimulation modulated event-related neural correlates normally associated with recollection. Relative to pre-stimulation, targeted network stimulation reduced late-positive potential amplitude and theta/alpha power at posterior electrodes. Greater amplitude reduction of the ERP post-stimulation was associated with larger precision improvement. Targeting the posterior hippocampal-cortical network with multiple-day rTMS therefore improved memory precision and modulated oscillatory and ERP neural correlates of recollection. These findings provide evidence for a causal role of posterior hippocampal-cortical networks in precision of episodic memory.

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Poster

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Topic: H.02. Human Cognition and Behavior

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John Templeton Foundation

Intel Labs

Title: Externalizing the internal process of context reinstatement through closed-loop neurofeedback

Authors: ***M. T. DEBETTENCOURT**, N. B. TURK-BROWNE, K. A. NORMAN; Princeton Univ., Princeton, NJ

Abstract: Memory retrieval is strongly influenced by mental context: The extent to which the context at retrieval matches the context that was present during encoding is predictive of retrieval

success. Here, we sought to manipulate context using closed-loop neurofeedback; specifically, we set out to "externalize" contextual reinstatement by increasing the visibility of studied pictures when participants' brain state at recall matched their brain state at encoding. We hypothesized that this would amplify fluctuations in contextual reinstatement, thereby making it easier to detect them neurally and relate them to subsequent recall. In two studies, participants completed multiple runs of a memory task during real-time fMRI. For each run, they first studied lists of words interleaved with photographs from a category (e.g., scenes) that served as the context. After encoding, they were cued to one list and instructed to reinstate its context by thinking about the photographs that had appeared with that list. These photographs were then presented, overlaid with photographs from a different category (e.g., faces). Reinstatement was measured with a classifier applied to patterns of BOLD activity across voxels in temporal and occipital cortex. Feedback about how well a participant reinstated context was returned by manipulating the proportion of the categories in the composite photograph. If there was more classifier evidence for the category from the cued list, the proportion of that category increased. After reinstatement, participants were probed to freely recall as many words from the cued list as possible. As predicted, in this feedback regime, neural evidence for reinstatement of the study context closely tracked behavioral recall performance. This relationship was not found when participants were asked to recall words from a different list (not associated with the reinstated context). To test whether feedback is necessary to detect a relationship between classifier evidence and recall, we ran a follow-up study comparing the feedback condition above to a no-feedback condition in which the context photographs were consistently visible during the reinstatement period, providing a perfect retrieval cue. We hypothesized that decoupling the visibility of the photographs from neural reinstatement would weaken our ability to track contextual reinstatement and predict recall. Indeed, we replicated our prior findings for the feedback condition, but did not find a relationship for the no-feedback condition. This suggests that neurofeedback can help to make "visible" subtle fluctuations in contextual reinstatement that would otherwise be difficult to track.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Cooperative Agreement N66001-14-2-4032

Title: Targeted brain stimulation to modulate memory in humans

Authors: ***Y. EZZYAT**[1], J. E. KRAGEL[1], J. F. BURKE[3], D. F. LEVY[2], L. O'SULLIVAN[1], P. WANDA[1], M. R. SPERLING[4], G. A. WORRELL[5], M. T. KUCEWICZ[5], K. A. DAVIS[6], T. H. LUCAS[6], C. S. INMAN[7], B. C. LEGA[8], B. C. JOBST[9], S. A. SHETH[10], K. ZAGHLOUL[11], J. M. STEIN[6], S. R. DAS[6], R. GORNIAK[4], D. S. RIZZUTO[1], M. J. KAHANA[1];
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Abstract: People often forget information because they fail to effectively encode it. Brain activity during encoding predicts remembering, which suggests that memory is better when the neural mechanisms of learning are efficiently deployed. Here, we test the hypothesis that targeted electrical stimulation can modulate encoding efficiency and subsequent memory outcomes. Using recordings from neurosurgical patients with intracranially-implanted electrodes, we trained machine learning classifiers to discriminate spectral activity during learning that predicted remembering from activity that predicted forgetting. We then used the classifiers to decode neural activity in later sessions in which we applied electrical stimulation during learning. Stimulation increased recall performance if delivered when the classifier indicated low encoding efficiency but tended to decrease recall if delivered when the classifier indicated high encoding efficiency. Across individuals, classifier decoding during the stimulation sessions predicted the stimulation-induced change in memory and showed this effect was most prominent for medial temporal lobe targets. In identifying the conditions under which stimulation modulates memory, the data suggest strategies for therapeutically treating memory dysfunction.

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Poster

353. Human Cognition and Memory II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.15/HHH22

Topic: H.02. Human Cognition and Behavior

Title: Pre-stimulus oscillatory activity reveals a preparatory form of episodic retrieval orientation

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Abstract: It is well established that the neural activity elicited during an episodic memory task will differ based on the type of information that is being targeted for retrieval. Such *retrieval orientation* (RO) differences have been shown with event-related potentials (ERPs) to onset within about 300 ms after item onset and to also be evident for new test items. The effects have thus been suggested to indicate the strategic orienting of retrieval processes intended to maximize overlap between test items and potential memory traces. One form of RO effect that has been somewhat difficult to observe is that evident prior to test-item onset, as would be presumed to occur if RO reflects preparatory processes engaged to support episodic retrieval. Previous studies have limited the detection of such differences, however, by focusing ERP analyses on post-stimulus amplitude effects that necessitate baseline correction in the pre-stimulus interval. The current study resolves this issue by testing for differences in oscillatory EEG power to determine whether RO effects take shape prior to item presentation. Subjects encoded a list of intermixed materials (pictures and words) and then completed memory tests that targeted retrieval attempts for only one type of material at a time in separate blocks. Oscillatory power at high frequencies (beta and gamma) differed according to the targeted material over frontal/central scalp and as early as 500 ms prior to item onset. These results provide novel evidence that the deployment of RO processes occurs in preparation of upcoming items. The findings are discussed in regard to their implications for increasing the statistical power to test RO, by including *all* test items (instead of only new items), and for using such preparatory effects to predict retrieval success.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA RAM N66001-14-2-4032

Title: Large-scale assessment of the effects of direct electrical stimulation on brain network activity

Authors: *M. J. KAHANA[1], Y. EZZYAT[1], B. C. LEGA[2], J. W. GERMI[2], G. A. WORRELL[3], M. T. KUCEWICZ[3], M. R. SPERLING[4], C. S. INMAN[5], P. C. HORAK[6], K. A. DAVIS[7], K. ZAGHLOUL[8], S. A. SHETH[9], J. M. STEIN[7], S. R. DAS[7], R. GORNIAK[4], D. S. RIZZUTO[1]; [1]Dept Psychol, Univ. Pennsylvania, Philadelphia, PA; [2]Univ. of Texas Southwestern, Dallas, TX; [3]Mayo Clin., Rochester, MN; [4]Thomas Jefferson Univ. Hosp., Philadelphia, PA; [5]Emory Univ. Hosp., Atlanta, GA; [6]Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [7]Hosp. of the Univ. of Pennsylvania, Philadephia, PA; [8]NIH, Bethesda, MD; [9]Columbia Univ., New York, NY

Abstract: Direct electrical stimulation (DES) of the human brain is often used to treat symptoms of motor dysfunction in Parkinson's Disease and has been increasingly applied to modulate high-level cognitive processes. However, studies that have used DES in the medial temporal lobe and hippocampus to modulate episodic memory function have reported that DES leads to disruption in memory function at least as often as it leads to improvement. This inconsistency is at least partly due to the fact that the mechanisms of influence of DES on neural activity remain poorly understood. Here, we report data from a large study (N = 180) of DES designed to identify how medial temporal lobe stimulation influences neural activity across the brain. In patients undergoing clinical monitoring for medically refractory epilepsy, we applied DES trains of varying frequency, amplitude and duration to subfields of the hippocampus, the medial temporal lobe cortex and other cortical control areas distributed across the brain. We simultaneously recorded local field potentials from implanted electrodes distributed across the brain. We analyzed the effects of stimulation on brain activity by first measuring changes in power across the frequency spectrum in our recording electrodes. Hippocampal stimulation tended to evoke simultaneous increases in high-frequency activity and decreases in low-frequency activity in electrodes distributed across the temporal and frontal lobes, a pattern that has been identified in prior work as reflecting successful memory function. In patients that also performed free recall memory tests in separate sessions, we trained a logistic regression classifier to discriminate brain-wide encoding activity predicting memory success from memory failure. We then applied the classifiers to decode the multivariate patterns of activity evoked during the stimulation task. We found that stimulation frequency modulated brain activity in a way that increased classifier output, suggesting that higher frequency stimulation generally leads to brain states that predict successful memory encoding. These data shed light on how to optimally select stimulation parameters to modulate neural activity associated with successful memory function.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 MH061975

Title: Electrophysiological biomarkers of successful spatial memory encoding

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Abstract: Recent studies of verbal episodic memory have revealed that successful encoding exhibits a distinct electrophysiological signature in the human brain: Throughout the medical temporal lobe (MTL), prefrontal cortex, and several other memory related regions, increased high frequency activity (HFA, 64-95 Hz) and decreased low frequency activity (LFA, 3-9 Hz) during memory encoding predicts that a word will be subsequently recalled on a delayed memory test (Burke, 2014). This pattern differs from spatial memory tasks wherein LFA has been shown to exhibit increases during virtual movement and wayfinding to known targets (Caplan, 2003). Because spatial and verbal memory tasks differ along many dimensions, it remains unknown whether good memory encoding of spatial information correlates with one or the other of these two patterns.

To address this question, we investigated electrophysiological biomarkers of good memory encoding in the context of a spatial navigation task in which subjects drove through a virtual town and were aurally presented with items they were asked to recall later. We used intracranial encephalography (iEEG) to record subdural and deep brain neural activity of neurosurgical epilepsy patients that required chronically implanted electrodes recordings from widespread brain regions. We first compared the changes in iEEG spectral power surrounding the presentation of items that were recalled versus those that were not. Consistent with the work of verbal episodic memory tasks (Burke, 2014), we found that surrounding the encoding of recalled items, there was increased HFA and decreased LFA. The increases in HFA were mainly localized to the MTL, while the decreases in LFA were more widespread throughout the brain. These effects were most prevalent over a 0 to 2 second time epoch after item presentation, but the differences in HFA could be seen as early as 750 ms before item presentation. Our findings suggest that the increased HFA and decreased LFA associated with successful verbal episodic memory encoding translate to free recall in virtual navigation as well.

Burke, J. F., Sharan, A. D., Sperling, M. R., Ramayya, A. G., Evans, J. J., Healey, M. K., . . . Kahana, M. J. (2014). Theta and high frequency activity mark spontaneous recall of episodic

memories. Journal of Neuroscience, 34 (34), 11355{11365. doi: 10.1523/JNEUROSCI.2654-13.2014
Caplan, J. B., Madsen, J. R., Schulze-Bonhage, A., Aschenbrenner-Scheibe, R., Newman, E. L., & Kahana, M. J. (2003). Human theta oscillations related to sensorimotor integration and spatial learning. Journal of Neuroscience, 23 , 4726{4736.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Restoring Active Memory (RAM) program (Cooperative Agreement N66001-14-2-4032

Title: Identifying biomarkers of spatial memory with direct brain recordings in the Treasure Hunt task

Authors: *J. MILLER[1], A. WATROUS[1], C. NOVICH[1], S. LEE[1], M. SPERLING[2], A. SHARAN[2], G. WORRELL[3], B. BERRY[3], B. LEGA[4], B. JOBST[5], K. DAVIS[6], S. SHETH[7], S. DAS[6], J. STEIN[6], R. GORNIAK[2], D. RIZZUTO[8], J. JACOBS[1];
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Abstract: The ability to remember where objects are located in the world is a vitally important skill for both humans and animals. Using electrocorticographic data from epilepsy patients with implanted electrodes, we investigated the neural correlates of object-location binding in “Treasure Hunt”. Treasure Hunt is a video game-like task that measures humans’ ability to remember links between objects and locations in a rich 3D virtual environment. In the navigation phase of the task, patients explored a virtual beach and periodically opened treasure chests to reveal hidden objects, with the goal of encoding the location of each encountered item. During the subsequent retrieval phase, patients were probed with an object and were asked to identify the location where the object was originally presented. We used signal processing and univariate

statistics to identify brain signals whose amplitude predicted successful spatial memory encoding. In addition, we applied machine learning methods to classify successful vs unsuccessful spatial memory encoding on an item-by-item basis. We found that good spatial memory encoding was associated with neural biomarkers at multiple time points and frequencies, including an "early" signal present before the object was revealed, and a "late" signal present after the object identity was known. We also identified brain signals that related to individuals' subjective confidence about their memory responses. Lastly, we used these techniques to infer the scale of patients' spatial memory representations. Our work demonstrates that there are multiple types of neural signals that relate to spatial memory in humans, and that these signals can be successfully measured using advanced 3D paradigms that are on par with modern video games.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA

Title: Rostral-caudal and hemispheric differences in human hippocampal theta oscillations during episodic memory encoding

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Abstract: In rodents, the hippocampal 4-9 Hz theta oscillation demonstrates a reliable power increase to predict successful memory encoding. In human studies of episodic memory and spatial navigation, power increases in this frequency range during mnemonic processing have demonstrated a mixed set of effects, with some evidence of power increases in the 2-5 Hz range in the setting of a broad power decrease. We sought to further explicate the data for human hippocampal theta activity by taking advantage of the emerging technique of stereo EEG to obtain simultaneous recordings from the anterior and posterior portion of the human

hippocampus, as well as from both cerebral hemispheres. Across 114 electrodes in 17 patients, we examined oscillatory activity and connectivity obtained via stereo EEG as participants performed an episodic memory task. Using precisely targeted rostral and caudal hippocampal electrodes (defined relative to the superior colliculus), we demonstrate that 2-5 Hz power increases during memory encoding preferentially occur in the tail of the hippocampus in the dominant hemisphere and both the head and tail in the non—dominant hemisphere. A persistent gamma band power increase, as previously described, is present throughout anatomical regions. Rostral—caudal and bilateral hippocampal connectivity at 3-5 Hz predicts successful item encoding as well. Our data suggest some of the heterogeneity within existing data for human hippocampal activity during memory encoding is attributable to rostral—caudal and hemispheric differences in activity.

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Topic: H.02. Human Cognition and Behavior

Support: DARPA RAM 564000

Title: Architecture of a whole-brain ECoG memory network reveals asynchronous activity of MTL during encoding.

Authors: ***E. A. SOLOMON**[1], M. R. SPERLING[3], G. A. WORRELL[4], B. M. BERRY[4], K. A. DAVIS[5], C. S. INMAN[7], B. C. LEGA[8], B. C. JOBST[9], S. A. SHETH[10], K. ZAGHLOUL[11], J. M. STEIN[6], S. R. DAS[5], R. GORNIAK[3], D. S. RIZZUTO[2], M. KAHANA[2];
[2]Psychology, [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Thomas Jefferson Univ., Philadelphia, PA; [4]Mayo Clin., Rochester, MN; [5]Neurol., [6]Radiology, Hosp. of the Univ. of Pennsylvania, Philadelphia, PA; [7]Emory Univ., Atlanta, GA; [8]Neurosurg., UT Southwestern Med. Ctr., Dallas, TX; [9]Neurol., Dartmouth Giesel Sch. of Med., Lebanon, NH; [10]Neurosurg., Columbia Univ., New York City, NY; [11]Neurosurg., Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Spectral phase from electrocorticography (ECoG) has long been used to study the neural substrates of human memory, a cognitive process that is vulnerable to disruption by common disease states. Less well known is the structure of brain-wide networks derived from phase synchronization. Prior studies of phase synchronization networks focus either on small subsets of brain regions involved in human memory, or observe connectivity only between entire

hemispheric lobes which may be too spatially coarse to reflect true memory encoding dynamics. To determine the high-resolution connectivity profile between memory regions and other brain structures, we assessed gamma and theta phase synchronization during a free recall memory task in a large dataset of 276 human subjects. We used graph-theoretic tools to parse the architecture of the derived connectivity patterns, and the resulting spatio-temporal networks represent the first detailed whole-brain ECoG networks constructed. During memory encoding, hippocampus and parahippocampus (collectively, MTL) exhibit a robust desynchronization from the rest of the brain in gamma-band frequencies and a synchronization in theta-band. Specifically, right/left MTL tend to strongly desynchronize from each other and bilateral temporal cortex. These results suggest that gamma activity reflects asynchronous neural activation of memory regions, with maintenance of lateral vs. medial temporal desynchronization playing a particularly important role in encoding. Detailed connectivity analyses like these provide a framework for (1) identifying connectivity biomarkers of brain states conducive to good memory encoding and (2) identifying targets for electrical stimulation to enhance memory performance.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Restoring Active Memory (RAM) program (Cooperative Agreement N66001-14-2-4032)

Title: Boundary-related neural oscillations in the human hippocampal formation

Authors: ***S. LEE**[1], J. MILLER[2], A. WATROUS[2], M. SPERLING[3], A. SHARAN[3], G. WORRELL[4], B. BERRY[4], B. LEGA[5], B. JOBST[6], K. DAVIS[7], R. GROSS[8], S. SHETH[9], S. DAS[7], J. STEIN[7], R. GORNIAK[3], D. RIZZUTO[10], J. JACOBS[2];
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Abstract: Environmental boundaries play a major role in spatial representation across a wide range of tasks, from early navigation abilities in children, to place learning in virtual reality environments, to 2D visual scene recognition. The neural correlates of boundary representations (i.e., boundary/border cells) have thus far been most widely studied in the rodent hippocampal formation. Nevertheless, converging evidence from a wide range of species and methodologies suggests that the neural correlates of spatial navigation are widely shared across vertebrates. Therefore, while the human correlate of boundary cells have not yet been discovered, it may be possible to detect boundary-specific neural activity in the human brain, particularly in the entorhinal cortex (EC) and subiculum where boundary-related neurons have been found in rodents. In this study we tested neurosurgical patients implanted with intracranial electrodes in a virtual navigation task with goal locations that varied across trials.
Here we present direct neural recordings from the hippocampal formation of 22 patients, comparing trials in which subjects encoded goal locations near environmental boundaries to trials in which they encoded goals in the central region of the arena. We find significantly higher theta power for trials in which the goal locations were near the boundaries of the arena. These results are not accounted for by differences in difficulty between near-boundary vs. central locations. We further investigate these effects in specific hippocampal subregions in which boundary cells have been found in rats (i.e., EC and subiculum) and discuss their implications for boundary representation in the human brain.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Restoring Active Memory (RAM) program (Cooperative Agreement N66001-14-2-4032)

Title: Direct brain recordings reveal patterns of theta and alpha oscillations related to spatial navigation and memory

Authors: ***U. R. MOHAN**[1], J. MILLER[1], A. WATROUS[1], S. LEE[1], M. SPERLING[2], A. SHARAN[3], G. WORRELL[5], B. BERRY[5], B. LEGA[6], B. JOBST[7], K. DAVIS[8], R. GROSS[10], S.

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Abstract: The goal of this project is to characterize the brain signals that distinguish moving through space and remembering particular locations. We collected human electrocorticographic recordings from patients who performed a spatial memory task where they were moved through space in a consistent sequence of rotating towards an object, moving towards an object, and then stopping at the object location. We characterized the neural correlates for each of these types of movement as well as distinguishing the brain signals that predict memory for the target object. We found that amplitudes of alpha and theta oscillations related to movement during navigation such that they increase in amplitude when the person arrived at the target location and more so when the person's memory of the location was strong. Furthermore, these signals were stronger in the right hemisphere than in the left hemisphere. These findings support the view that the right hemisphere plays a particularly important role in spatial cognition. Moreover, our work suggests that a distinct oscillatory network activates when a person tries to remember their current location in space, as demonstrated by the large right hemisphere activation that appears while stopping and that relates to successful memory.

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Poster

353. Human Cognition and Memory II

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Program#/Poster#: 353.23/HHH30

Topic: H.02. Human Cognition and Behavior

Title: Strategic orienting of retrieval processes toward simulated memories of different artificial remoteness

Authors: *E. K. LEIKER[1], E. A. GRIFFITHS[2], E. N. WRIGHT[1], J. D. JOHNSON[1]; [1]Psychological Sci., Univ. of Missouri, Columbia, MO; [2]Univ. of Surrey, Guildford, United Kingdom

Abstract: A ubiquitous finding in episodic memory research is that successful retrieval diminishes as targeted memories become more remote. Interpretations of this finding often point to mechanisms that operate on the memory trace, such as those involving decay or interference. The likelihood of retrieval, however, can also depend on the strategic orienting processes that subjects adopt to allow them to restrict their search space. Recent studies have demonstrated that these orienting differences are also evident in retrieval tasks designed to target memories encoded at distinct times (remote vs. recent). Here, we employ a novel encoding paradigm to investigate time-related retrieval orientations for memories encoded in a single experimental session. Subjects first studied words in the context of imagining autobiographical details from three different periods in their lives: one month ago, one year ago, and five years ago. Blocked retrieval tests then employed exclusion task procedures in which the words from only one period were targeted, while subjects had to reject words from the other periods. Event-related potentials (ERP) elicited by new items indicated differences across the different test blocks, whereby the ERPs over frontal scalp were more positive for the 5-years-ago condition than for the other two conditions. These results suggest that subjects are able to strategically orient retrieval processing toward memories that are even artificially associated with different times. The findings are also consistent with the literature on episodic future simulation whereby subjects can flexibly place events in different time periods.

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Topic: H.02. Human Cognition and Behavior

Support: DoD Cooperative Agreement N66001-14-2-4032

Title: Effects of electrical brain stimulation location on interictal epileptiform activity

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Abstract: Electrical brain stimulation has been used as a therapeutic intervention for some neurological disorders including refractory epilepsy. The effects of brain stimulation on seizure and interictal epileptiform discharge (IED) rates have been studied. However, the influence of different stimulation parameters remains unclear. We analyzed intracranial EEG data recorded from 30 epilepsy patients undergoing clinical monitoring to elucidate the effect of stimulation location on IED rates. Data were collected for 63 half-hour stimulation sessions as part of the Restoring Active Memory (RAM) project. Stimulation sites included both medial-temporal-lobe (MTL) structures (n = 42) and neocortical regions (n = 21). Stimulation frequencies were 10, 25, 50, 100, and 200 Hz. Clinicians confirmed stimulation amplitudes for individual subjects and brain locations. Overall, we did not observe a significant change in IED rates between the 2 minutes before and after stimulation sessions (median change = +0.03 IEDs/minute, p = 0.2, signed-rank test). However, there were 4 clear outliers, in which IED rates changed by over +/-2 IEDs/minute. Each of these 4 sessions involved stimulation of MTL structures (hippocampus and perirhinal cortex). Considering all MTL stimulation sessions did not reveal a significant change in IED rate (p = 0.07, sign-rank test) or a difference relative to all non-MTL sessions (p = 0.08, U test). However, as suggested by the 4 outliers, the variability of IED rate changes was significantly higher for MTL stimulation than non-MTL stimulation (p = 0.01, Ansari-Bradley test). That is, stimulation of MTL structures lead to generally larger increases and decreases in IED rates. These results suggest that stimulation of MTL structures has the potential to influence IED rates in epilepsy patients more dramatically than stimulation of other areas does. The factors affecting the direction of the change remain to be determined.

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Poster

353. Human Cognition and Memory II

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.25/HHH32

Topic: H.02. Human Cognition and Behavior

Support: Diamond Foundation

DOD Cooperative Agreement N66001-14-2-4032

Title: Electrocorticographic changes in hippocampal oscillations during memory tasks and spatial navigation in ambulatory humans

Authors: *S. MEISENHELTER[1], M. E. TESTORF[1], P. C. HORAK[1], N. R. HASULAK[2], T. K. TCHENG[2], D. S. RIZZUTO[3], M. J. KAHANA[3], B. C. JOBST[1];
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Abstract: Intracranial electrophysiology of memory has been primarily studied in rodents using spatial navigation paradigms, leading to the momentous discoveries of place cells, grid cells, and a relationship between spatial memory and theta oscillations in the hippocampus. However, these discoveries have proven difficult to reproduce in humans. Human electrocorticography-based (ECoG-based) studies are typically conducted in a perioperative setting, where alterations of subjects' medication regimens and side effects of acute implantation injury confound studies of brain activity.

In this study, we recruited subjects with a chronically implanted RNS® Neurostimulator (NeuroPace, Inc.) to perform real world spatial navigation tasks and free recall tasks. Because subjects are not in a perioperative setting, we were able to remove many confounding factors that are present in studies using traditional ECoG methods. We developed hardware and software tools to allow for interaction of the neurostimulator with an external task and to allow us to synchronize the neurostimulator with the task computer by adjusting for differences in clock speed and irregularity.

We found a significant decrease in low frequency activity while subjects were walking compared to standing still. Subjects also performed free recall memory tasks, during which the effects of various electrical stimulation paradigms (stimulation of abnormal epileptiform activity versus scheduled stimulation) on memory performance were measured. Effects of stimulation were variable by subject and enrollment of more subjects is ongoing.

Disclosures: **S. Meisenhelter:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NeuroPace, Inc.. **M.E. Testorf:** None. **P.C. Horak:** None. **N.R. Hasulak:** A. Employment/Salary (full or part-time): NeuroPace, Inc. **T.K. Tcheng:** A. Employment/Salary (full or part-time): NeuroPace, Inc.. **D.S. Rizzuto:** None. **M.J. Kahana:** None. **B.C. Jobst:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NeuroPace, Inc..

Poster

353. Human Cognition and Memory II

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Topic: H.02. Human Cognition and Behavior

Support: DARPA grant N66001-14-2-4032

Title: Human memory enhancement through stimulation of middle temporal gyrus.

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Abstract: Direct stimulation of the human brain can elicit sensory and motor perceptions as well as recall of memories. Stimulating higher order association areas of the temporal cortex in particular was shown to activate multi-modal memory representations of past experiences (Penfield and Perot 1963, Brain 86). Hypothetically, this could be utilized to develop new treatments for cognitive deficits. Most of the recent attempts at memory enhancement in human patients have focused on the hippocampus and the associated medial temporal lobe structures reporting mixed results in small subject groups (Kim et al. 2016, Neurobiology of Learning and Memory, in press). Here we used a large dataset of intracranial recordings with stimulation in epilepsy patients to investigate the effect of targeted local stimulation on memory performance across a range of brain structures.
In total, 40 patients implanted with intracranial electrodes for seizure monitoring were stimulated during encoding of word lists for subsequent recall in two verbal memory tasks. In each task subjects remembered a list of 12 words for subsequent free recall following a math distractor problem. 50Hz continuous bipolar stimulation was delivered during epochs of word presentation through a pair of neighboring electrode contacts selected based on their anatomical localization and neurophysiological patterns of activity established from preceding passive task recordings. 22 distinct brain structures were stimulated to assess the effect on memory recall.
We report memory enhancement in two out of two cases of stimulation in the left posterior middle temporal gyrus, which resulted in significantly increased number of remembered words on stimulated versus non-stimulated lists ($p<0.05$, permutation test) with subjective experience of improved remembering of words in one of the patients. The effect of stimulation was

correlated with univariate changes in spectral power, coherence and phase synchrony, as well as by a multi-variate classifier analysis of spectral power changes characterizing successful word recall. There was no positive effect found in any other of the structures tested in this study, which included areas of the prefrontal cortex, hippocampus and the associated medial temporal neocortex.
To our knowledge, this is the first report of memory enhancement induced by superficial stimulation of the temporal neocortex. Our results support intelligent biomarker-driven mapping and targeting of specific cortical networks for development of novel neurotechnologies to modulate memory functions and treat cognitive deficits in humans.

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Poster

353. Human Cognition and Memory II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.27/HHH34

Topic: H.02. Human Cognition and Behavior

Title: Phase synchronization in the human medial temporal lobe predicts the precision of spatial memory encoding: Evidence from direct brain recordings

Authors: *A. WATROUS[1], J. MILLER[1], S. LEE[1], M. SPERLING[2], R. GORNIAK[2], A. SHARAN[2], G. WORRELL[3], B. BERRY[3], B. JOBST[4], K. DAVIS[5], R. GROSS[6], B. LEGA[7], J. STEIN[5], S. DAS[5], S. SHETH[1], D. RIZZUTO[5], J. JACOBS[1];
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Abstract: Lesion and fMRI studies have identified the hippocampus and adjacent cortical areas in the medial temporal lobe (MTL) as essential areas for spatial memory encoding, although the electrophysiological basis for spatial encoding is less clear. Prior invasive MTL studies have implicated oscillatory phase as a possible mechanism for coordinating activity within and between different MTL structures during episodic encoding. Here, we asked whether similar phase based mechanisms support spatial encoding by testing patients with pharmaco-resistant epilepsy on a virtual Morris water maze task. Patients were able to successfully encode an object

location in the environment and return to this location during a retrieval test. Assessing phase synchronization between all MTL electrodes in more than 25 patients in the low and high theta bands, we found that particular phase relations between MTL subregions was related to the precision of spatial encoding. These observations were statistically robust both on individual electrode pairs and in many patients. Moreover, phase synchronization was strongest in the slow theta/delta band. These findings further implicate slow theta phase as a mechanism of information representation and transmission in the human MTL.

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Poster

353. Human Cognition and Memory II

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Topic: H.02. Human Cognition and Behavior

Support: DARPA Restoring Active Memory (RAM) program (Cooperative Agreement N66001-14-2-4032)

NIH Grant MH55687

Title: Core episodic encoding and retrieval processes revealed by dynamics of neural activity

Authors: ***J. E. KRAGEL**[1], Y. EZZYAT[1], J. F. BURKE[2], J.-J. LIN[3], J. M. STEIN[4], S. R. DAS[4], R. J. GORNIAK[5], R. E. GROSS[6], K. A. DAVIS[4], M. R. SPERLING[5], B. C. JOBST[7], S. A. SHETH[8], K. A. ZAGHLOUL[9], G. A. WORRELL[10], D. S. RIZZUTO[1], M. J. KAHANA[1]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of California, San Francisco Med. Ctr., San Francisco, CA; [3]Univ. of Texas Southwestern, Dallas, TX; [4]Hosp. of the Univ. of Pennsylvania, Philadelphia, PA; [5]Thomas Jefferson Univ. Hosp., Philadelphia, PA; [6]Emory Univ. Hosp., Atlanta, GA; [7]Dartmouth Med. Ctr., Lebanon, NH; [8]Columbia Univ. Med. Ctr., New York, NY; [9]NIH, Bethesda, MD; [10]Mayo Clin., Rochester, MN

Abstract: The capacity to remember the past is dependent on neural processing that occurs not only when an event is experienced but also when it becomes reinstated into awareness at a later point in time. As a result, memory can fail due to deficient encoding or retrieval operations. Neuroimaging studies of episodic memory have identified cortical regions that commonly

predict memory success during encoding and retrieval, including the medial temporal lobe (MTL) and prefrontal cortex. In contrast, neural processing has been observed that is specific to either encoding or retrieval operations, such as retrieval-related engagement of parietal systems spanning the inferior parietal lobe and precuneus. To elucidate whether common or distinct processes during encoding and retrieval best predict memory performance, we administered a delayed free recall task to 187 neurosurgical patients fitted with subdural or intraparenchymal depth electrodes and analyzed the recorded spectral activity to identify multivariate signatures of good memory encoding and retrieval. We identified biomarkers of memory success that were jointly predictive of both successful memory formation and recall, in the form of increased high frequency activity (HFA) in prefrontal, MTL, and inferior parietal cortices, accompanied by a decrease in low frequency power. In addition, we identified a signal of successful retrieval that was not informative of successful memory formation, marked by a distinct pattern of increased HFA in the hippocampus. These results suggest that while distinct neural mechanisms accompany the encoding and retrieval of episodic information, processes that are common to both operations are sufficient to predict mnemonic success.

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Poster

353. Human Cognition and Memory II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.29/HHH36

Topic: H.02. Human Cognition and Behavior

Support: NINDS IRP

DARPA RAM

Title: Studying the effects of direct subdural electrical stimulation in human subjects during a verbal associative memory task

Authors: ***T. SHEEHAN**[1], R. YAFFE[1], J. WITTIG, Jr[1], S. INATI[1], G. WORRELL[2], M. KUCEWICZ[2], K. DAVIS[3], M. KAHANA, PhD[3], M. SPERLING[4], S. A. SHETH[5], B. JOBST[6], B. LEGA[7], K. ZAGHLOUL[1];
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Abstract: Electrical stimulation of the human entorhinal cortex can improve spatial associative memory. Here we examine whether stimulation can improve non-spatial associative memory in volunteers with drug-resistant epilepsy who were implanted with intracranial electrodes for seizure monitoring. Participants (n=34) completed 1-4 sessions of a verbal paired associates task on a laptop computer while intracranial EEG (iEEG) signals were digitally recorded. Lists of six word pairs were presented sequentially for four seconds each, and approximately 45 seconds later one of the words from each pair was presented and the participant was instructed to say the missing partner word. Twelve volunteers also performed the task while subdural electrical stimulation was applied during pseudo-randomly selected word pairs.

Stimulation had no consistent across-subject effect on memory performance, as measured by overall differences in percent correct for stimulated versus non-stimulated word pairs. However, one subject showed significant memory impairment for word-pairs presented during hippocampal stimulation, and another subject showed a significant memory improvement for word-pairs presented during stimulation of the frontal cortex. These findings left two open questions: (1) for the subjects that did show significant memory changes due to stimulation, could we use brain-wide iEEG signals to predict, on a trial-by-trial basis, whether stimulation was going to be effective, and (2) for all other subjects, could a similar trial-by-trial analysis reveal consistent across-subject effects of stimulation on memory performance?

We built an L2-regularized logistic regression classifier using the spectral power of iEEG recordings to predict, on a trial-by-trial basis, whether a word-pair would be remembered. The optimized classifier accurately predicted performance during non-stimulated word pairs in sessions with stimulation, but did not accurately predict performance during stimulated word pairs. This classifier-based approach did not reveal a consistent across-subject effect of stimulation on memory performance. For the subjects highlighted above who demonstrated significant performance changes due to stimulation, we used the classifier to characterize brain-wide physiological responses to stimulation. We found that stimulation caused robust increases and/or decreases in high frequency power at locations that were highly predictive of memory performance. These preliminary findings suggest that machine learning algorithms can successfully be used to predict memory performance based on brain-wide iEEG signals in some subjects.

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Poster

353. Human Cognition and Memory II

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 353.30/HHH37

Topic: H.02. Human Cognition and Behavior

Title: Another View on Deja vu - a memory illusion that results from a failure of reality monitoring

Authors: *P. WALLISCH[1], M. J. GODDARD[2];
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Abstract: The deja vu experience is one of the most striking memory illusions encountered in everyday life. Whereas its phenomenology and prevalence is well established, the deja vu experience remains fundamentally unexplained. Several proposed hypotheses as to its nature and origin cannot account for the full range of phenomenological reports and - perhaps as a consequence - deja vu experiences cannot yet be reliably elicited in the lab. To gain a deeper understanding of this experience, we asked several thousand participants questions online about the typical properties of their deja vu experiences such as frequency and duration as well as questions about their lifestyle and habits.The emerging picture from our well-powered approach is consistent: The more someone reports to watch movies, travel or remember their dreams, the more likely they are to experience deja vu, whereas all other reported habits show no clear trends. In other words, the more someone engages in behavior that makes it likely to create a confusion between the source of new and old experiences, the more probable deja vu becomes. We conclude that deja vu is perhaps due to a recognized mismatch between an association from episodic memory and knowledge from semantic memory. This effect could be driven by exposure to patterns of environmental stimulation for which the brain is not prepared in evolutionary terms (e.g. travel, movies). These could evoke a sense of familiarity owed to the activation of patterns from episodic memory. This explanation accounts for all known aspects of the experience and opens up avenues for studying it in a lab setting in the future.

Disclosures: P. Wallisch: None. **M.J. Goddard:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.01/HHH38

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH097061

Title: Orbitofrontal cortex neurons encode confidence in an auditory decision

Authors: *P. MASSET[1], M. LAGLER[2], J. SANDERS[1], T. KLAUSBERGER[2], A. KEPECS[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Ctr. for Brain Res., Med. Univ. of Vienna, Vienna, Austria

Abstract: The ability to assign appropriate levels of confidence to decisions is essential to perform behaviors in complex and changing environments. Previously neural correlates of decision confidence have been found in the orbitofrontal cortex (OFC) in an olfactory decision task (Kepecs et al, 2008) and OFC inactivation was shown to diminish the ability for confidence reporting in rats (Lak et al, 2014). Confidence is often conceptualized as a form of metacognition, an ability to monitor cognitive processes. Therefore, a brain region computing a metacognitive decision confidence signal would be expected to represent confidence irrespective of the modality used to make the decision. Here we set out to test this hypothesis by recording OFC neural activity during an auditory decision task. Rats were trained to perform an auditory decision task (Kepecs and Sanders, 2012, Brunton et al, 2013) complemented with a behavioral report of their decision confidence. Rats listened to a binaural stream of Poisson clicks and had to decide, by leaving the sampling port, whether the left or the right stream had a faster click rate. Once they reported their choice by entering the left or right choice ports they had to wait for a delayed water reward delivered at randomized timings. The time rats were willing to wait for an uncertain reward was proportional to decision confidence, as predicted by theory, and served as a behavioral report of confidence. We recorded OFC neuronal activity using tetrodes while rats (n=2) performed this task. We found 2 populations whose activity was strongly modulated by decision confidence. The first population showed a strong modulation at the beginning of the anticipation period, once the rat has entered the choice port but is waiting for the outcome of his choice. The second population showed a strong modulation from the onset of the movement period to the beginning of the anticipation period. The activity of those OFC neurons showed three signatures of decision confidence (or it's inverse, decision uncertainty): (i) firing rates monotonically predicted accuracy, (ii) firing rates increased (decreased) with stimulus discriminability for correct choices and decreased (increased) with discriminability for error choices and (iii) firing rates predicted accuracy beyond stimulus discriminability (i.e. psychometric functions were steeper (shallower) for trials with higher firing rates), similar to the

confidence encoding found during olfactory decisions. These results suggest that OFC carries information about decision confidence based on multiple sensory modalities and could be the seat of computation of a "metacognitive" confidence signal.

Disclosures: **P. Masset:** None. **M. Lagler:** None. **J. Sanders:** None. **T. Klausberger:** None. **A. Kepecs:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Program#/Poster#: 354.02/HHH39

Topic: H.01. Animal Cognition and Behavior

Support: Charles H. Revson Foundation

Title: Neural signals in the anterior cingulate cortex during effort-based decision-making

Authors: *S. E. MORRISON;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: A functionally intact anterior cingulate cortex (ACC) is essential for overcoming high effort requirements to obtain larger rewards. However, it remains unclear how neural signaling in the ACC supports effortful choices. Although the bulk of work on effort-based choice has been performed in rodents, few studies have examined neuronal activity in the rodent ACC during decision-making incorporating varying effort. In particular, it is unknown how neurons in the rodent ACC represent effort at the moment of decision.
In order to shed light on this question, we recorded the activity of single ACC neurons during a task in which we systematically varied the effort - i.e., number of lever presses - required to obtain a large reward. Rats chose between a low-effort/low-reward (LR) option, in which one lever press resulted in the delivery of 2 sugar pellets, and a high-effort/high-reward (HR) option, in which a variable number of lever presses - ranging from 1 to 8 - resulted in 4 sugar pellets. Trials were presented at unpredictable intervals, and each trial was initiated by an auditory cue and the extension of one or both levers. The effort level was varied in a block-wise fashion; each block consisted of 20 forced choice trials, in which only one option was available, and 10 free choice trials. We found that rats allocated their choices based on effort: when effort levels were equal (at 1 lever press) they virtually always chose the HR lever, but as the effort requirement increased, they reduced their choices of the HR lever accordingly.
Individual neurons in the ACC had a variety of response profiles during this task, including a subset that exhibited phasic excitation or inhibition in response to cue presentation. By

examining forced choice trials, we found that many cue-responsive neurons encoded anticipated effort, most commonly by reducing their cue-evoked response as effort levels increased. Notably, this was often accompanied in the same block by an increased response to the cue when lower effort was expected. On free choice trials, some neurons tracked the effort associated with an upcoming choice, while others appeared to encode the effort associated with the HR option whether or not it was chosen. Together, these findings provide a neural signaling basis for the essential role of the ACC in decision-making based on effort.

Disclosures: S.E. Morrison: None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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Topic: H.01. Animal Cognition and Behavior

Support: CAS Hundreds of Talents Program

STCSM Grant 15JC1400104

Title: Representations of probabilistic evidence in the prefrontal cortex during decision making

Authors: *T. YANG, Y. ZHANG, Y. CHEN;
Inst. of Neurosci., Shanghai, China

Abstract: We often make decisions based on pieces of evidence that are processed sequentially. For optimal results, we need to combine them according to the laws of probability. Previously, we have shown that neurons in the lateral intraparietal area (LIP) encode accumulated evidence from multiple pieces of evidence in the form of log likelihood ratio (logLR) during a sequential probabilistic categorization task involving eye movements. It is unclear, however, where in the brain are logLRs for individual pieces of evidence encoded. In the current study, we recorded from the orbitofrontal (OFC) and lateral prefrontal (LPFC) areas in a monkey that has been trained to perform a similar probabilistic categorization task as used in our previous study. The monkey viewed four shape cues presented sequentially near a central fixation point and had to choose between a red or green peripheral choice target by making a saccade toward it when the fixation point was turned off. The shapes were drawn with replacement from a pool of 10, furnishing evidence of correct targets. Each shape was presented for 300ms with a 200ms gap in between shape presentations. After training, the monkey was able to base its choices on the combined logLR from the four shapes by learning the appropriate weights of the shapes. We

recorded single- and multi-unit activity from both OFC and LPFC. Our preliminary results showed that neurons in both orbitofrontal and lateral prefrontal areas encoded logLRs of individual shapes. In addition, we found that some neurons in both areas encoded absolute values of logLR of individual shapes. Contrary to what was previously observed in LIP, few neurons in either OFC or LPFC encoded total logLR. These findings suggest that these prefrontal areas may play a different role than LIP during decision making.

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Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Program#/Poster#: 354.04/III1

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 15K16570

Title: Decision related activities of anterior insular and orbitofrontal cortex in a gambling behavior of rats

Authors: ***H. ISHII**[1], Y. KAIZU[1], S. TAKAHASHI[1], S. OHARA[1], P. N. TOBLER[2], K.-I. TSUTSUI[1], T. IIJIMA[1];
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Abstract: To survive the competitions, sometimes we should take risks even if they could wreak losses. However excessive risk taking can result in ruin. Thus, the point is to balance between risk taking and avoiding. How does the brain use the two strategies? We are focusing on the roles of the two brain regions, the anterior insular cortex (AIC) and the orbitofrontal cortex (OFC). We previously found that inactivating AIC reduced risk preference of the rats in a gambling task whereas inactivating OFC increased it (Ishii et al., 2012), suggesting that these two regions play opposite functions in risky decision making; AIC promotes risk taking whereas OFC suppresses it. Here, to reveal how the AIC and OFC activate at the timing of the decision, we have recorded the single unit activities in the AIC and the OFC during the performance of the gambling task. We used five adult male Wistar rats and implanted the multichannel electrodes into their AIC and OFC. The gambling task offered water deprived rats the choice between a risky option which possibly provided 4 drops of water but in 50% and a sure option which guaranteed 2 drops of water. These two options were associated with left and right levers respectively. For the instruction of the relationship between the lever locations and the two options, the first 40 trials

of a session were set as forced choices trial. The following 160 trials were forced and free choice trials. In addition, to test the selectivity of neuronal activity to the lever location, the rats were re-tested on the gambling task, where the combination of the options and the levers was switched. To date, we recorded 230 single unit activities in the AIC and 167 neurons in the OFC. For the analysis, we used two-way ANOVA [risk/sure × right/left] to determine whether the activity was correlated with choice type or lever location. Here we focus on the activity during 500ms before the lever press. In the AIC, we found 18 neurons (8%) correlated with choice type, 45 neurons (20%) correlated with lever location, and 12 neurons (5%) correlated with both. In the OFC, we found 9 neurons (5%) correlated with choice type, 52 neurons (31%) correlated with lever location, and 18 neurons (11%) correlated with both. We next analyzed the neurons, which correlated with choice type and both, whether they fired for risky choice or sure choice. In the AIC, 13 neurons increased the firing and 17 neurons decreased the firing preferentially for risky choice. Meanwhile, in the OFC, 11 neurons increased the firing for risky choice and 16 neurons increased the firing for sure choice. These differences might contribute to the functional difference between the AIC and the OFC in risky decision making.

Disclosures: **H. Ishii:** None. **Y. Kaizu:** None. **S. Takahashi:** None. **S. Ohara:** None. **P.N. Tobler:** None. **K. Tsutsui:** None. **T. Iijima:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.05/III2

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R01MH062349

STCSM 14JC1404900

STCSM 15JC1400104

Title: A circuit model for the interplay between orbitofrontal cortex and lateral prefrontal cortex in value-based economic decision-making

Authors: ***M. Y. YIM**[1], X. CAI[1], X.-J. WANG[1,2];
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Abstract: Previous work (Padoa-Schioppa and Assad 2006, Cai and Padoa-Schioppa 2014) showed that economic decisions can be made independently of the spatial configuration of the

offers and the action necessary to implement the choice (in goods space). In addition, neurons in the orbitofrontal cortex (OFC) encode the choice outcome in goods space. Furthermore, a recent study (Cai and Padoa-Schioppa 2014) demonstrated that such abstract choice outcome encoded in the OFC may be transformed into an action plan (good-to-action transformation) through the lateral prefrontal cortex (LPFC), a cortical output target of OFC. In that study, a substantial fraction of OFC neurons encoded the choice outcome in the presence of the offer cues, but the encoding faded away once the offer cues were turned off which constituted a memory period before the action plans were revealed to the animal. Therefore, OFC neurons did not appear to maintain the memory of choice outcome. On the other hand, the choice memory trace was observed in the LPFC, where neuronal activity underwent a transition from encoding choice outcome in goods space to representing the action plan for obtaining the outcome. To examine how such good-to-action transformation may take place in LPFC, we construct a network model based on neurophysiological and behavioral observations. The network consists of the following main modules: 1) a memory module for storage of abstract choice outcome information from OFC; 2) a spatially-selective module receiving input from the choice memory module and saccade target information (potential action plans) from visual areas. Combining abstract choice and saccade target information, together with the synaptic interactions within the spatially-selective module, the chosen target signal emerges in the network. The experimental and computational modeling studies together suggest that LPFC is a potential neural substrate for memory maintenance of a value-based choice and good-to-action transformation during economic decision-making.

Disclosures: **M.Y. Yim:** None. **X. Cai:** None. **X. Wang:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Program#/Poster#: 354.06/III3

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant F31 MH107111-01

Title: Properties of value adaptation in orbitofrontal cortex

Authors: *K. CONEN, C. PADOA-SCHIOPPA;
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Abstract: The subjective values that guide economic decision making can vary by orders of magnitude across behavioral contexts. To generate effective choices in different circumstances,

neural circuits must account for the value scale while remaining sensitive to small (relative) value differences. Previous work showed that value-encoding neurons in the orbitofrontal cortex (OFC) and other brain regions undergo range adaptation (Tobler et al., 2005; Padoa-Schioppa, 2009; Bermudez and Schultz, 2010; Kobayashi et al., 2010; Cai and Padoa-Schioppa, 2012). However, it remains unclear exactly what features of the value distribution guide adaptation in any behavioral context. In this study, we examine the hypothesis that the maximum and minimum values of the distribution provide reference points for value encoding. During the experiments, rhesus monkeys chose between two juices (A and B) offered in variable amounts. Each session included 2-3 blocks of ~250 trials. Within each block, we kept the range of values constant for each juice while varying offers pseudo-randomly across trials. Between blocks, we varied the range (minimum and maximum values) for one or both juices. We recorded and analyzed the activity of 492 neurons from the OFC of one animal. Previous work in similar conditions indicated that OFC neurons encode variables offer value A, offer value B, chosen juice and chosen value (Padoa-Schioppa and Assad, 2006). Following established procedures, we analyzed neuronal firing rates in seven time windows and defined a neuronal response as the activity of one cell in one time window. Neuronal responses that passed a one-way ANOVA (factor: trial type, $p<0.05$) in at least two consecutive blocks were assigned to one of the four variables based on linear regressions. Focusing on responses encoding the offer value ($n = 266$) or the chosen value ($n = 224$), we compared the tuning slope, activity for maximum value, and activity for minimum value across trial blocks. Neurons adapted systematically to changes in both the maximum and minimum values. In blocks with a narrow range of values (high minimum or low maximum), neurons encoded value more steeply (higher slope), showing greater sensitivity to small differences. Neurons consistently remapped their maximum response to the maximum value in each context, suggesting that it served as a reference point for encoding. In contrast, changes in the minimum value produced less consistent effects across neurons. Interestingly, adaptation to the minimum value appeared to be stronger when the maximum and minimum values changed simultaneously, suggesting that a change in the maximum value may serve as a contextual cue to trigger adaptation.

Disclosures: **K. Conen:** None. **C. Padoa-Schioppa:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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NIH Grant T32 EY007125

NARSAD Young Investigator Award from the Brain and Behavior Research Foundation

Title: Rule encoding in orbitofrontal cortex and striatum

Authors: *G. LOCONTE, B. SLEEZER, M. CASTAGNO, B. HAYDEN;
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Abstract: The brain's reward system is often distinguished, both functionally and anatomically, from its executive control system. While reward processing is most often attributed to the orbital surface of the cortex and the ventral and medial parts of the striatum, executive control is most often attributed to dorsal and lateral structures (especially dorsomedial and dorsolateral prefrontal regions). Recent research, however, suggests that the orbitofrontal cortex (OFC) and its striatal targets may also contribute to executive processes, such as flexible rule-based decision-making, but their respective contributions are largely unclear. We hypothesized that OFC and striatum contribute to flexible decision-making by maintaining and signaling changes in task requirements. To test this hypothesis, we examined firing rates of single neurons in OFC and two striatal regions, dorsal (DS) and ventral (VS) striatum, in a modified version of the Wisconsin Card Sorting Task. We found significant encoding of both specific rules and rule category in all three regions. Rule also affected phasic responses to choice offers following rule acquisition, indicating that the acquisition of rule information influences response selectivity. These effects were not explained by differences in behavioral performance across rules or rule-specific changes in reward expectation. Thus, our results challenge the common notion that OFC and its striatal targets have pure reward functions and instead endorse the hypothesis that OFC, VS, and DS are a critical part of the brain's control system, albeit one that happens to show strong activity modulations for reward.

Disclosures: G. Loconte: None. **B. Sleezer:** None. **M. Castagno:** None. **B. Hayden:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.08/III5

Topic: H.01. Animal Cognition and Behavior

Title: Comparing neural activity patterns in striatum and orbitofrontal cortex during set shifting using a dynamics model

Authors: *P. BALASUBRAMANI[1], B. Y. HAYDEN[2];
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Abstract: Single unit activity patterns in reward regions are highly heterogeneous, and thus gaining insight into the mechanism or activity structure of the neural responses is highly challenging. Dimensionality reduction of population activity to a handful of explanatory variables has been successful in explaining the single trial phenomena in various cognitive activities involving learning and decision making. Those explanatory variables provide a denoised version of the underlying shared neural processes of a population.
Here we used Gaussian Process Factor Analysis to explore the single trial activity patterns during different stages of rule learning and decision making. This dynamics model helps to characterize the population activity through time in a low dimensional space.
Our dataset consists of single unit recordings in three brain regions (204 cells in dorsal striatum, 103 cells in ventral striatum and 115 cells in orbitofrontal cortex) while macaques performed a Wisconsin Card Sorting Task. On each block, monkeys were required to learn one of six rules (three color and three shape rules) and then make a saccadic response according to one of three colored shapes matching the correct rule. Rules changed randomly every 15 correct trials in a block.
We first compute the latent factors that characterize the covariance among neurons in reward regions after removing their inherent spike variability. Then, we extract the low dimensional trajectories for specific trial types of interest by projections on the identified factors. We use Gaussian mixture models to classify these low dimensional responses. The results show that these low dimensional features generated from factor analysis dynamics model can be effectively used to understand the neural code as a function of task variables. The trajectories differentiate various phases within a single trial, such as stimulus presentation, choice and outcome. We obtain higher efficiency of clustering the activity in any reward region- for classifying trial accuracy while using their neural pattern during choice and outcome epoch, and -for classifying rule learning while using their stimulus presentation epochs. The study aims to find the probability of classifying activity patterns from various reward regions for distinguishing stages of rule learning by referring to the subject's behavioral response. To summarize, we show that a dimensionally reduced form of ensemble neural activities can be effectively used to label and classify task variables across reward regions. Such strategies for single unit recordings could be an essential step to infer their simple underlying neural pattern.

Disclosures: P. Balasubramani: None. **B.Y. Hayden:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.09/III6

Topic: H.01. Animal Cognition and Behavior

Support: R01 DA037229

Title: Shared economic roles of subgenual and dorsal anterior cingulate cortices in decision making

Authors: *H. AZAB[1], B. Y. HAYDEN[2];
[1]Brain and Cognitive Sci., [2]Dept. of Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Most models of subgenual anterior cingulate cortex (sgACC) emphasize its emotional, limbic, and arousal-related roles, while models of dorsal ACC (dACC) emphasize its cognitive and regulatory functions. To compare these areas directly, we recorded activity in both regions in a two-alternative, value-based decision-making task where monkeys gambled for tokens, allowing us to compare neural responses to wins and losses. We found that, while sgACC was more weakly driven, both regions encoded several basic economic variables; including offer values, remembered values, chosen values, and outcome values. Overall, sgACC showed enhanced firing for losses, while dACC showed enhanced firing for higher outcome magnitudes - regardless of valence. While choice-related variables were significantly encoded in both areas, this encoding arose earlier in dACC, suggesting a pre-decisional role for this region, as opposed to a purely evaluative one. These results highlight the common economic functions of the anterior cingulum, and suggest there may be some specialization for option selection and evaluation.

Disclosures: H. Azab: None. **B.Y. Hayden:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.10/III7

Topic: H.01. Animal Cognition and Behavior

Support: DA027127

Title: Adolescent alcohol use increases risk preference and alters dopamine receptor expression in OFC.

Authors: *S. D. CORWIN, E. JACOBS-BRICHFORD, J. D. ROITMAN;
Univ. of Illinois At Chicago, Chicago, IL

Abstract: Adolescence is a critical period for neural development of the Prefrontal Cortex (PFC), an area that is implicated in reward encoding to support decision-making. The continuing maturation of PFC during adolescence makes it susceptible to toxins, such as alcohol, that may alter its anatomy and function, leading to behavioral deficits. Repeated exposure to alcohol during adolescence has been associated with disrupted reward processing in adulthood, which may manifest as elevated risk-preference. To determine a potential mechanism underlying alcohol-induced changes in neural encoding and corresponding risk-preference, we measured the expression of PFC dopamine (D1, D2) and acetylcholine receptors (nAChrnaα4β2, nAChrnaα7). Rats were given access to alcohol in a gelatin medium for 1h daily during adolescence. As adults, they performed daily sessions of a risk task where preference for small-certain vs. large-risky rewards was measured. Subsequent to behavioral task performance, gene expression analysis of the Orbitofrontal region of PFC (OFC) was done using RT-qPCR. The results showed that male rats who consumed high amounts of alcohol in adolescence exhibited higher preference for large-risky rewards than either control or low alcohol consuming male counterparts. Furthermore, female rats who consumed high levels of alcohol did not show an elevated risk preference compared to control and low alcohol consuming female counterparts, indicating that alcohol's effects on risky behavior in adulthood is sexually dimorphic. RT-qPCR results revealed an increase in D2 receptor expression in low alcohol consuming male and female rats, suggesting that reduced expression of D2 receptors may play a role in increased vulnerability for the risky behavior phenotype.

Disclosures: S.D. Corwin: None. **E. Jacobs-Brichford:** None. **J.D. Roitman:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 4R00AA021780-02

Title: The role of the orbitofrontal cortex in incentive learning

Authors: E. T. BALTZ, *C. M. GREMEL;
Univ. of California San Diego, La Jolla, CA

Abstract: The orbitofrontal cortex (OFC) has been shown to control goal-directed actions dependent on outcome value. However, whether the OFC is directly involved in the updating of action value is unclear. Previous research suggests that incentive learning, or updating the value of a reward triggered by a new motivational state, is mediated by the basolateral amygdala (BLA). The OFC has excitatory projections to BLA, which has been implicated in updating value, and to other regions involved in goal-directed actions. To probe the role of OFC in updating outcome value, we adapted an incentive learning test for mice. In order to target excitatory projection neurons in the orbitofrontal cortex, we used a chemogenetic approach and used rAAV5/hSyn-DIO-hM4D- mcherry and rAAV5/CamKIIa-GFP-Cre to express inhibitory DREADD hM4Di receptor in CamKIIa expressing OFC neurons. During training, mice under minimal food restriction (2 h) learned to press one lever to gain access to a secondary lever that resulted in delivery of sucrose (20% w/v). Mice were then food restricted for either extended (16 h) or minimal (2 h), following which mice had the opportunity to consume randomly delivered sucrose. Minimally food- restricted control mice licked significantly less than those under extended restriction. Administration of the hM4Di agonist clozapine-*n*-oxide (CNO) and subsequent attenuation of OFC projection neuron activity did not alter the extended restriction-induced increase in licking behavior. The following day, in the same food restricted state but with no CNO pretreatment, mice were given a 5 min non-reinforced probe test. In this probe test, control mice with minimal restriction maintained baseline response rates while control mice with extended restriction substantially increased their response rate over baseline. In contrast, mice previously treated with CNO (prior to sucrose exposure) did not show increased responding following either 2 or 16 h food restriction. This finding suggests that OFC projection neuron activity specifically is necessary for the ability to update the value of sucrose. Lastly, in the same food restricted state, mice were given a 60 minute rewarded test to investigate real-time updating of value. Extended restriction mice that had been previously treated with CNO resumed responding at the rates of the extended restriction controls. These results point to the involvement of the orbitofrontal cortex in mediating the updating of outcome value and further parse out what information OFC is sending downstream.

Disclosures: **E.T. Baltz:** None. **C.M. Gremel:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Topic: E.04. Voluntary Movements

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Title: A hippocampal-posterior parietal cortex circuit for memory-based decision making

Authors: *U. RUTISHAUSER[1,3,2], T. AFLALO[3], N. POURATIAN[4], C. Y. LIU[5], A. N. MAMELAK[1], R. A. ANDERSEN[3];
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Abstract: Memories allow us to utilize previous experiences for making decisions. The medial temporal lobe (MTL) is essential for episodic memories, but how memories influence decision making is poorly understood. The posterior parietal cortex (PPC) is thought to support memory-based decision making by integrating memory-based information provided by the MTL. We recorded single neurons in two groups of patients to study this process: the MTL in epilepsy patients implanted with depth electrodes for localizing seizures and the PPC (area AIP) in tetraplegic patients implanted with Utah arrays for a brain-machine interface clinical trial. Patients in both groups performed the same new/old recognition memory task. First, in a learning session, participants viewed a sequence of 100 novel images. Second, following a 30min break, participants viewed the same images (old) again intermixed with novel images (new). For each image, participants reported whether the image was new or old on a 6-point confidence scale. Patients in the MTL and PPC group had good memory with an accuracy of 71±7% and 75±4%, respectively. We recorded: i) 1065 MTL neurons from 28 patients in 44 sessions, and ii) 916 PPC (Area AIP) neurons from 2 patients in 7 sessions. We compared responses between correct and error trials as a strategy to differentiate memory from decision signals. We first identified memory-selective (MS) neurons in both areas by comparing responses between new and old stimuli in correct trials. We show three new findings. First, units differentiating new from old stimuli were prominent in PPC: 169 (18%) of units were MS cells, with some firing more to new (85) and some more to old (84) stimuli. Secondly, similar to the MTL, PPC MS neurons were

strongly modulated by decision confidence (ROC analysis, new vs. old trials for high and low confidence was 0.58±0.02 and 0.54±0.0, respectively, p<1e-6). Thirdly, during error trials, PPC MS neurons indicated the erroneous choice made by the subject rather than the ground truth (Defining the preferred tuning based on correct trials only, we found that Non-preferred>Preferred, p<1e-5). In contrast, MTL MS neurons (9% of all units) continued to signal the ground truth (Preferred>Non-preferred, p=0.008) despite the incorrect decision. Together, this shows the first single-neuron evidence of memory-based decision making in human PPC. PPC decision cells were modulated by memory strength (confidence) and indicated the choice the subject made regardless of its correctness. In contrast, MTL cells provided the evidence (input) for the decision. This is the first human single-neuron evidence for a MTL-PPC circuit for episodic-memory based decision making.

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Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.13/III10

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal theta entrains and reconfigures prefrontal single-unit activity during delay in a navigational task

Authors: *M. V. MYROSHNYCHENKO, C. C. LAPISH;
Indiana Univ., Indianapolis, IN

Abstract: The ability to flexibly direct cognitive resources towards task-relevant variables and transiently hold this information in memory is critical to guide decision-making. To examine underlying processes facilitating spatial memory-guided decision-making, neural recordings were simultaneously obtained from the prefrontal cortex (PFC) and hippocampus (HC) of rats performing a well-validated model of working memory, the delayed spatial win-shift (DSWS) task on the radial arm maze. Each trial consisted of a study phase followed by a 60-second delay, where this information is held and/or manipulated to guide a behavior in the final (test) phase. Behavioral experiments suggest that rats are capable of using both retrospective (e.g. where have I been?) and prospective (e.g. where should I go?) strategies to solve this task. A transition may occur at some point during task performance, probably the delay, where retrospective information is integrated into a prospective plan. Consistent with the role of theta entrainment in stabilization of representations in cortical circuits, theta power and HC-PFC coherence were

highest during delay. Simultaneously, some prefrontal units became entrained to hippocampal theta, and their subset gradually increased firing rate. These units were more entrained to theta during the second half of the delay than first. Only 20% of prefrontal units carried information helpful in study-test phase classification, and the content of spike trains transitioned from study to test configuration during delay. All of these characteristics were present only in trials where the animals would go on to perform well after the delay. Delay PFC activity in DSWS features a transition from a state where the HC and PFC are relatively decoupled to a phase-locked configuration, the emergence of which is predictive of optimal behavioral performance. Moreover, the emergence of phase-locking coincides with the hypothesized transition to prospective coding. Thus, it suggests that HC-mPFC communication facilitated by the theta band coherence and phase locking is important for remapping prefrontal networks to facilitate optimal test phase performance. These findings contribute to our understanding of computational role of delay-active neurons in spatial working memory.

Disclosures: M.V. Myroshnychenko: None. **C.C. Lapish:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Program#/Poster#: 354.14/III11

Topic: H.01. Animal Cognition and Behavior

Support: NIDA/NIH Intramural

Title: Hippocampal contributions to neural representations in OFC during decision making

Authors: *A. M. WIKENHEISER[1], Y. MARRERO-GARCIA[1], G. SCHOENBAUM[1,2,3]; [1]Natl. Instutite on Drug Abuse, Baltimore, MD; [2]Dept. of Anat. and Neurobio., Univ. of Maryland, Sch. of Med., Baltimore, MD; [3]Solomon H. Snyder Dept. of Neurosci., The Johns Hopkins Univ., Baltimore, MD

Abstract: The hippocampus and orbitofrontal cortex (OFC) have both been linked to model-based behavior, yet there is relatively little research testing how these areas interact to support such responding. To begin to address this question, we recorded single unit activity in OFC in rats performing a decision making task while inactivating the ventral subiculum, an output of the hippocampal processing stream. In each trial, rats sampled one of three odor cues. Two cues instructed rats to respond either left or right to earn liquid reward. A third cue indicated that responses in either direction would be rewarded. The reward available at each fluid well differed in both flavor (chocolate or vanilla milk) and magnitude (3 drops or 1 drop). The locations of the

large, 3-drop reward and the small, 1-drop reward reversed positions several times in each session, forcing rats to update their behavior. Electrodes were implanted in OFC, and a virus expressing halorhodopsin (halo) was infused into the ventral subiculum, where optical fibers were then implanted. In halo sessions, light was delivered for the duration of each trial to suppress output from the ventral subiculum. In control sessions, light outside of halo's frequency sensitivity was delivered over the same epoch. Halo stimulation slowed adaptation to the new reward contingency following block switches. This behavioral deficit was accompanied by a marked reduction in the proportion of OFC neurons active in anticipation of the outcomes in the halo sessions. In addition, the neurons that were active in this period in the halo sessions were less likely to represent information about the direction of rat's response on that trial relative to neurons recorded in the same position in the same rats during control sessions. Interestingly, neurons represented information about the identity and size of impending outcomes similarly in both halo and control sessions. Thus, hippocampal processing appears to facilitate the formation of integrated, direction-specific outcome predictions in OFC, while encoding of other features of the upcoming outcomes may not require hippocampal input.

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Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Program#/Poster#: 354.15/III12

Topic: H.01. Animal Cognition and Behavior

Title: Orbitofrontal cortex lesions improve performance in a go/no-go reversal learning task

Authors: *M. H. RAY[1], M. CRABTREE[2], C. PICKENS[2];
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Abstract: Previous research from our laboratory has shown that, after alcohol access during adolescence, the level of alcohol consumption during early adulthood is negatively correlated with reversal learning errors in a go/no-go task, such that higher drinking rats make fewer commission errors. Likewise, repeated anesthetic doses of ketamine also decrease the number of commission errors in the same task 3 weeks after the last ketamine injection. These patterns were found in a reversal learning task in which the rats were reinforced for withholding responses on no-go trials, and the reversal learning criteria required several consecutive sessions of reaching a daily performance criterion in order to pass reversal. In both experiments, the drug effects/correlations were found in the sessions that occurred after the rat's first session to reach the daily performance criterion, but before they had passed for several consecutive days (which

could be considered regressive errors). The neurobiological cause of these effects is unclear. As an initial step, we examined the effects of orbitofrontal cortex (OFC) lesions on a similar reversal learning task in male Long Evans rats. Rats were required to press a "go" lever and withhold responding from a "no-go" lever on 9 consecutive trials for one session to pass discrimination. During reversal, the identity of the levers was switched and the rats were required to make 9 correct responses in a row for 4 consecutive days in order to pass reversal. We found that OFC lesions caused rats to commit fewer commission and omission errors during reversal learning, and that these differences occurred in the sessions before the rats reached the daily criterion for the first time, but not in the sessions after this (perseverative or new learning errors). As the pattern of errors does not resemble that seen in the alcohol and ketamine experiments, it does not appear that our drug-related effects are related to orbitofrontal cortex function. Future experiments will investigate other potential neurobiological substrates of our drug-related effects.

Disclosures: **M.H. Ray:** None. **M. Crabtree:** None. **C. Pickens:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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NIH Grant 1R01DA038063-01

Title: Temporal context and decision-making: Behavioral and neural mechanisms of choice adaptation in rhesus macaque

Authors: *J. ZIMMERMANN, P. GLIMCHER, K. LOUIE;
Ctr. for Neuroeconomics, New York Univ., New York, NY

Abstract: Any organism has to react and adapt to a constantly changing environment, requiring its nervous system to encode broad ranges of information efficiently within finite constraints of coding capacity. In sensory systems, this problem is widely believed to be addressed by adaptive coding mechanisms like temporal adaptation and spatial normalization. Recent work has demonstrated that temporal adaptation occurs in reward-processing and decision-related brain areas, but the computational mechanisms and behavioral consequences of this temporal adaptation are largely unknown.Here, we present data from a saccadic choice task. Trained

monkeys were offered a choice between two options differing in reward magnitude and juice type. Blocks of trials were composed of a mixture of “adaptor trials” and “measurement trials”. In measurement trials, fixed across all blocks, monkeys were asked to choose between an unvarying reference reward (fixed reward magnitude and juice type) and one of 5 variable rewards. These responses allowed us to plot the monkey’s probability of choosing the reference reward as a function of the magnitude of the variable reward; a choice curve. Across blocks, we varied the structure of the adaptor trials and examined the effect of changes in the standard deviation of the adaptor magnitudes on the slopes of choice curves. While monkeys performed this task, single-unit activity from orbitofrontal cortex (area 13) was recorded. We found that adaptor variability significantly effects measurement trial choices. To account for these effects of adaptor variability, we implemented a two-stage model of adaptive value coding based on dynamic normalization. This model relies on two cascaded divisive-normalization networks, which we refer to euphemistically as “OFC” and “LIP”. Adaptation is implemented via divisive normalization and the excitatory output of the OFC network is used as an additional source of gain control in the LIP network. The time constants of the networks, however, differ by several orders of magnitude (OFC being much slower). This allows the OFC network to adapt the sensitivity of the LIP network.Observed adaptor-driven changes in choice curve slope and single trial level choices can be accounted for by our model. Our model predicts daily variability in choice stochasiticity which a non-dynamic model can not. We are currently in the process of relating monkey OFC single cell neuronal dynamics to our behavioral and model predictions and demonstrate initial evidence of value coding variability changes due to adaptation. Our findings suggest that divisive normalization may underlie adaptive value coding like that seen in previous work.

Disclosures: J. Zimmermann: None. **P. Glimcher:** None. **K. Louie:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.17/III14

Topic: H.01. Animal Cognition and Behavior

Title: Navigation and decision in a virtual foraging task for monkeys

Authors: *R. AKAISHI, B. HAYDEN;
Univ. of Rochester, Brain & Cognitive Sci., Rochester, NY

Abstract: In animal experiments, especially in rodents, decisions are known to occur with prospective information processing about the future trajectories of actions and its outcomes in

the space. However, it has not been known whether a similar prospective information processing is taking place in primates. This is partly due to a lack of the adequate experimental paradigm that can induce such future-oriented representations in the realistic space. We developed the virtual reality foraging environment to examine the behavior and neural activity of monkeys performing navigation and foraging decisions in a realistic space. In order to promote a natural sense of the space, we have arranged the coordination of the monkey's natural orienting behavior and the change of the orientation in the virtual space. With this experimental setup, we have begun to conduct two kinds of experiments: one with more controlled setting similar to the rodent experiments and another with open space to investigate the mechanisms of foraging decisions in a more naturalistic setting. We first investigate the basic mechanisms of prospective decision making in the maze tasks with a clear decision point and alternative paths with defined trajectories of movements to reach to the targets. This experimental setting helps to identify the neural representations of the spaces/events that are to be experienced in the course of action. In the second kind of experiment, monkeys freely navigate in a virtual open field that is akin to the environment some animals face in their natural foraging behavior. The clusters of the reward objects ("food") are distributed in the VR space to mimic the "patches" of the natural environment. We examine how the neural representations change when the monkeys leave the patch and go to the other patches and how the representations are maintained when the monkeys stay in the same patch. These analyses and interpretations are aided by the insight obtained in the first set of the experiments conducted with the more controlled VR space.

Disclosures: R. Akaishi: None. **B. Hayden:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.18/III15

Topic: H.01. Animal Cognition and Behavior

Support: Klingenstein Foundation

Templeton Foundation

Title: Prospective evaluation involves reactivating neural response patterns associated with outcome monitoring

Authors: *Z. WANG, B. Y. HAYDEN;
Univ. of Rochester, Rochester, NY

Abstract: Decision-makers must assign values to options before choosing between them. We hypothesized that prospective evaluation relies on reactivating patterns of neural activity elicited by outcomes. To test this idea, we compared neural responses to reward outcomes with offer-period responses in orbitofrontal area 13 (OFC) in a choice task. Consistent with the reactivation hypothesis, we found strong quantitative similarities between offer and outcome ensemble response patterns. A similar match was observed in ventromedial area 14 (vmPFC) but not ventral striatum (VS), suggesting the reactivation principle may be cortically specific. We interleaved trials in which offers values were indicated symbolically (described offers) and communicated by water aliquots (experienced offers). We found no overlap in the neural patterns elicited by these two types of offers, suggesting that our results are not attributable to a single value coding scheme in OFC. These results support the hypothesis that prospectively evaluating rewards involves simulating their receipt. We further extended these ideas in an observational learning task. We recorded from dorsal anterior cingulate cortex (dACC) while monkeys took turns with either another monkey or a computer algorithm to reveal and receive rewards in a changing environment. Neural responses in dACC for prospective evaluation, outcome monitoring, action control, fictive reward, and social learning were then compared.

Disclosures: Z. Wang: None. **B.Y. Hayden:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 17022052

KAKENHI 22300138

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KAKENHI 26119504

KAKENHI 26330266

KAKENHI 16H03301

Title: The role of the monkey orbitofrontal cortex during value-based decision-making

Authors: *T. SETOGAWA[1,2], T. MIZUHIKI[2,3], F. AKIZAWA[3,4], R. KUBOKI[3], B. J. RICHMOND[1], N. MATSUMOTO[5], M. SHIDARA[2,3];
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Abstract: When we choose one item from several alternatives, we consider their values from amount of reward and the amount of work needed to obtain them. To study the neuronal mechanism of such a decision-making, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC), a cortical region that the neurons carry information about rewards and their values. Two monkeys were initially trained to perform a reward schedule task. In this task, the monkey had to complete the schedule composed of 1, 2 or 4 trials of visual discriminations to earn 1, 2 or 4 drops of liquid reward. After the monkey learned this task, the decision-making schedule task was introduced. The decision-making schedule task had a decision-making part and a reward schedule part. In the decision-making part, two choice targets (CT) were presented sequentially at the center of a computer monitor. Brightness and length of the CT were proportional to the amount of liquid reward (1, 2, or 4 drops) and the number of the visual-discrimination trials (1, 2, or 4 trials) needed, respectively. After both first and second CTs had been presented, the same two CTs simultaneously reappeared side by side of the fixation point. The monkey was required to choose one of the two CTs by touching the corresponding bar in the chair. Then, the chosen reward schedule task was started. We recorded 256 neurons in the monkey OFC during the decision-making schedule task (137 and 119 neurons from each monkey). The CT values were estimated from the monkey's choice behavior using an exponential discounting model of reward value. To analyze the relation between the firing rate in the second CT period and the first and the second CT values, we performed a generalized linear model analysis. 26.6% (68/256) of the neurons showed a significant correlation between the neuronal firing and the first and the second CT values. 37/68 neurons had different signs for the coefficients of 2 CT terms, and 31/68 neurons had coefficients with the same signs. Moreover, 14.8% (38/256) of recorded neurons coded currently presented CT values. Finally, to examine the role of OFC neurons for this choice behavior, we injected muscimol into the recording sites bilaterally to inactivate the tissue locally. Following injection, choice accuracy and speed were degraded in the CT value dependent manner. These results suggest that OFC not only encodes information about reward value, but is critical for normal choices to occur.

Disclosures: **T. Setogawa:** None. **T. Mizuhiki:** None. **F. Akizawa:** None. **R. Kuboki:** None. **B.J. Richmond:** None. **N. Matsumoto:** None. **M. Shidara:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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Topic: H.01. Animal Cognition and Behavior

Support: S.E.C is supported by the Middlesex Hospital Medical School General Charitable Trust

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L.T.H is supported by a Sir Henry Wellcome Fellowship (098830/Z/12/Z)

Title: Autocorrelation structure at rest predicts value correlates of single neurons during decision-making

Authors: *S. E. CAVANAGH, S. W. KENNERLEY, L. T. HUNT;
UCL Inst. of Neurol., London, United Kingdom

Abstract: Value correlates can be found ubiquitously throughout the prefrontal cortex (PFC) during reward-guided choice. However, at the level of individual neurons - including those simultaneously recorded from within the same cortical area - there is substantial variability in the strength with which decision variables are 'coded'. We tested the hypothesis that the autocorrelation in neuronal spiking at rest, which likely provides an insight into a neuron's position within an underlying network architecture, could explain the variability of value correlates across single PFC neurons.
We analysed neuronal data recorded whilst four monkeys (Macaca mulatta) performed a cost-benefit decision making task (Hosokawa et al. 2013). Importantly, choice and reward were separated by a cost epoch (1500-5400ms), which allowed us to probe the maintenance and reactivation of neuronal representations across time. Resting neuronal activity was determined from a fixation period (1000ms) which preceded each trial. This period was subdivided into non-overlapping, 50ms bins and spike-count autocorrelation was calculated (Ogawa and Komatsu, 2010). The decay of autocorrelation with increasing bin separation was captured by an exponential equation (Murray et al. 2014). This equation was fitted to the autocorrelation of individual PFC neurons (Dorsolateral PFC (DLPFC) = 195; Orbitofrontal Cortex (OFC) = 99; Anterior Cingulate Cortex (ACC) = 152). A τ parameter (time constant) indexed the persistence of resting autocorrelation for each cell.
Throughout PFC, we observed a large degree of variability of both the single-neuron time constants and the strength of decision variable correlates. We found that responses of neurons with higher time constants - hence those exhibiting a more temporally sustained autocorrelation at rest - showed a stronger correlation with chosen value during choice. Within OFC, such

neurons sustained coding of chosen value from choice through to the experience of reward delivery, providing a potential neural mechanism for credit assignment. These findings reveal that within the prefrontal microcircuit, variability across neurons in time constant predicts their involvement in specific decision-making computations. Neurons exhibiting temporally extended autocorrelation at rest are more involved in the temporal integration and maintenance of evidence during choice.

Disclosures: S.E. Cavanagh: None. **S.W. Kennerley:** None. **L.T. Hunt:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 354.21/III18

Topic: H.01. Animal Cognition and Behavior

Support: NIDA R01 DA19028

NIMH R01 MH097990

Title: Prefrontal coding of strategies to reduce working memory load

Authors: *F.-K. CHIANG[1], J. D. WALLIS[1,2];
[1]Psychology, Univ. of California Berkeley Dept. of Psychology, Berkeley, CA; [2]Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Working memory is limited to a capacity of about 3 items but we rarely notice this constraint as we are able to implement strategies, such as chunking, that can overcome this limitation. However, precisely how these strategies lower capacity constraints remains unclear. We trained two monkeys to perform a spatial self-ordered search task with six targets. Each trial began with the subject fixating a central cue, after which a configuration of six targets appeared. The subject was required to saccade to each target one at a time, returning to fixation after each target, thereby requiring him to use working memory to keep track of which targets he has already visited. We identified two strategies that subjects employed to lower the working memory demands of the task and perform the task with fewer errors. The first strategy was to search each location in a stereotyped sequence e.g. responding to each item in a clockwise pattern. The second strategy was to chunk the targets into two groups of three and search within one group before moving to the second group. We recorded the activity of 1077 single neurons (368 from subject Q and 709 from subject R) that were widely distributed across dorsolateral prefrontal cortex (DLPFC). Across epochs, around 35% of DLPFC neurons showed changes in

firing rate that depended on the type of strategy being followed. In addition, the strategy modified the amount of information encoded in the firing rate. Specifically, stereotyped search patterns were associated with reduced spatial information being encoded in DLPFC neurons suggesting that these strategies can indeed reduce the load on working memory. In sum, our findings support a role for DLPFC in implementing strategies that can improve working memory performance.

Disclosures: F. Chiang: None. **J.D. Wallis:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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Topic: H.01. Animal Cognition and Behavior

Support: DARPA/ARO Contract # W911NF-14-2-0043

NIDA R21DA041791

Title: Biasing decision making through stimulus-outcome specific microstimulation of orbitofrontal cortex

Authors: *E. B. KNUDSEN, J. D. WALLIS;
Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Addiction is a disease of aberrant learning. Addictive substances and associated cues hijack normal reward circuitry in the brain and become overvalued relative to all else causing drug craving, seeking, and intake at the expense of healthy behaviors. These aberrant stimulus-outcome (SO) associations are manifested in frontolimbic (FL) networks that normally drive a wide range of healthy behaviors. An emerging therapeutic avenue, deep brain stimulation, has the potential to treat addiction through the direct targeting of maladaptive SO associations. However, modifying SO associations for the treatment of addiction requires understanding how SO associations are formed in FL networks and how these formations can be manipulated through targeted electrical stimulation. Here, we trained one non-human primate to perform a value-based decision task in which optimal SO associations must be learned and maintained. On each trial the subject is tasked with choosing the most valuable option of any pair of 3 total options, or forced to choose a single available option. After learning, options are driven to new stable values through random-walks. We implanted bilateral 96 channel semi-chronic devices to record and stimulate neurons throughout the FL network. We targeted orbitofrontal cortex (OFC)

as a candidate stimulation target due to its documented role in valuation of environmental stimuli. First we tested the effect of different stimulation parameters (frequency, amplitude) on subsequent value encoding when delivered prior to reward. We found that stimulation systematically modified value coding of neurons in the FL network in a frequency-dependent manner, while the amplitude influenced the numbers of neurons recruited downstream of OFC. Second, we paired stimulation to a single option and were able to reliably induce positive choice biases towards the stimulated option in 5 of 8 sessions. In the remaining 3 sessions, stimulation drove choices to chance levels. We found that the direction and magnitude of biasing effects were a function of the degree to which the subject needed to track drifting values. Further, we found that the FL network transitions from stimulus-independent to stimulus-dependent value coding as more precise SO associations were required, such that value coding for one option was not predictive of other options. Together, these results demonstrate preliminary evidence that learned SO associations within the FL network can be modified in real time through microstimulation and highlight several key issues that must be considered when moving towards systems-based therapies for neuropsychiatric disorders such as addiction.

Disclosures: E.B. Knudsen: None. **J.D. Wallis:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH095894-04

Title: Social context influences decision signals in primate ACC

Authors: *W. S. ONG[1,2], M. L. PLATT[2];
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Abstract: Effective social communication is essential for mediating cooperation. Human fMRI studies have shown brain areas, including the anterior cingulate cortex (ACC) that respond to cooperation; however the neural processes are unclear.To test this, we developed a new task based on the classic “chicken game”. 2 monkeys (M1&M2) face each other across a shared monitor showing 2 colored annuli framing dot motion arrays and 4 response targets. On some trials, the larger reward (denoted by visual tokens) lies opposite M1 behind the opponent (M2)’s annulus; Smaller rewards lie to the left. To obtain the larger reward, M1 goes straight, but if M2 also goes straight the annuli collide and neither monkey gets reward. On other trials, a

"cooperation bar" allows both to obtain larger rewards only if both choose to go left; If only one yields he receives a smaller reward. Dot motion coherence is randomized on some trials to obscure intention signals.Our 4 trained animals maximized juice intake by attending to the reward tokens as well as the choices of their opponent. Monkeys' strategies depended on their opponent: the dominant animal in the dyad preferentially aggressed and required more incentive to cooperate, while the subordinate preferentially yielded. Collisions were more frequent when a computer player replayed past trials in the presence of a 'decoy' monkey, compared with playing a computer in the absence of a decoy monkey or playing an active monkey. Players initiated cooperation for small rewards with an active player quickly, distinguishing between active players and decoys within about 15 trials.We next recorded the activity of 481 neurons from the ACC of two monkeys in these 3 conditions. In the decoy condition, neuronal responses scaled with the amount of juice received for selfish choices (ie the other monkey gets a tiny amount), but did not when the same reward was delivered for cooperation. This demonstrates that neurons in the ACC respond differentially to the presence and behavior of (non-)interactive agents. ACC neurons signaled the full value of the cooperative reward for an active cooperator but did not do so for decoy cooperators, suggesting ACC plays a role in the integration of social cues, actions, and outcomes to guide strategic decisions.

Disclosures: W.S. Ong: None. **M.L. Platt:** None.

Poster

354. Decision Making: Orbitofrontal, Anterior Cingulate, and Hippocampal Cortices

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Project 5R01NS086104

Title: Token asset effect on monkey's decision making involving risky gains and losses.

Authors: *Y.-P. YANG[1], X. LI[2], V. STUPHORN[1,2,3];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Neurosci., Johns Hopkins University, Sch. of Med., Baltimore, MD; [3]Zanvyl Krieger Mind/Brain Inst., Baltimore, MD

Abstract: The descriptive economic theories of decisions under risk postulate that humans: (1) evaluate the outcomes of decisions as gains or losses relative to their current wealth level, and (2) show different risk-attitude when facing gains or losses. However, the neural mechanisms underlying these effects are not known. We therefore designed a risk-based decision making task, in which the monkeys received token as a secondary reinforcer. Across multiple trials, they

had to accumulate six tokens to earn a standard fluid reward. This allowed us to test gamble options that resulted in a gain or a loss of token. In general, the monkeys showed an overall tendency of risk-seeking in both the gain and the loss condition. However, they displayed more preference for the gamble option when facing a risky gain than when facing a risky loss. This is the opposite of the typical risk preferences found in human studies. In a relative framework, the SV of the reward options is independent of the absolute number of tokens that the monkey starts or ends up with and solely depends on the relative change in token number. Such a pure relative SV model did not fit the behavioral data well. However, the model was better able to fit the data, when the starting token number was taken into account. With increasing token assets, monkeys were prone to choose the gamble option less often in the gain domain, but more or equally often in the loss domain. In an absolute framework, the SV of all reward option is independent of whether the relative change of tokens represents a gain or a loss. Instead, SV solely depends on the number of token the monkey will have at the end of each trial. We found the absolute SV model provided a good fit to behavior. We also found that the probability of getting reward in the near future strongly influenced monkey's choice behavior. Nevertheless, we also found different preferences for the gamble option in the gain and loss domain, even if the same absolute token number was the outcome (i.e., framing effects). Altogether, our behavioral findings indicate that the monkey's choices depend both on relative changes in wealth (gain/loss) and the absolute number of tokens that the monkey owns at the end of the trial. SV estimation is therefore best explained by a model that combines aspects of the relative and absolute framework.

Disclosures: **Y. Yang:** None. **X. Li:** None. **V. Stuphorn:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

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NIH grant NS073974

Title: Computational model of a positive BDNF feedback loop in hippocampal neurons following inhibitory avoidance training

Authors: ***Y. ZHANG**[1], P. SMOLEN[1], C. M. ALBERINI[2], J. H. BYRNE[1];
[1]Neurobio. & Anat., Univ. of Texas at Houston Dept. of Neurobio. and Anat., Houston, TX; [2]Ctr. for Neural Sci., New York Univ., New York City, NY

Abstract: Establishing new long-term memory (LTM) relies on multiple interacting molecular processes that operate on time scales ranging from minutes to days. As these molecular processes evolve over time, LTM becomes increasingly stable and insensitive to disruption for prolonged time periods (days to lifetimes), a process referred to as consolidation. Although extensive progress is being made in identifying the molecular mechanisms that underlie LTM, the dynamical properties of these molecular pathways are not well understood. To gain insights into molecular underpinnings of consolidation, a differential-equation based model was developed of a positive feedback loop that is activated by inhibitory avoidance (IA) training and that regulates the expression of brain-derived neurotrophic factor (BDNF) in hippocampal neurons. Constraints for the model were derived from data in published literature. The simulated feedback loop initiates with a training-induced release of BDNF. Increased release of BDNF leads to a rapid increase in phosphorylated cAMP response element-binding protein (pCREB), which in turn leads to a delayed increase in the level of the β isoform of CCAAT/enhancer binding protein (C/EBPβ). Subsequently, in accordance with data, C/EBPβ reciprocally increases *bdnf* expression. The model simulated the complete time courses of transcription and translation for BDNF and the time courses of CREB phosphorylation and C/EBP expression after training, and fit the empirical measurements up to 7 days after training. Simulations suggest that at least two independent self-sustaining signaling pathways downstream of the BDNF feedback loop may play substantial roles in long-term memory formation and persistence. The simulation results suggested that the delayed initiation of the BDNF positive feedback loop may play a substantial role in the dynamics of time windows for disruption of memory by protein synthesis inhibition (PSI) and other treatments. If in a given system memory consolidation depends on a positive feedback loop with little delay of activation, then there may be a significant early window of decreased sensitivity to PSI. An advance in initiation of a positive feedback loop, and therefore of maximal PSI sensitivity, might result from a stronger stimulus intensity. The putative biochemical elements that determine the delays of initiation, and of termination, of the BDNF feedback loop were also delineated.

Disclosures: **Y. Zhang:** None. **P. Smolen:** None. **C.M. Alberini:** None. **J.H. Byrne:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: R01-MH074736

Agalma Foundation

Title: Latent, long-lasting memory traces are stored during the infantile amnesia period in rats

Authors: *A. TRAVAGLIA, R. BISAZ, C. M. ALBERINI;
New York Univ., New York, NY

Abstract: Episodic memories formed during the first postnatal period are rapidly forgotten, a phenomenon known as *infantile amnesia*. Infantile amnesia is conserved throughout evolution, as it has been described in rodents as well as humans. In rats infantile amnesia is believed to last until approximately postnatal day 23 (PN23). It remains to be understood whether this amnesia is the result of immaturity of the infant brain, impaired memory retrieval or failure in memory storage. Furthermore, because several studies have shown that early life experiences can influence adult behavior, it also remains to be determined how and which mechanisms underlying infantile learning and amnesia have long-lasting effects. Using inhibitory avoidance (IA) in infant rats, we first recapitulated the infantile amnesia at PN17. In fact, at PN17 IA memory was acquired and expressed immediately after training, but then rapidly lost, while rats at PN24 expressed long-lasting memory similar to adult rats. However, exposing PN17 trained rats to a later reminder (comprised of an unpaired presentation of context and shock) reinstated a robust, context-specific and long-lasting memory. We also found that the acquisition of this latent, long-lasting memory trace was dependent on activity and BDNF-dependent mechanisms in the dorsal hippocampus. Furthermore, administration of BDNF into the hippocampus immediately after training rescued the infantile amnesia, as, in fact, it resulted in long-lasting IA memory expression. We then extended our finding to a non-aversive hippocampal-dependent learning paradigm, by employing the object location (OL) task. We found that, similarly to IA, OL memory was expressed immediately after training at PN17, but lost 2 hours later. Conversely, rats trained at PN24 retained and expressed long-term memories similar to those of adult rats. Like with IA, BDNF injected bilaterally into the dorsal hippocampus immediately after OL training at PN17 rescued the rapid memory decay and, in fact, led to significant memory expression 2h after training. We conclude that the hippocampus is critically engaged in the formation of latent, long-lasting memories during the period of infantile amnesia, and that infantile amnesia of both aversive and non-aversive experience is rescued by BDNF.

Disclosures: A. Travaglia: None. **R. Bisaz:** None. **C.M. Alberini:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: 5T32MH019524-23

R01-MH100822

Title: The role of glycogenolysis and astrocyte-neuronal coupling in mechanism in memory formation during development.

Authors: *E. CRUZ, A. TRAVAGLIA, C. M. ALBERINI;
Ctr. for Neural Sci., New York Univ. Ctr. for Neural Sci., New York, NY

Abstract: Previous studies from our laboratory showed that hippocampal glycogenolysis and astrocyte-neuronal lactate shuttling are necessary for the consolidation of inhibitory avoidance (IA) memory as well as for *in vivo* hippocampal long-term potentiation in adult rats. Here we investigated the role of hippocampal glycogenolysis and astrocyte-neuronal lactate metabolic mechanisms during memory consolidation in early development. Specifically, we focused on rats at postnatal day 24 (PN24), a pre-adolescent age at which rats have acquired the ability to form strong and long lasting hippocampal-dependent memories. We found that the hippocampal levels of the lactate dehydrogenase A, (LDHA), but not of LDHB, were significantly elevated in PN24 rats compared to adult rats (PN80). Furthermore, as lactate is transported via monocarboxylate transportes (MCTs), we quantified the hippocampal relative levels of MCT1, which is expressed in astrocytes, oligodendrocytes and in microvessel endothelial cells, MCT2, which is concentrated in neurons and MCT4, which is preferentially expressed in astrocytes. We found that MCT1 and MCT4, but not MCT2, were significantly elevated in PN24 rat hippocampus compared to the hippocampus of adult rats (PN80). Together, these results support the conclusion that hippocampal lactate metabolism and transport are higher during development. In addition, we found that hippocampal infusions of the inhibitor of glycogen phosphorylase and synthase activity 1,4-dideoxy-,4-imino-d-arabinitol (DAB), which blocks glycogenolysis, or the antisense-mediated knockdown of the MCT1, MCT2, and MCT4 at PN24 impairs long-term memory. L-lactate rescued the memory impairment produced by DAB or by the knock down of MCT1 or MCT4, but not of MCT2. These data suggest that, similarly to what we previously found in adult rats, learning-dependent lactate production from glycogen stored in astrocytes and lactate transport into neurons are required for long-term memory formation in early development. However, we found that higher levels of L-lactate were required to rescue the memory DAB and MCT knock down-induced impairments elicited at PN24 compared to adults, suggesting that the developing brain requires higher levels of lactate metabolism to form long-lasting memories. We conclude that hippocampal astrocyte-neuronal lactate metabolism is critical for memory formation during development, and that lactate requirement is developmentally regulated. Future studies will investigate the lactate-mediated mechanisms that differentiate the developing brain from the adult one during memory consolidation.

Disclosures: **E. Cruz:** None. **A. Travaglia:** None. **C.M. Alberini:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Support: NIMH R01 MH074736 to C.M.A

Title: A critical role of NPAS4 in the medial prefrontal cortex in context retrieval-mediated memory enhancement

Authors: *D. KAPELLER-LIBERMANN, X. YE, C. M. ALBERINI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Understanding the biological mechanisms that in the brain underlie long-term memory formation and enhancement is important for both healthy conditions and diseases. Using inhibitory avoidance (IA), a context-dependent fear memory task in rats, we have previously shown that multiple brief contextual reactivations of a recent IA memory lead to memory enhancement. Further analyses have revealed that this memory enhancement is accompanied by bidirectional changes of excitatory and inhibitory synapse markers in the prelimbic cortex (PL) a sub-region of the medial prefrontal cortex (mPFC). Neuronal PAS domain protein 4 (NPAS4) is an activity-dependent immediate early gene expressed in the brain that acts as a transcription factor, and regulates inhibitory/excitatory synapse balance during development. We hypothesized that NPAS4 may regulate inhibitory and excitatory synapse formation during context retrieval-dependent memory enhancement. We first examined whether and in which neuronal subtypes of the mPFC the levels of NPAS4 change following training and following the brief contextual reactivations. Using immunohistochemical double staining, we found that the reactivations of a recent IA memory significantly increase NPAS4 level in excitatory but not inhibitory neurons in the mPFC compared to untrained and non-reactivated controls, which had similar levels. We then used antisense oligodeoxynucleotide (ODN) targeting NPAS4 to knock down NPAS4 expression induced by memory reactivation. Our preliminary results show that bilateral NPAS4 antisense injection into the PL before each IA memory reactivation blocks memory enhancement, suggesting a causal link. To establish that the retrieval-mediated induction of NPAS4 is general across species, we employed a similar paradigm in mice. In agreement with this study, we found that mice that underwent IA training followed by a single context reactivation had significant memory enhancement. Furthermore, similarly to what we have found in rats, memory reactivation in mice also produced NPAS4 increase in the mPFC. Further studies will determine the causal role of this NPAS4 induction. In conclusion, our data suggest that mPFC NPAS4 might be a critical regulator of context retrieval-mediated memory enhancement.

Disclosures: **D. Kapeller-Libermann:** None. **X. Ye:** None. **C.M. Alberini:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: R37-MH065635

Title: Cell type mapping of insulin-like growth factor 2 mRNA expression in the adult rat brain in basal conditions and following learning

Authors: ***S. L. SHENG**[1,2], C. M. ALBERINI[1];
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Abstract: Memory consolidation, the process by which newly formed memories are stabilized, is dependent on *de novo* gene transcription and translation. Insulin-like growth factor 2 (IGF2) is required for memory consolidation and administration of recombinant IGF2 with training or memory retrieval promotes significant memory enhancement in both rats and mice (Chen et al., 2011; Stern et al., 2014a and b). We have previously shown that IGF2 mRNA and protein levels are augmented in total extracts of dorsal hippocampus (dHC) following inhibitory avoidance (IA) training (Chen et al., 2011). However, the brain cell-type origin of IGF2 in basal conditions and following learning is not known. Previous studies have shown high IGF2 mRNA expression in the choroid plexus and leptomeninges of the adult rodent brain, but it is unclear whether neurons or glia also express IGF2, and whether learning regulates IGF2 expression in one or more of these cell types. Here we used the RNAscope Multiplex Fluorescent assay system (ACD Bio; Wang et al., 2012), to investigate and quantify the cell-type expression levels of IGF2 mRNA in the adult rat brain in basal (naïve) conditions and following IA training. In addition to confirming high expression levels in the choroid plexus and leptomeninges, we have found that IGF2 mRNA is expressed at relatively high levels in the (Pecam1-expressing) endothelial cells of the blood vessels, and at relatively low levels in (CaMKII-alpha-expressing) excitatory and (Gad1/Gad67 expressing) inhibitory neurons, and in (Aldh1l1-expressing) astrocytes. This cell type distribution was similar in the dHC, anterior cingulate cortex, prelimbic and infralimbic cortices. Furthermore, we found that the expression level of IGF2 in the endothelial cells and inhibitory neurons was increased following IA training, whereas no significant changes were found in excitatory neurons. Together, these results suggest a learning-dependent regulation of

IGF2 expression in specific cell types, which include endothelial cells of the blood vessels and inhibitory neurons.

Disclosures: **S.L. Sheng:** None. **C.M. Alberini:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Support: NIMH R01 MH074736 to C.M.A

NARSAD Young Investigator Grant to X.Y.

Title: Dissecting prelimbic cortical circuits and mechanisms in retrieval-mediated fear memory enhancement

Authors: ***X. YE**, D. KAPELLER-LIBERMANN, A. TRAVAGLIA, C. M. ALBERINI; Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Memories guide our decisions and behaviors, as well as influence our emotional regulation. Impairments or maladaptive modulations of memory strength, persistence and inflexibility are commonly observed in many neuropsychiatric disorders, including anxiety, depression, schizophrenia, autism and post-traumatic stress disorder. Understanding the neural circuit and mechanisms modulating memory strength is crucial for developing effective treatments that can rescue memory dysfunction. Modulation of fear memory strength occurs naturally with memory retrieval: Brief re-exposure to the context (context retrieval) in which a threat was recently experienced leads to strengthening of the fear memory. In contrast, re-exposure to the context for prolonged period or at a remote time point favors extinction, which, through a new memory, decreases the conditioned response. These opposite behavioral outcomes raise the following questions: how does fear memory fear strengthening *vs.* extinction occur? Are the mechanisms that strengthen or extinguish the memory mutually exclusive? Or do they crosstalk, thus regulating the final outcome? We explored these questions with the inhibitory avoidance (IA) task in rats. First, using Designer Receptors Exclusively Activated by Designer Drugs (DREADD), we examined the role of direct projections from dorsal hippocampus (dHC) to medial prefrontal cortex (mPFC) in retrieval-mediated memory modulation. We found that direct dHC input to the prelimbic cortex (PL), a subregion of the mPFC, is necessary for retrieval-mediated memory enhancement, but not for extinction, which, in contrast, required a

direct input from dHC to the infralimbic subregion (IL). Second, in the mPFC, IA training increased the levels of cell adhesion molecules specific for excitatory and inhibitory synapses, neuroligin 1 (NLGN1) and neuroligin 2 (NLGN2), respectively, during the first week after training through brain-derived neurotropic factor (BDNF)-dependent mechanisms. Blocking PL NLGN1 prevented retrieval-mediated memory strengthening. In contrast, blocking PL NLGN2 facilitated extinction through increase of IL activity. Finally, we found that at 4 weeks after IA training, a time point when context retrievals led to extinction rather than memory strengthening, mPFC NLGN1 and NLGN2 levels were significantly lower than those in naïve rats. Collectively, these findings suggest that brief context retrieval produces memory strengthening recruiting direct dHC to PL projections as well as excitatory and inhibitory synaptic changes in the PL that, by a concerted action, lead to fear memory strengthening and suppression of extinction.

Disclosures: X. Ye: None. **D. Kapeller-Libermann:** None. **A. Travaglia:** None. **C.M. Alberini:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Title: Expression of ribosomal RNA gene variant 4 is activated by learning and required for memory consolidation of a spatial learning task in mice.

Authors: *K. D. ALLEN[1], M. J. TROY-REGIER[2], C. HSIEH[2], P. TSOKAS[2], C. OKEZUE[3], J. WOLK[4], A. FENTON[5], T. C. SACKTOR[2,6], A. I. HERNANDEZ[3,7];
[2]Physiol. and Pharmacol., [3]Pathology, [1]SUNY Downstate Med. Ctr., Brooklyn, NY; [4]Physics, [5]Ctr. for Neural Sci., [6]New York Univ., New York, NY; [7]SUNY Downstate Med. Ctr., The Robert F. Furchgott Ctr. for Neural and Behavioral Sci., Brooklyn, NY

Abstract: Persistent experience-evoked changes in synaptic efficacy are widely believed to form the basis of learning and memory. The transition from short-term to long-term forms of synaptic plasticity requires protein synthesis and new gene expression. Most efforts to understand experience-induced changes in neuronal gene expression have focused on the transcription products of RNA polymerase II—primarily mRNAs and the proteins they encode. Using an *in vitro* mouse model, we showed for the first time that RNA polymerase I (Pol I) directed ribosomal RNA (rRNA) synthesis is essential for the maintenance of hippocampal long-term potentiation (LTP). Furthermore, by using a specific Pol I inhibitor *in vivo*, we showed for the first time that learning-induced, activity-driven rRNA gene expression is required for

consolidation of the Active Place Avoidance spatial memory task. De novo rRNA synthesis is a prerequisite to the production of new ribosomes. We found that if rRNA synthesis is blocked by intra-hippocampal injection of the Pol I inhibitor, CX5461, mice still learn the task but exhibit a memory deficit 24 hours later. Surprisingly, pre-existing ribosomes do not compensate for the lack of learning-induced new ones. Given this data, we hypothesize that *new and qualitatively different* ribosomes are required for long- term plasticity and memory consolidation. Consistent with this hypothesis, we tested the seven mouse rRNA gene variants previously described and found that only one, rRNA variant 4, is significantly upregulated after learning. This observation constitutes the first evidence that differential rRNA gene expression and *new and qualitatively different* ribosomes play a role in learning and memory.

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Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: CNPq

CAPES

FAPEMIG

Title: De novo transcription conversely modulates the late consolidation of contextual fear remote memory in prefrontal cortex and hippocampus

Authors: *L. M. PEREIRA[1], C. M. CASTRO[2], J. T. MARQUES[3], G. S. PEREIRA[2]; [1]Univ. Federal De Minas Gerais, Belo Horizonte, Brazil; [2]Fisiologia e biofísica - Núcleo de Neurociências, [3]Bioquímica - Lab. RNAi, Univ. Federal de Minas Gerais, Belo Horizonte, Brazil

Abstract: Contextual fear-memory consolidation has its molecular basis well grounded by several years of research. However, it remains to be explored if fear-memory persistence has a similar molecular basis. Here we tested the hypothesis that memories that last longer, named remote memory, rely on *de novo* transcription during the late consolidation, in dorsal hippocampus (dHIP) and dorsomedial pre-frontal cortex (dmPFC). Male adult C57/BL6 mice were submitted to contextual fear conditioning using weak training (1US: 1 foot-shock; 0.7mA)

or strong training (5US: 5 foot-shocks; 0.7mA). Our first result showed that fear memory induced by 1US lasted less than 7d, while 5US prolonged fear memory to more than 30d. Next, we implanted bilateral cannulae into the CA1 region of dHIP and administered actinomycin (ACTD: 2.5ng/side), a transcription inhibitor, immediately (early consolidation) or 12h (late consolidation) after conditioning and tested animals 48h (long-term memory; LTM) and 30d (remote memory; RM) after. Our results showed that *de novo* transcription in the dHIP, during late consolidation, is necessary to RM, but not LTM expression. However, ACTD impaired LTM in both 1US and 5US groups, if administered during early consolidation. Accordantly, *bdnf* gene expression increased 12h after training only in 5US group. Next, we performed a similar experiment, though using animals with cannulae into the dmPFC. Both, LTM and RM, were impaired by trancription inhibition during the early consolidation, similar to what we observed in the dHIP. Surprisingly, ACTD administration during late consolidation, impaired LTM in 1US group, but enhanced both LTM and RM in 5US group. Our results showed that the strength of training dictates the persistence of fear memory. We corroborate literature showing that, in the hippocampus, 12h seems to be a key time point for memory persistence. Interestingly, dmPFC is especially affected by transcription inhibition during the late consolidation of both LTM and RM triggered by 5US. We are currently performing q-PCR to evaluate *Bdnf* expression in mPFC 12h after conditioning and also the expression of other important genes to synaptic plasticity, such as *Arc*.

Disclosures: **L.M. Pereira:** None. **C.M. Castro:** None. **J.T. Marques:** None. **G.S. Pereira:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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AA06420

DA034140

Title: Contribution of newly born progenitors generated during the proliferative burst to emotional memory deficits associated with alcohol dependence in male and female rats

Authors: M. FANNON[1], J. WILLIAMS[1], K. MYSORE[1], R. MORALES[1], M. STAPLES[2], H. CAMERON[3], *C. D. MANDYAM[4];
[1]VMRF, San Diego, CA; [2]TSRI, San Diego, CA; [3]NIH, Bethesda, WA; [4]Skaggs Sch. of Pharm., UCSD, LA Jolla, CA

Abstract: The hippocampus is a critical region involved in emotional behavioral tasks such as trace fear conditioning (TFC). Prolonged ethanol exposure has been shown to impair hippocampal functioning and could be resulting from neuroplastic and neuroadaptive changes in the hippocampus. Such neuroplastic changes could involve the proliferative burst in the neural progenitor cells in the dentate gyrus 72 hours into withdrawal, a timeframe associated with negative affect. The effect this proliferative burst has on hippocampal functioning, particularly emotional behaviors dependent on the hippocampus, remain unclear. Therefore, the purpose of this study was to use a genetic approach to temporally ablate these progenitors born during the proliferative burst prior to TFC and evaluate performance. To target the specific progenitors, a transgenic model was utilized in which the Herpes Simplex Virus Thymidine Kinase (HSV-TK) is expressed under the control of the GFAP promoter (GFAP-TK rats). A total of ninety-seven 8-12 week old male and female GFAP-TK rats were exposed to a 7 week chronic intermittent ethanol (EtOH) vapor exposure (CIE, 14 hours EtOH, 10 hours air). Three days before removal from EtOH vapor, rats were treated with the antiviral drug valganciclovir (VGCV), to ablate proliferating cells. The rats were trained on a TFC paradigm 72 h into withdrawal and tested for recall 24h later. Hippocampal tissue was collected and stained for the proliferation marker Ki67. Male and female rats that received VGCV showed a distinct reduction in Ki67+ cells suggesting that VGCV abolished the proliferative burst. During the training session of the TFC paradigm, all animals were able to acquire the association of the pairing of conditioned stimulus (CS, 80db tone) with unconditioned stimulus (US, 0.5mA footshock), however male rats that were exposed to EtOH showed an exaggerated response compared to their female counterparts. Additionally, during recall, male rats only exposed to EtOH vapors showed impairments on CS retention while the males that received EtOH exposure and VGCV did not show the same deficits. Female rats did not show the same susceptibility to hippocampal impairments following EtOH exposure. This data suggests that exposure to EtOH may create a higher vulnerability for hippocampal impairment in male subjects when compared with female subjects. Additionally, the proliferative burst in neural progenitors that is seen 72 h into withdrawal from EtOH plays a mechanistic role in hippocampal deficits.

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Poster

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Title: Hippocampal circadian clock regulates time-dependent memory retrieval and spine morphology of CA1 neuron

Authors: *M. MIYAHARA[1], S. HASEGAWA[1,2], S. KIDA[1,2];
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Abstract: Circadian rhythm in suprachiasmatic nucleus (SCN) is generated through the transcriptional regulation by circadian transcription factor BMAL1. Interestingly, peripheral tissues also show similar circadian transcription rhythms and circadian clocks in peripheral tissues have been shown to play critical roles in tissue-specific functions. However, roles of circadian clocks in forebrain, especially hippocampus, still remain unknown. We have tried to understand roles of forebrain clock in memory performance using double transgenic mice expressing a dominant negative BMAL1 exclusively in the forebrain under the control of tetracycline-dependent transcription factor (dnBMAL1 mice) and found that dnBMAL1 mice show time-dependent impairments in hippocampus-dependent memory retrieval around ZT10, suggesting that hippocampal clock regulates memory retrieval efficiency. In this study, to understand possible mechanisms of deficits in memory retrieval observed in dnBMAL1 mice, we analyzed spine morphology of neurons in hippocampus, prefrontal cortex and amygdala using triple transgenic mouse generated by crossing dnBMAL1 mouse with Thy1-GFP mouse expressing GFP in the neurons. dnBMAL1 mice showed a significant decrease in spine density in apical dendrites of CA1 pyramidal neurons and furthermore, show a significant decrease in the number of thin spines, but show normal number of mushroom and stubby spines, suggesting that the decrease in spine density of dnBMAL1 mice is due to reduced number of thin spine. On the other hand, dnBMAL1 mice exhibit normal spine density in the prefrontal cortex, amygdala and the dentate gyrus area of hippocampus. These observations suggest that BMAL1 contributes to the regulation of dendritic spine morphology in the CA1 area of hippocampus. To further understand the molecular mechanisms the regulation of dendritic spine morphology by BMAL1,

we analyzed gene expression profiles of forebrain of dnBMAL1 mice using next generation sequencing. We found that dnBMAL1 mice show decrease in expression levels of several synaptic protein in hippocampus. We are now analyzing the relationship between morphology of dendritic spine and expression levels of synaptic protein in dnBMAL1 mice.

Disclosures: **M. Miyahara:** None. **S. Hasegawa:** None. **S. Kida:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Title: Comparisons and discrimination of fear and extinction neurons at the molecular and cellular levels

Authors: *R. ISHIKAWA[1], S. KIDA[1,2];
[1]Tokyo Univ. of Agr., Tokyo, Japan; [2]CREST, JST, Saitama, Japan

Abstract: Brief fear memory retrieval triggers fear responses, whereas long-time or repeated retrieval extinguishes fear memory. Memory circuits contributing to fear and extinction have been identified, respectively. However, comparisons and discrimination of fear and extinction neurons have not well examined. We have characterized “fear” and “extinction” neurons by comparing memory phases of reconsolidation and extinction using contextual fear conditioning and inhibitory avoidance tasks (Suzuki et al., 2004; Mamiya et al., 2009; Fukushima et al., 2014). Here we compare and discriminate fear and extinction neurons at the molecular and cellular levels using catFISH by detecting Arc and Homer1a (H1a) mRNAs following retrieval of contextual fear or inhibitory avoidance memory. We first confirmed previous findings (Vazdarjanova et al., 2002) by showing that in the amygdala, Arc mRNA is quickly transcribed 2~10 min following retrieval of contextual fear memory, whereas H1a mRNA appears in the nucleus 25~40 min after this retrieval. We next tried to identify fear and extinction neurons using contextual fear conditioning. Mice were re-exposed to the context to induce fear

(reconsolidation) and extinction, respectively, 24 hrs after the contextual fear conditioning using several experimental groups. Significant increases in Arc mRNA-positive cells were observed in the basolateral amygdala (BLA) 5 min after brief or prolonged re-exposure to the context inducing fear or extinction, respectively. Similarly, significant increase in H1a mRNA-positive cells was observed 30 min after the brief re-exposure. Importantly, prolonged re-exposure to the context for 30 min showed no increase in H1a mRNA-positive cells at 5 min after the re-exposure, suggesting that gene expression (H1a and Arc expressions) is activated just after termination of re-exposure (off-CS). Finally, an experimental group inducing both fear and extinction by the re-exposure to the context twice showed no increases in double positive cells of Arc and H1a mRNAs although this group showed significant increases in single positive cells of Arc and H1a mRNA. These observations suggest that BLA show distinct populations of fear and extinction neurons at the molecular levels. We are now trying to compare fear and extinction neuron populations in other brain regions using this strategy using contextual fear conditioning and inhibitory avoidance tasks.

Disclosures: R. Ishikawa: None. **S. Kida:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Title: Paired and unpaired conditioning during aging: BDNF, GABA, glutamate and serotonin changes after memory consolidation

Authors: *C. E. VASQUEZ[1], V. MITCHELL[2], R. COSSIO[1], J. FORNAGUERA[3], G. BRITTON[1];
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Abstract: Excitatory/inhibitory processes in the hippocampus (HPC) and medial prefrontal cortex (mPFC) have been found to be required in cue discrimination of aversive and non-aversive stimuli. Moreover, these mechanisms in HPC and mPFC activity seem to be altered during aging. The main objective of the present work was to explore age interactions in fear responses and levels of brain derived neurotrophic factor (BDNF) protein, serotonin (5-HT), 5-Hydroxyindoleacetic acid (5-HIAA), gamma-aminobutyric acid (GABA), glutamate (Glu) and glutamine (Gln) in the HPC and mPFC after memory consolidation. Adult (3 months) and aged rats (12 months) were trained in paired and unpaired fear paradigms. Paired fear conditioned rats

were trained with tone and footshock pairings. Unpaired fear conditioned rats received tones and shocks explicitly unpaired. Fear memory (freezing) was measured during tone presentations in the training context in the absence of shock. Behavioral testing results from adult and aged rats showed a reduction of freezing during the tone in the unpaired group. In paired groups, adult and aged rats expressed higher levels of fear to the tone. We further evaluated the levels of BDNF, 5-HT, 5-HIAA, GABA, Glu and Gln in the HPC and mPFC 24 h after paired and unpaired fear learning. In both HPC and mPFC control adult rats showed higher levels of GABA and GABA/Glu than control aged rats. In HPC control adult rats showed higher levels of BDNF than control aged rats. We found a decrease in the levels of BDNF, Glu, Gln, GABA, Glu/Gln and GABA/Gluin HPC of adult rats that received unpaired conditioning relative to adult controls. These results suggest a reduction in both glutamate and GABA processing 24 h after unpaired fear conditioning. In mPFC, the levels of 5-HT and 5-HIAA were increased and the levels of GABA were decreased in adult animals after unpaired conditioning. However, no differences in glutamate or GABA/glutamate ratio were found, which could indicate a different mechanism occurring in GABA processing in HPC after unpaired conditioning. In aged rats we found an increase of 5-HT and 5-HIAA 24 h in the HPC after paired conditioning. We did not find differences in HPC or mPFC of adult rats 24 h after paired conditioning. Taken together, although we found similar behavioral responses between adult and aged rats in paired and unpaired fear conditioning, there were distinct age dependent hippocampal patterns in serotonin levels. Age dependent differences in PFC and HPC neurochemistry after unpaired memory acquisition could be mediating fear learning and the expression of fear.

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Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Title: Using viral translating ribosome affinity purification to study associative recognition memory consolidation

Authors: *J. R. GAUNT[1], H. SCOTT[2], L. MARUCCI[2], S. SHEARDOWN[4], E. C. WARBURTON[3], J. B. UNEY[2];
[1]Biomed. Sci., [2]Sch. of Clin. Sci., [3]Physiol. & Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [4]Takeda Pharmaceut. Co., Cambridge, United Kingdom

Abstract: Associative recognition memory is a hippocampal-dependent process enabling the determination of whether a particular configuration of stimuli has been seen before. The consolidation of these memories into long term storage is thought to involve protein synthesis. However, little research to date has addressed the identities of the proteins synthesised on a genome-wide scale. Messenger RNAs (mRNAs) undergoing translation into protein at the ribosome were profiled at a range of timepoints during associative recognition memory consolidation in CA1 neurons with the aim of furthering understanding of the gene regulatory networks involved in this process.
Translating Ribosome Affinity Purification (TRAP) was used to profile translating mRNAs. Using this technique, the ribosomal protein L10a is tagged with EGFP in a genetically defined cell population, enabling the ribosomes and bound mRNA to be immunoprecipitated. A range of viral approaches to TRAP were developed for use in the rat hippocampus. Expression of the transgene EGFP-L10a was achieved in rodent hippocampal neurons both in-vivo and in-vitro and sufficient RNA was extracted using the TRAP method for NGS.
TRAP was subsequently combined with the paired viewing paradigm, which presents novel and familiar visual stimuli to rodents simultaneously, for comparison of novel and familiar conditions and an additional no-image control. Bioinformatic analyses conducted on Next Generation Sequencing (NGS) data from these experiments will be presented and discussed in the context of understanding molecular mechanisms of associative recognition memory consolidation.

Disclosures: **J.R. Gaunt:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Pharmaceutical Company. **H. Scott:** None. **L. Marucci:** None. **S. Sheardown:** A. Employment/Salary (full or part-time): Takeda Pharmaceutical Company. **E.C. Warburton:** None. **J.B. Uney:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: mTOR-dependent mechanisms in the persistence of contextual fear memory

Authors: P. MACCALLUM[1], T. KENNY[1], K. FALLON[1], *J. J. BLUNDELL[2];
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Abstract: Reconsolidation parallels many of the same molecular features as consolidation, which act to stabilize and maintain the engram through the synthesis of new proteins. One such common intracellular feature required for long lasting, experience-driven behavioural plasticity in consolidation and reconsolidation is the activation of the mechanistic target of rapamycin (mTOR) kinase. mTOR and its downstream effectors facilitate de novo protein synthesis by carefully integrating activity-dependent upstream signals, for example brain-derived neurotrophic factor and glutamate, to regulate mRNA translation. Beyond initial memory formation and stabilization after reactivation, the role of mTOR in memory persistence is relatively unknown. We have previously shown biphasic rapamycin-sensitivity immediately and 12 hours after auditory fear training or reactivation. Further, it was recently shown that PF-4708671, a specific inhibitor of the mTOR downstream effector S6K1, administered immediately after reactivation destabilized post-reactivation memory for an auditory fear memory 10 days later, but not at 24 hours. As such, we sought to determine whether a similar temporal pattern of mTOR blockade could attenuate associative contextual fear memory consolidation, reconsolidation, and persistence. Male C57BL/6 mice received intraperitoneal injections of rapamycin or vehicle (40 mg/kg) at various time points following contextual fear conditioning or fear memory retrieval. Fear memory recall was tested at either 48 hours, 7 days, or 21 days following training and 48 hours or 7 days following reactivation. Systemic rapamycin treatment immediately or 3 hours, but not 12 hours after conditioning disrupted memory consolidation and persistence. Blockade of mTOR with rapamycin 3 hours after reactivation attenuated contextual fear memory when tested 7 days later, but not 2 days post-reactivation. Unlike consolidation, systemic rapamycin treatment 12 hours after memory reactivation impairs the persistence of reconsolidated contextual fear memory when tested 7 days later. We are currently investigating whether reconsolidated memory is affected from this treatment at 48 hours post-reactivation. Moreover, we are systematically examining the time course of PF-47086721-sensitivity during mTOR-dependent consolidation and reconsolidation-like events that contribute to contextual fear memory. Identifying the mechanisms underlying fear memory persistence will increase our understanding of the precise requirement of mTOR and its effectors in different types of memory (context vs. cue) and phases (consolidation vs. reconsolidation).

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Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Support: NHMRC Grant 1083569

Title: Dissociation of contextual fear expression and hippocampal Arc expression following immediate shock

Authors: *J. A. LEAKE[1,2], R. ZINN[1,3], L. H. CORBIT[2], B. VISSEL[1,4,3];
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Abstract: In a contextual fear conditioning paradigm, mice require around 30s in the context prior to shock to display substantial conditioned fear at test. This period of time is necessary for animals to form an integrated representation of the context, which comes into association with the shock. In the present study, we examined the effect of the duration of the placement-shock interval (PSI) on conditioned freezing and hippocampal activity, as measured by the expression of the immediate early gene product Arc.
Mice were placed in the conditioning chamber for 30s, 180s or 720s prior to the administration of footshock. Conditioning at all PSIs increased dorsal hippocampal (DH) Arc expression relative to controls, corresponding with the ability of mice to acquire conditioned freezing responses under these conditions. We next sought to determine which aspects of the experience were contributing to these increases. Exposure to the context in the absence of shock was sufficient to induce Arc expression in the DH, consistent with a role for this region in processing contextual information. However, a similar increase was observed after immediate shock, a condition that does not support context-dependent freezing. We hypothesised that Arc increases in immediately shocked animals could be due to exposure to extrachamber cues, preceding placement in the chamber. To test this possibility, mice were habituated to handling and transport cues for ten days prior to immediate shock. In this case, immediate shock did not significantly alter Arc expression. These findings indicate that contextual learning drives Arc expression in the DH and that, under some conditions, the context encompasses extrachamber cues that the mice experience prior to shock.
Given that extrachamber cues were sufficient to induce hippocampal activation, we wondered whether they also formed part of the contextual conditioned stimulus during fear conditioning at different PSIs. To test this, we conditioned mice at a 30s or 180s PSI, and then kept the extrachamber cues the same or changed them significantly. In both conditions, intrachamber cues

remained the same. We found that the switched condition led to profoundly reduced freezing at the 30s PSI but not the 180s PSI. Thus animals utilize different aspects of the experience leading up to shock to predict future shock, depending to the duration of the PSI. Under conditions where opportunity to encode the shock environment itself is limited, animals use, and demonstrate fear to other elements of the experience including handling and transport cues. These findings have potential theoretical and practical implications for contextual fear conditioning paradigms.

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Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Title: Lowering Fkbp5 expression in the ventral hippocampus enhances fear conditioning without affecting anxiety levels

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Abstract: Memories of traumatic events are strengthened by glucocorticoid signaling. Excessive glucocorticoid signaling may contribute to the enhanced fear to traumatic reminders seen in patients with post-traumatic stress disorder (PTSD). FKBP5 expression inhibits glucocorticoid signaling and could, therefore, modulate the acquisition or extinction of conditioned fear. Since the hippocampus provides information that determines in what context a stimulus predicts danger, we hypothesized that FKBP5 expression in the hippocampus modulates fear conditioning or extinction in rodents. To test this, we first used western blot to determine whether FKBP5 protein levels change after fear conditioning or extinction. We found that FKBP5 protein decreases after fear conditioning and remains low after extinction in the ventral hippocampus (VH). In contrast, FKBP5 expression in the dorsal hippocampus was stable. Next, we examined

whether reducing FKBP5 expression by infusing FKBP5 shRNA plasmids into the VH affects fear conditioning or extinction. Reducing FKBP5 in VH produced a PTSD-like phenotype with enhanced fear acquisition that led to higher fear during extinction learning and recall. Reducing FKBP5 in VH did not affect locomotion or anxiety levels. Our findings suggests the possibility that low levels of FKBP5 protein in the hippocampus may increase the risk of developing PTSD in humans by strengthening fear memories and making them more difficult to extinguish.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Israel Science Foundation (ISF) Morasha grant 604/15 to KR

Title: The role of quinone reductase 2 in hippocampal dependent learning

Authors: *K. ROSENBLUM[1], V. SHARMA[1], M. HLEIHEL, 3498838[2], E. EDRY[2], G. NATHANIEL[2];
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Abstract: Our laboratory identified that quinone reductase 2 (QR2) levels of expression are a muscarinic receptor dependent removable constraint on memory formation in the cortex (Rappaport *et al.,* 2015). Upon acquiring novel taste memories, QR2 mRNA levels in the rat insular cortex decrease. This reduction can be ablated by the use of a muscarinic receptor antagonist, which also impedes the formation of memory. The use of acetylcholine esterase inhibitors, permitting artificially higher acetylcholine (ACh) availability for muscarinic activation, mimics the effect of ACh release during novel taste learning on QR2 mRNA expression. Furthermore, the use of QR2 inhibitors improves cortical dependent learning. These results put QR2 in the center of the axis of novel information, neuromodulation via ACh and better cognitive performance. However, does QR2 act similarly in other brain regions underlying other forms of learning? By what mechanism does QR2 act? We thus analyzed the levels of QR2 mRNA levels in the mouse hippocampus following fear conditioning. Additionally, we used viral vector harboring shRNA targeting QR2 and also QR2 inhibitor S29434 injection directly to the CA1 region of the hippocampus, to determine the effect on hippocampal dependent memory strength and underlying cellular processes.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CONACYT (237570)

PAPIIT (IN201415)

Title: Effects of transcription inhibition in dorsal striatum on gene expression and memory consolidation after moderate and enhanced training

Authors: ***A. C. MEDINA**[1], E. ALVARADO-ORTÍZ[1], M. I. HERNÁNDEZ GUITÉRREZ[1], S. GONZÁLEZ-SALINAS[1], G. L. QUIRARTE[1], A. ANTARAMIAN[2], R. A. PRADO-ALCALÁ[1]; [1]Neurobiologia Conductual y Cognitiva. Inst. de Neurobiologia-UNAM, Queretaro, Mexico; [2]Unidad de Proteogenomica. Inst. de Neurobiologia-UNAM, Queretaro, Mexico

Abstract: During memory consolidation there are molecular events that regulate transcription, such as activation of immediately early genes (IEGs), including *c-fos*, *c-jun*, *zif268*, and *egr-3*, and of effectors IEGs such as *arc*, *narp*, *homer*, *cox-2*, and *rheb* that act directly upon cells producing different effects, including plastic changes. It is known that the striatum is necessary for memory formation of moderate training, but not for enhanced training. Gene expression in this structure has not been studied under these training conditions. We aimed to study the effects of transcription inhibition in the striatum during consolidation and to measure the expression of *c-fos, zif268* and *arc*, using moderate and enhanced training. We administered 40 µg/µl of DRB (5,6- Dicloro-1-β-Dribofuranosilbenzimidazol, transcription inhibitor) or its vehicle (DMSO, 1%), bilaterally into the dorsal striatum of male Wistar rats, 30 min before training of inhibitory avoidance task with a footshock of 1.5 or 3.0 mA. Forty-eight hours later, their retention latencies were measured. In other groups, under the same training conditions, we measured *c-fos, zif268* and *arc* expression using Quantitative Real-Time PCR, 30 min after training. The results showed that pre-training DRB administration produced amnesia in the 1.5 mA group while the 3.0 mA group showed an intact memory. We found that *c-fos* and *zif268* increased significantly in striatum in the 1.5 mA-vehicle group as compared with the sham group, and DRB blocked this effect. The enhanced training only diminished the expression of *zif268*; *arc* increased its

expression in a control group that received a non-contingent footshock of 3.0 mA. These data indicate that enhanced training of inhibitory avoidance training blocks the DRB-induced amnesia in dorsal striatum; this is consistent with previous experiments from our laboratory. The changes in gene expression suggest that the memory consolidation in the striatum involves molecular activity which is dependent upon the intensity of training.
The authors thank the excellent technical assistance of Norma Serafín, Martín García, Alejandra Castillo, Adriana González, Alberto Lara, Omar González and Ángel Méndez. Supported by CONACYT (237570) and PAPIIT (IN201415).

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: PAPIIT IN201415

CONACyT 237570

Title: Structural changes in medium spiny neurons of dorsal and ventral striatum associated with retrieval of over-reinforced inhibitory avoidance learning

Authors: ***P. BELLO-MEDINA**[1], G. FLORES[2], G. L. QUIRARTE[1], R. A. PRADO-ALCALA[3];
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Abstract: Retrieval is a process that involves the recovery of stored information. There is evidence that retrieval induces changes in gene expression and protein synthesis. However, it is not yet known whether retrieval produces structural changes in the medium spiny neurons (MSN) of the dorsal and ventral striatum. In this work we investigated if there are changes in the total number (density) and shape (thin, stubby and mushroom) of dendritic spines in MSN in dorsal (dorsomedial and dorsolateral) and ventral striatum (nucleus accumbens core and shell) produced during retrieval of IA that had been trained with moderate (1.0 mA) and strong (3.0 mA) foot-shocks The results showed that in dorsomedial and dorsolateral striatum there was an

increase in the density and in mushroom spine ratio, which were proportional to the intensity of training. On the other hand, in the ventral striatum there was an increase in the density and in mushroom spine ratio associated with the non-contingent administration of the foot-shocks. These results are probably due to the dorsal striatum is processing contextual and procedural information from the cortex and hippocampus, whereas the ventral striatum seems to process information derived from the aversive stimulation These results probably reflect processing, during retrieval, of contextual and procedural information of moderate and intense IA training in dorsomedial and dorsolateral striatum, whereas the ventral striatum seems to process information derived from the aversive stimulation. Acknowledgements: We thank Martín García, Ángel Méndez, Cristina Medina, Norma Serafín, Alexander Gonzalez, Leonor Casanova, and Nydia Hernández for excellent technical and experimental assistance. This research was supported by grants PAPIIT IN201415 and CONACyT 237570.

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Poster

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CONACyT

Title: Glucocorticoid receptor phosphorylation in hippocampal and striatal neurons after inhibitory avoidance training

Authors: *D. A. GONZALEZ FRANCO, A. M. CRUZ-QUIROZ, R. PEGUEROS-MALDONADO, P. BELLO-MEDINA, R. A. PRADO-ALCALA, G. L. QUIRARTE;
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Abstract: Corticosterone (CORT), the principal glucocorticoid in rodents, is released after stressful experiences such as training with high foot-shock intensities in the inhibitory avoidance task (IA). After being released into the bloodstream, CORT reaches the glucocorticoid receptor (GR) located in almost all brain cells; subsequently, GR is phosphorylated at serine 211 (pGRser211). This phosphorylation has been reported to be an indicator of GR ligand-dependent activation, and is required for GR to translocate into the nucleus and work as a transcription

factor. The GR is present in the hippocampus with a high concentration in CA1 and dentate gyrus (DG), and a smaller proportion in CA3; also the GR is sparsely present in the dorsal striatum; both structures are involved in memory consolidation of IA. In order to determine the participation of glucocorticoids in this task, we quantified the nuclear pGRser211 in both hippocampus and dorsal striatum (CA1, CA3 and DG from hippocampus, and dorsolateral, dorsomedial, ventrolateral and ventromedial regions of the dorsal striatum) of rats trained in IA, using different foot-shock intensities (0.5, 1.0, and 2.0 mA); we also studied three control groups: the first one was placed in the training context but without foot-shock (0.0 mA), the second received only the foot-shock (2.0 mA) without training, and the last one was a home-cage group. Brains were dissected 60 min after training and cryosectioned for immunodetection of pGRser211+ cells. The results show that the groups trained with 1.0 and 2.0 mA had higher retention latencies than the 0.0 mA or 0.5 mA groups; the 2.0 mA group showed stronger resistance to extinction than the other groups. In hippocampus the highest proportion of pGRser211 cells was found in CA1 in the 2.0 mA trained group. No changes were observed in CA3 and DG; for the whole dorsal striatum the 2.0 mA group showed an increase in the pGRser211 cells proportion, however at a regional level the increase was only observed in ventral regions. These findings suggest that activation of GR in CA1 and in the ventral regions of the dorsal striatum is involved in the consolidation of a stronger memory of IA, possibly through the modulation of gene expression. We thank the technical assistance of Nydia Hernández, Norma Serafín, Cristina Medina, Leonor Casanova, Lourdes Lara, Martín García, and Ramón Martínez. Supported by PAPIIT-UNAM (IN202414) and CONACyT.

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Poster

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PAPIIT-UNAM IN214111, IN202414 and IN208812.

Title: Glucocorticoid receptor phosphorylation in the amygdala and hippocampus after acquisition of contextual fear conditioning

Authors: *R. PONCE, M. CARRANZA, N. SERAFIN, R. A. PRADO-ALCALÁ, G. L. QUIRARTE;
Inst. De Neurobiología, UNAM, Queretaro, Mexico

Abstract: Glucocorticoid release during the acquisition process facilitates memory consolidation, especially of highly emotional or stressful experiences. This effect depends on several factors, among them is glucocorticoid receptor (GR) activation. The GR is a transcriptional factor whose transcriptional activity and cellular localization depends on its phosphorylated state. The present work aimed to investigate the effect of contextual fear conditioning training on the phosphorylation of GR at serine 211(Ser 211) and serine 226 (Ser 226) in the amygdala and the hippocampus. Male Wistar rats (250-350 g) were trained in contextual fear conditioning in a single session (11 min) under different foot-shock intensities (0.0, 0.5, or 1.5 mA). There were two groups for each intensity, in the first one the subjects were tested for memory retention at 48 h and the other group was sacrificed 1 h after training, and the amygdala and the hippocampus were extracted. Total GR and GR phosphorylated at Ser 211 and Ser 226 levels were measured by SDS-PAGE/WB. Rats trained with 0.5 and 1.5 mA learned the task. The optical density of total GR was the same for all the intensities in the amygdala and the hippocampus, with higher levels in the hippocampus. Density of GR phosphorylated at Ser 211 was higher than Ser 226 in the two brain areas and there were no differences among intensities. Phosphorylation at Ser 211 was higher in dorsal hippocampus and amygdala, whereas phosphorylation at Ser 226 was higher in ventral hippocampus. Phosphorylation ratio Ser 211/Ser226 showed that dorsal hippocampus and amygdala had the highest levels. These results indicate that in the acquisition phase of the contextual fear conditioning task, GR phosphorylation is not a process that depends on the stressor intensity during training, and that those areas involved in context-footshock association (dorsal hippocampus and amygdala) have the highest levels of GR phosphorylated at Ser 211, which probably could be related to an increase of transcription of molecules involved in plasticity. We acknowledge the technical assistance of, Cristina Medina, Bertha Islas Rivas Martín García, Leonor Casanova, Omar González, Sandra Hernandez and Lourdes Lara. This work was funded by CONACYT (Grant 130524, Scholarship to RP-L 342154) and PAPIIT-UNAM IN214111, IN202414 and IN208812.

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Poster

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NDSEG

Title: Retrieval of a context fear memory involves sex-specific recruitment of hippocampus and amygdala

Authors: *A. A. KEISER, M. A. DARIAN, L. PAN, D. TCHESSALOVA, K. M. COLLETTE, N. C. TRONSON;
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Abstract: Disorders of fear and anxiety such as post-traumatic stress disorder (PTSD) are more prevalent in women than in men, possibly due to sex differences in retrieval of trauma-related contexts and cues. Our previous studies demonstrated that female mice show greater context fear generalization and suggest sex differences in retrieval of context information. Here we determined sex differences in (1) the roles of hippocampus and amygdala in retrieval of context fear conditioning and (2) interactions of context-shock associations with new learning using blocking. Immunohistochemical experiments quantified cFos and Arc activation in dorsal hippocampus and basal amygdala during retrieval of context-fear in male and female mice. To confirm the differential roles of dorsal hippocampus and basal amygdala, we used pre-test inhibition of these regions in males and females. Males showed stronger activity in dorsal hippocampus after retrieval, whereas females showed stronger amygdalar recruitment. Together with data showing greater context fear generalization in females, these data suggest that males and females retrieve qualitatively different information. Using a blocking paradigm, we assessed sex differences in the interaction of the retrieved context fear memory with new learning of a tone-shock association. Despite strong context fear memory in both sexes, only males showed robust impairments of the new tone-shock memory, whereas females showed a small enhancement in tone-fear conditioning. This male-specific blocking effect suggests that males retrieve a precise context-shock association, but females do not. Exactly what information is retrieved by females after context fear conditioning remains an open question. Our data demonstrating sex differences in context generalization, hippocampal and amygdala activation, and blocking suggest that males and females use differential memory processing strategies and retrieve different information in context fear conditioning. The hippocampal and associative processes required by males have been well described over many years of research. Determining *how* and *what* information is retrieved by females after a traumatic event is of critical importance for sex-specific treatments for disorders of anxiety and fear.

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Poster

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NSERC CGS M

Title: Concentration of PKMzeta in the basolateral amygdala correlates with fear memory strength

Authors: *M. BERNABO, K. NADER;
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Abstract: PKMζ is a persistently active atypical protein kinase C isoform, critical for long-term memory maintenance. Specifically, PKMζ prevents endocytosis of GluA2 subunit-containing AMPA receptors from the postsynaptic membrane. Considerable research has demonstrated that stable long-term memory requires an increased presence of these AMPA receptors at the membrane- with amnesia and forgetting involving a net loss of these receptors. We previously demonstrated that the concentration of these GluA2-containing AMPA receptors at the membrane positively correlates with memory strength in an auditory fear conditioning paradigm (Migues et al. 2010). Given the critical role of PKMζ in maintaining GluA2-containing AMPA receptors at the membrane, we asked if PKMζ specifically correlates with memory strength in this protocol. Rats underwent either naïve (one tone, no shock), weak (one tone-shock pairing), or strong (ten tone-shock pairings) training. Rats' memory was tested 24 hours post-training and rats were sacrificed 24 hours post-test. Concentration of PKMζ within the basolateral amygdala was quantified via Western blotting. Results indicate that postsynaptic concentration of PKMζ does increase with stronger training protocols. Furthermore, the concentration of PKMζ positively correlates with freezing behaviour regardless of whether the rat received weak or strong training. These data suggest that PKMζ is intimately involved in the regulation of differential memory strength, likely through its regulation of GluA2-containing AMPA receptors. Additionally, these results offer direct evidence for a continuous (rather than discrete) relationship between PKMζ concentration and memory performance.

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Poster

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MH094757

Title: Gene expression and DNA methylation dynamics in the mouse amygdala during threat consolidation

Authors: *S. SHARMA[1], S. MADDOX[2], L. LIN[3], Y. LI[3], P. JIN[3], K. RESSLER[2];
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Abstract: The necessity of DNA methylation alterations in the consolidation, reconsolidation, extinction, and retrieval of fear/threat memories is well established. Herein we present a genome-wide view of transcriptional dynamics and DNA methylation dynamics by high throughput RNA sequencing, and immunoprecipitation of 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), followed by high-throughput sequencing. We find the majority of dynamic methylation sites are within introns, exons, and intergenic regions. In particular, we find a large number of "poised" 5-hmC sites in introns, where high levels of the modification are lost upon threat consolidation. We further probe the causal nature of the relationship between DNA methylation and gene regulation by employing CRISPR-Cas9 fusion proteins.

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Poster

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Evelyn F. McKnight Brain Institute

Civitan International

Title: Repressive histone methylation regulates mTOR activation in the hippocampus during fear memory reconsolidation

Authors: *T. J. JAROME, R. M. HAUSER, M. C. RICH, F. D. LUBIN;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Epigenetic mechanisms play a critical role in gene transcription changes necessary for long-term memory formation and consolidation. While epigenetic mechanisms are both dynamically and persistently regulated during the consolidation of long-term fear memories, the role of epigenetic mechanisms in the regulation of gene transcription or protein synthesis during memory reconsolidation remains unclear. Protein synthesis mediated by the mTOR-translational control pathway is critical for memory reconsolidation following retrieval. Phosphatase and tensin homolog (PTEN) is a protein phosphatase widely implicated as an upstream inhibitor of mTOR signaling. However, it is currently unknown if PTEN is epigenetically regulated during the reconsolidation process. Trimethylation of histone H3 at lysine 27 (H3K27me3) is an epigenetic mechanism strongly associated with heterochromatin and transcriptional repression. This histone lysine methylation mark is catalyzed by the histone lysine methyltransferase EZH2, the functional enzymatic component of the polycomb repressive complex 2. PTEN has been shown to be a target of EZH2 in neurons, yet whether H3K27me3 regulates transcriptional repression of PTEN necessary for mTOR-mediated translational processes during memory reconsolidation remains equivocal. Here, we found that PTEN levels were significantly decreased in the CA1 region of the dorsal hippocampus 1hr after retrieval of a contextual fear memory. Additionally, we found that H3K27me3 levels were significantly increased in bulk histone extract from the dorsal hippocampus, concurrently with increased EZH2 expression. Furthermore, chromatin immunoprecipitation analysis revealed significant increases in H3K27me3 levels at specific memory-related genes. Interestingly, H3K27me3 levels were significantly decreased at 24hrs after retrieval, well after completion of the reconsolidation process, suggesting long-term regulation of this repressive histone methylation mark as a result of retrieval. Using in vivo siRNA and CRISPR-dCas9 technology we will further determine whether retrieval-induced EZH2-H3K27me3 increases are directly coupled to mTOR regulation during memory reconsolidation. Collectively, these findings indicate that PTEN mediated inhibition of mTOR may be a target of EZH2-H3K27me3 pathway triggered by retrieval and may be a process necessary for memory reconsolidation

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Poster

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UAB Neuroscience T32 Training grant

Evelyn F. McKnight Brain Institute

Civitan International

Title: Context fear memory formation is regulated by Neat1 long non-coding RNA mediated histone lysine methylation changes in the hippocampus

Authors: *A. A. BUTLER, F. D. LUBIN, A. W. CHANG;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Histone lysine methylation is critical for the formation and maintenance of long-term memories. Manipulation of specific histone lysine methyltransferases and demethylases modulate histone methylation and is sufficient to either improve or impair the formation of long-term memories. However, initiation of histone lysine methylation mechanisms during long-term memory formation or "consolidation" is poorly understood. In recent years, long noncoding RNAs (lncRNAs) have been implicated in the targeting of epigenetic modifiers to gene loci. Indeed, a single lncRNA may target hundreds or thousands of genomic loci for epigenetic regulation, resulting in semi-permanent and functionally important changes in gene transcription changes necessary for neuronal activity. New studies have identified expression of a large population of lncRNAs in the rodent hippocampus, a region critical for the conversion of short-term memories to long-term memories. Here, we examined the role of one such lncRNA, Neat1, in the process of long-term memory consolidation. Using RNA immunoprecipitations, we found multiple chromatin modifying enzymes associated with Neat1 in cultured cells, including the repressive histone methyltransferases Ehmt2 and Ezh2, which mediate H3K9me3 and H3K27me3, respectively. Blocking Neat1 expression via siRNA significantly enhanced memory retention in contextual fear conditioning memory task. Inversely, we found that Neat1 overexpression using CRISPR-dCas9 technology significantly interfered with long-term memory formation. These findings suggest for the first time a unique role for Neat1 as a "molecular brake" on the formation of long-term memories. Using an informatics approach, we identified several memory-related gene targets for Neat1 and publicly available sequencing data revealed that Neat1 is upregulated in the hippocampus of aged animals relative to young adults, and after

chronic stress -both of which are associated with memory deficits. Collectively, these results support the hypothesis that the Neat1 lncRNA is a powerful negative regulator of long-term memory formation, and further indicate that targeting Neat1 activity may have both clinical relevance and therapeutic potential for the treatment of memory deficit disorders associated with normal aging and stress.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Title: Translational profiling of CA1 projection neurons after fear learning

Authors: *A. L. JONES[1], L. REIJMERS[2];
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Abstract: The ability to encode and store long term fear memories allows us to remember and subsequently avoid dangerous stimuli. The synthesis of new proteins is one of the primary molecular mechanisms for the long-term storage of these memories. In mouse models of contextual fear learning, in which the animal learns to associate a novel environment with an aversive stimulus, protein synthesis is initiated within the hippocampus and blocking protein synthesis within that region interferes with storage of the fear memory. Recent studies have begun to identify for which mRNAs translational activity is modulated by contextual fear conditioning within the hippocampus. A major challenge left unaddressed by these studies however is deciphering which cell types are responsible for translational changes at the level of individual genes. Furthermore, because these studies do not account for cell type specificity, they may have missed changes that occur in genes that are expressed in multiple cell types but that are only translational regulated in specific cell types. To address these challenges, we utilize a transgenic mouse line that expresses EGFP tagged ribosomal fusion protein (EGFP-L10a) in Camk2a positive neurons. Because this mouse line expresses EGFP-L10a in Camk2a expressing neurons only within the CA1 of the hippocampus, we are additionally able to address translational changes specific to the CA1. The Translating Ribosome Affinity Purification (TRAP) technique allows isolation of ribosome bound mRNA from cell types expressing the EGFP-L10a fusion protein, however these ribosome-bound mRNAs may include genes that are not actively translating. By combining contextual fear conditioning with a modified version of TRAP and downstream RNA sequencing (RNAseq) we are able to determine the full set of

genes that are both ribosome bound and actively translating in Camk2a expressing neurons within the CA1 of the hippocampus. Preliminary RNAseq results suggest that this approach can be used to isolate the effects of fear learning on translational regulation at the genome-wide level with complete cell type specificity.

Disclosures: **A.L. Jones:** None. **L. Reijmers:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 355.28/III49

Topic: H.01. Animal Cognition and Behavior

Support: Leir Foundation fellowship

Title: Cell-type specific gene profiling from amygdala & cortex during long-term taste aversion memory formation

Authors: *D. LEVITAN[1], D. B. KATZ[2], S. B. NELSON[3];
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Abstract: The formation of associative memories requires activation of gene programs that change the intrinsic and synaptic properties of neurons. The molecular changes accompanying learning have typically been studied at the level of whole regions using tissue homogenates, rather than on anatomically or genetically identified cell types, an approach that lacks the resolution necessary for identifying changes that are cell type-specific. We instead used Next-Generation sequencing from genetically identified neurons to study the molecular mechanisms underlying conditioned taste aversion (CTA), a canonical form of simple learning that requires transcription in the basolateral amygdala (BLA) and gustatory-cortex (GC). Specifically, we profiled global gene expression in projection neurons and fast-spiking parvalbumin-positive interneurons in GC and BLA 4 hours following CTA (compared to control conditions), validating hits (genes differentially expressed during learning) *via* qPCR and immunofluorescence. In BLA pyramidal neurons we observed a cell-type specific pathway composed of the transcription factor c-fos and its direct target (the trans-synaptic protein Nptx2 which is known to regulate excitatory to inhibitory synapses). We went on to show that these same c-fos expressing neurons project to the GC, a finding predicted by the fact that the connection between the BLA and the GC is important for taste coding during learning. Thus suggests engagement of a cell-type specific gene pathway controlling BLA to GC network activity during taste learning.

Disclosures: **D. Levitan:** None. **D.B. Katz:** None. **S.B. Nelson:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant T32 Sleep Genetics Post doctoral fellowship

NIH Grant DP2MH104119

Title: Cell type specific translational profiling during sleep dependent memory consolidation

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Abstract: Sleep plays a crucial role in memory consolidation and sleep deprivation causes cognitive impairment. However the molecular mechanism by which sleep mediates structural changes in synapses and functional changes in neuronal networks is not well understood. Since contextual fear memory (CFM) consolidation depends on both post-training sleep and hippocampal protein synthesis, we hypothesized that sleep promotes synaptic plasticity in the hippocampus via cell type-specific protein translational changes. We used translating ribosome affinity purification (TRAP) to assay these changes in pyramidal neurons within the dorsal hippocampus and prefrontal cortex. Cell type specificity was achieved by crossing Ribo-tag transgenic mice (expressing a floxed construct with HA-tagged RPL22 protein) with mice expressing Cre-recombinase in excitatory neurons (Camk2a-CRE). This genetic strategy allowed us to rapidly affinity purifies translating ribosomes and associated mRNAs from these specific neuronal populations, after a period of post-CFM-training sleep. Our preliminary qRT-PCR gene expression studies showed that TRAP combined with tissue sample pooling significantly increased the ratio between glutamatergic neuronal markers and glial/GABAergic neuronal markers (signal: noise ratio) in the mouse hippocampus. To profile ribosome-associated mRNA during CFM consolidation we used three behavioral manipulations, control sleeping mice (sham training+3h sleep), CFM sleeping mice (CFM training+3h sleep)and CFM sleep-deprived mice (CFM training+3h sleep deprivation). Sleep-dependent translational profiling during CFM consolidation was achieved by comparing CFM sleeping and control sleeping mice and specific translational changes during sleep was achieved by comparing CFM sleeping and CFM sleep-deprived mice. Ribosome-associated mRNAs were quantified and identified using Affymetrix microarray (Mouse gene ST2.1) and confirmed by quantitative real time RT-PCR. Compared to

the CFM sleeping group, mRNAs associated with synaptic plasticity, metabolic, epigenetic, cell cycle, circadian, inflammatory, transcription factors and miRNAs showed differential association with ribosomes in CFM sleep-deprived group, in both hippocampus and prefrontal cortex. Surprisingly, however, there were no single mRNAs changed in CFM sleeping group compared to control sleeping group. We hope that by clarifying which mRNAs are translated during sleep-dependent memory consolidation, these studies will open a new window on sleep function in the brain.

Disclosures: T. Kariharan: None. **S. Aton:** None.

Poster

355. Animal Cognition: Memory Consolidation and Reconsolidation

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH099730

NIH Grant MH102703

NIH Grant AG017628

Title: Phosphorylation of eukaryotic translation initiation factor 4E binding protein 2 (4EBP2) is required to rescue memory impairments caused by sleep deprivation

Authors: *J. C. TUDOR, C. W. CHUNG, E. SORENSEN, T. ABEL;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Protein synthesis is required for the consolidation of long-term memories. We recently demonstrated that sleep deprivation impairs protein synthesis required for hippocampus-dependent memory consolidation. A specific subset of the insulin signaling pathway, AMPK-mTORC1-4EBP2 signaling, is affected by sleep deprivation in the hippocampus. Here, we developed a mutant phosphomimetic 4EBP2 adeno-associated virus (AAV) with a CamKII alpha promoter fragment to selectively express in excitatory neurons of the hippocampus. This virus contains selective mutations to aspartate residues at the phosphorylation sites of 4EBP2 to mimic phosphorylation. Expression of phosphomimetic 4EBP2 in the hippocampus was sufficient to prevent the memory deficits associated with sleep deprivation in the object place recognition task. To further support our findings, we also developed a phosphodefective 4EBP2 AAV with selective mutations at its phosphorylation sites to alanine residues to prevent phosphorylation at these sites. Expression of phosphodefective 4EBP2 did not rescue memory impairments induced

by sleep deprivation. Interestingly, mice that were not sleep deprived had memory impairments similar to those mice that were sleep deprived. Together, we find that phosphorylation of 4EBP2 is required to rescue the memory impairments caused by sleep deprivation. Furthermore, our findings highlight a vital role for protein synthesis and mTOR signaling on long-term memory formation.

Disclosures: **J.C. Tudor:** None. **C.W. Chung:** None. **E. Sorensen:** None. **T. Abel:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 356.01/III52

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal sharp-wave ripple characteristics during delay periods in the rodent automated search task

Authors: *K. TAHON[1,2], D. A. JACKSON[2], W. H. DRINKENBURG[2];
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Abstract: The hippocampus is strongly associated with spatial working memory: the capacity to briefly hold and recall information associated with visuospatial information. Hippocampal sharp-wave ripples (SWRs) are shown to be important in memory consolidation, although the significance of these events in working memory functions is not well understood. In the present study the working memory dependency of an automated search task was increased by the incorporation of intertrial intervals (ITI). Male Long-Evans rats (n = 30) were trained on the search task in operant boxes modified with a touch screen opposite to the reward site with a food-pellet dispenser. Finding a hidden location on the screen was rewarded and repeated for 10 trial bins. Each session consisted of 8 different locations. Disruption of working memory abilities was investigated using scopolamine (0.05 and 0.1 mg/kg s.c.), as has been evidenced in other working memory tasks. Simultaneous EEG recording while the animals performed the task allowed for the identification of SWR properties in the CA1 region during the task's ITIs associated with memory formation. Both the introduction of either 2 or 20 sec ITIs paradoxically resulted in significantly less search errors in later trial bins (7-10) when compared to a 0 sec ITI. Scopolamine (0.1 mg/kg s.c.) significantly increased the total number of search errors for trial bins 1-3 but only for longer (10 sec) delays, indicating that the memory processes for the initial acquisition stage are more sensitive to both the longer delays and the higher scopolamine dose tested. Preliminary data (n = 9) on SWR's characteristics in the automated search task using a

fixed 20 sec ITI indicate no significant correlations between the number of search errors on one hand and ripple amplitude, duration or frequency during the delay period on the other hand. However, we found a significant correlation between ripple duration and time within the ITI with longer ripples occurring more at the beginning of the delay period. These preliminary findings allow us to hypothesize that the SWR metrics used in this study do not simply relate to cognitive processes of working memory and suggest an assessment of these events in a more complex oscillatory framework linked with working memory.

Disclosures: **K. Tahon:** A. Employment/Salary (full or part-time): Department of Neuroscience, Janssen Research & Development, a Division of Janssen Pharmaceutica NV Beerse, Belgium. **D.A. Jackson:** A. Employment/Salary (full or part-time): Janssen Pharmaceuticals. **W.H. Drinkenburg:** A. Employment/Salary (full or part-time): Janssen Pharmaceuticals.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 356.02/JJJ1

Topic: H.01. Animal Cognition and Behavior

Support: OGSST

NSERC Discovery

NSERC CREATE VSA

Brain Canada

Krembil Foundation

Title: Experience-dependent enhancement of sharp wave ripples near visual targets

Authors: ***K. L. HOFFMAN**[1], T. K. LEONARD[2];
[1]Psychology, Neurosci. Grad. Diploma Program, York Univ., North York, ON, Canada; [2]York Univ., Toronto, ON, Canada

Abstract: Hippocampal sharp-wave ripples (SWRs) are highly synchronous oscillatory field potentials that are thought to facilitate memory consolidation. SWRs typically occur during quiescent states, when neural activity reflecting recent experience is replayed, but have more recently been described during decision making in rodents and visual exploration in macaques.

To better understand the role of SWRs in waking exploration, we recorded SWRs from the hippocampus of two macaques as they performed a memory-guided visual search task. Their goal was to locate a 'target' object embedded in a scene, revealing improved search performance between initial and repeated presentations. When SWRs coincided with target-object fixations during search, detection was more likely than when these events were decoupled. Ripples were also more likely to occur on repeated than initial image presentations, suggesting an association with memory-guided search. Furthermore, the location of visual fixations at the time of ripples was closer to the target on repeated than on novel trials, even after accounting for differences in fixations across trial types. These results reveal that SWRs are not limited to off-line states as conventionally defined; rather, they occur during active and informative performance windows including search on familiar scenes. The SWR in primates is an infrequent but influential phenomenon whose appearance during active search supports an extended role of SWRs during exploration in primates.

Disclosures: K.L. Hoffman: None. **T.K. Leonard:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

CIHR

Title: Single neuron signatures of the cognitive map in virtually navigating rhesus monkeys

Authors: *R. A. GULLI[1,2], G. DOUCET[1,3], B. W. CORRIGAN[1,2], L. DUONG[1], S. WILLIAMS[4], J. MARTINEZ-TRUJILLO[1,2,3];
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Abstract: The hippocampus has long been studied as a component of two functional systems in the brain: first, integrating information for episodic memory; and second, supporting spatial navigation. Both of these processes have been theoretically linked as forms of "cognitive mapping" (O'Keefe & Nadel, 1978; Buzsaki & Moser, Nat Neuro, 2013; Eichenbaum & Cohen, Neuron, 2014).

If networks of hippocampal neurons indeed support cognitive mapping, it has been proposed that three patterns should be observed in their activity. First, ensembles of neurons that support cognitive mapping should should contain space-dependent and non-spatial representations. Second, meaningful and tractable dimensions of relational cognitive maps should be contained within the neurons that support these maps. Third, network activity should reflect observed behaviour as one moves through the cognitive map (Schiller et al, JNeurosi, 2015).
In order to test these predictions, we recorded single-neuron spikes from the hippocampus of two non-human primates (*Macaca mulatta)*. Each monkey completed two tasks in the same virtual reality (VR) environment: 1) foraging for rewards, or 2) a context-object associative learning task. We found that 54.2% of 189 recorded single neurons show place-selective firing during virtual foraging. During the contextual learning task in the same VR environment, 67.2% of these neurons showed place-selective firing. Single neurons exhibited fewer place fields per neuron during foraging than contextual learning (median 1 and 3, respectively). Place fields are homogenously distributed throughout the VR environment during foraging, with additional place fields arising at the rewarded ends of the maze during contextual learning.
Concordantly, decoding of spatial position in the VR maze using all recorded neurons is more accurate during contextual learning than foraging. Importantly, prediction accuracy during contextual learning is further improved decoding trial period as monkeys move through the maze than in the contextual learning task as compared to spatial position per se. Finally, we find that single neurons are selective for non-spatial parameters of the contextual learning task (context; objects present on a single trial; value of objects), and that these parameters can be reliably decoded from population activity. These cross-task analyses suggest that previously stereotyped hippocampal activity patterns vary dynamically according to behavioural demands of the task are engaged in, supporting the theorized role of the hippocampus in cognitive mapping.

Disclosures: R.A. Gulli: None. **G. Doucet:** None. **B.W. Corrigan:** None. **L. Duong:** None. **S. Williams:** None. **J. Martinez-Trujillo:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Brain Canada

NSERC

Krembil Foundation

CRAINIA

Title: Closed-loop interruption of hippocampal ripples in macaque

Authors: *O. TALAKOUB[1], A. GOMEZ PALACIO SCHJETNAN[2], M. R. POPOVIC[1], T. A. VALIANTE[1], K. L. HOFFMAN[2];
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Abstract: Hippocampal sharp-wave ripples (SWRs) are thought to play a role in memory consolidation. Stimulation of the CA3 axon collaterals in the hippocampal commissure in rats interrupts sharp-wave ripples and leads to memory impairment. In primates, these commissural collaterals are limited, and it's unclear if any other pathways would control ripple production. To investigate whether fornix fiber stimulation could interrupt ripples, stimulating electrodes were implanted bilaterally alongside the fornix in the macaque, together with recording microelectrodes targeting the hippocampus. A subset of the stimulation pulse counts and intensities we tested evoked short-latency hippocampal responses, and were therefore selected for the interruption experiments. SWRs were detected in real-time from the hippocampal recording electrodes of a non-anesthetized macaque, triggering immediate fornix stimulation (Stim condition) or stimulation with a 1-second delay (Lag condition). Stimulation truncated SWRs and suppressed the ripple-associated multiunit responses, in a site-selective and time-specific manner. The post-stimulation suppression of multiunit activity suggests that the fornix could be a target for closed-loop stimulation and interruption of other types of hippocampal activity. Due to largely conserved connections in humans, this approach may be a means for modifying pathological hippocampal events affecting humans, such as mesial temporal lobe seizures.

Disclosures: O. Talakoub: None. **A. Gomez Palacio Schjetnan:** None. **M.R. Popovic:** None. **T.A. Valiante:** None. **K.L. Hoffman:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: EU FP7 grant no. 604102 (Human Brain Project)

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Title: Population activity during sharp wave-ripples depends on structured interactions and spontaneously generated sequential activity in a network model of hippocampal area CA3

Authors: *S. KALI[1,4], A. ECKER[1,4], E. VERTES[1,4,5], I. MIKLOS[2,3], T. F. FREUND[1,4], A. I. GULYAS[1];
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Abstract: Several distinct patterns of population activity can be recorded in the hippocampus in vivo, including theta-modulated gamma oscillations and low-rate irregular activity with periodically occurring large-amplitude sharp wave-ripple (SWR) events. During SWRs, neuronal populations in the hippocampus have been found to "replay" activity recorded during theta-gamma activity in the exploring animal. Such replay may be important for the establishment, maintenance and consolidation of long-term memory. Our aim was to develop a mechanistic understanding of cellular and network mechanisms underlying the generation of SWRs, sequence replay during SWRs, and the observed switching to other types of population dynamics such as gamma oscillations, based on in vitro and in vivo experimental observations. We built a large-scale network model of area CA3 of the hippocampus, and set single-cell and synaptic parameters according to in vitro data. When we used uniform or randomly varying synaptic conductances for all types of connection, there was no sequential activity, and sharp waves with moderate pyramidal cell firing rates and accompanying ripple oscillations were never observed. When recurrent excitatory weights were set by applying an additive spike timing-dependent plasticity rule during simulated runs in a circular maze, sharp wave-like activity with ripple oscillations, physiological rates, and accelerated sequential replay of learned activity patterns emerged spontaneously. All of these features of neural activity were robust to scaling the synaptic conductances in a relatively broad range. We then used systematic perturbations of the synaptic weight matrix to explore the links between these different aspects of the neural dynamics and the underlying functional connectivity. After shuffling the incoming and/or outgoing weights of individual neurons, sequential activity disappeared, and neither moderate rates nor ripple oscillations could be robustly maintained. On the other hand, binarizing the weights by replacing the strongest weights by their average, and the rest of the weights by their average, did not lead to any fundamental change in the dynamics. These results demonstrate that the distribution of synaptic weights is neither necessary nor sufficient for the observed physiological activity of the original network. Manipulations which destroyed embedded convergent paths in the weight matrix invariably led to the disappearance of both sequential activity patterns and SWR population dynamics, demonstrating a fundamental link between temporal representations (coding) and population dynamics in structured cortical networks.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: R01MH102394

Title: Hippocampal-prefrontal synchrony in spatial working memory

Authors: *A. EDSALL, A. L. GRIFFIN;
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Abstract: Spatial working memory (SWM) is hypothesized to depend on oscillatory synchrony between the hippocampus and medial prefrontal cortex (mPFC). Typically, tasks that are designed to assess SWM do not control for variations in sensorimotor behavior. In the present study, we used a novel task-comparison approach, which allowed us to directly manipulate SWM demand by using two variants of visuospatial conditional discrimination task on an elevated T-maze: one that is dependent on SWM (CDWM) and one that is not (CD). The tasks differ solely in the extent to which SWM is required, allowing us to identify SWM-specific neural activity. We recorded local field potentials from the dorsal hippocampus and mPFC of rats as they switched between the CDWM and CD tasks. We set a threshold for "good" performance at 75% correct, dividing all of the recorded sessions into four categories based on performance (good vs. poor) and task (CDWM vs. CD). We then compared phase coherence across these four session types. Differences in theta coherence between session types were restricted to the un-cued portion of the T-maze, where SWM is required. Specifically, of the four session types, the highest theta coherence was seen on good CDWM sessions, followed by poor CDWM sessions. There were also significant differences between good CDWM and CD sessions. Thus, theta coherence was highest in sessions in which SWM was required and used optimally to guide behavior. These results support the hypothesis of hippocampal-PFC synchrony increases with SWM demand.

Disclosures: **A. Edsall:** None. **A.L. Griffin:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Program#/Poster#: 356.07/JJJ5

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01AG043688

Title: How dentate gyrus place cells represent distinct place memories

Authors: *M. T. VAN DIJK[1], A. A. FENTON[2,3];
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Abstract: The dentate gyrus (DG) of the hippocampus is critical for discriminating between spatial memories for similar environments. Rate remapping of DG place fields has been reported for place representations across similar but distinct environments, and DG remapping was greater than in downstream place cells in Ammon's horn. Such forms of pattern discrimination are thought to be the substrate for memory discrimination, but the critical experiment that can link place field remapping to memory discrimination was never performed. We first replicated the remapping results in DG and Ammon's horn place cells by small and large environmental manipulations in the absence of an explicit memory discrimination task. We then investigated remapping in DG neurons while mice performed active place avoidance task variants on a rotating arena. The rotation dissociates place representations into stationary "room" and rotating "arena" spatial frames. Mice initially learn to avoid the location of a stationary shock zone, then in DG-dependent memory discrimination "conflict" trials, they learn the new location of the shock zone, 180° away. Firing field stability was indistinguishable across pairs of "matched" sessions (i.e. before changing the shock zone) and pairs of "memory discrimination" sessions (i.e. before and after changing the shock zone). Instead, the cell-pair discharge correlations measured by Kendall's tau increased across memory discrimination session pairs but not across matched session pairs. To further investigate whether the temporal organization of DG discharge reflects multiple representations of place we took advantage of the fact that the arena rotation dissociates locations into distinct room and arena spatial frames. During each 133 ms, the information about place in DG ensemble discharge tends to preferentially represent either the current room or the current arena location for a few seconds such that the ensemble discharge alternates between the two representations. This multistability, a non-remapping example of pattern discrimination, increases from the pretraining sessions to the training sessions when shock is on, consistent with the presence of shock reinforcing the need to discriminate between room and arena locations. This multistability mirrors spatial behavior because after training, DG

discharge preferentially represents room locations when the mouse is close to the to-be-avoided shock zone. These findings reject the hypothesis that DG place field remapping underlies memory discrimination and show that instead, memory discrimination is mediated by a multistable, temporally coordinated organization of DG ensemble discharge.

Disclosures: **M.T. Van Dijk:** None. **A.A. Fenton:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Program#/Poster#: 356.08/JJJ6

Topic: H.01. Animal Cognition and Behavior

Title: Mnemonic coding of place cells on the 8-arm maze

Authors: ***H. XU**, J. O'NEILL, J. CSICSVARI;
IST Austria, Klosterneuburg, Austria

Abstract: The hippocampus is a key brain region for memory and notably for spatial memory. Hippocampal place cells selectively discharge in specific location of the environment to form mnemonic representations of space. Previous studies have shown that place cells in the CA1 region can remap when animals learn new sets of goal location on an open-field apparatus. However, less is known about place cell activity supporting spatial learning in a maze with narrow paths. Therefore, we have developed a task on a radial 8-arm maze where, every day, only three arms were rewarded to examine how spatial memory traces are represented in the hippocampus. In this task reward locations of two arms were shifted from day-to-day to introduce a spatial memory configuration every day. We found that during learning, many CA1 place cells developed place fields at the newly-rewarded arms, and maintained the same in subsequent probe trials. Those place cells that fired at the newly-rewarded arms gradually shifted their place fields towards the new rewards at the end of the arm as the learning progressed. Moreover, the faster the animal learned the task, the faster this shift took place. These results demonstrate that hippocampal representations are updated when animals shift their visit preferences to new sets of goal arms to meet task demand.

Disclosures: **H. Xu:** None. **J. O'Neill:** None. **J. Csicsvari:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Program#/Poster#: 356.09/JJJ7

Topic: H.01. Animal Cognition and Behavior

Support: ANR

INSERM

Title: The Planar cell polarity pathway regulates the balance between pattern completion and pattern separation

Authors: *B. J.-A. ROBERT[1], M. M. MOREAU[1], M. CARTA[2], S. D.-S. CARVALHO[1], A. QUIEDEVILLE[1], R. PEYROUTOU[1], G. BARTHET[2], M. GARRET[3], B. ATCHAMA[1], S. FIÈVRE[2], L. BRAYDAT-BRUNO[1], C. GUETTE[1], C. RACCA[4], C. MEDINA[1], D. J. HENDERSON[5], A. DESMEDT[1], C. MULLE[2], A. MARIGHETTO[1], M. MONTCOUQUIOL[1], N. SANS[1];
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Abstract: Vangl2, one of the most upstream core planar cell polarity (PCP) proteins, is known for its role in the coordination of cellular cytoskeleton dynamics, participating in the global orientation of cells within many tissues, from drosophila to mammals. The embryonic mutation of Vangl2 leads to severe disruption of the nervous system in mammals, and neonatal lethality. We found an enrichment of vangl2 in hippocampus of adult mice, a structure intimately associated with memory. However, the requirement for vangl2 in learning and memory in adults has never been tested, nor more globally a role for PCP proteins in synaptic plasticity. Here we used a conditional genetic approach to delete vangl2 in specific postmitotic population of neurons in mice brains and tested their memories. Our results demonstrate that the global loss of vangl2 did not alter the acquisition of spatial memory, but the reorganization of spatial information and the computational information transmitted by the DG-CA3 network were affected. We show that conditional vangl2 knock-out mice (cKO) are impaired in a ‘pattern completion’ paradigm using the Morris water maze task, while they performed better than controls in a fear conditioning discrimination task, where mice have to distinguish two similar contexts. Our results suggest that a vangl2-dependent PCP signaling is necessary for optimal balance between ‘pattern completion’ and ‘pattern separation’ processes required for memory function, and demonstrate the implication of the protein in cognitive processes.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: NIH-R25MH059472

NIH-R01MH099128

NSF-IOS 1326187

Title: Molecular and synaptic mechanisms of learning and persistent hippocampal memory for an active place avoidance

Authors: ***R. M. HARRIS**[1,2], H.-Y. KAO[3,2], A. CHUNG[3,2], J.-M. ALARCON[4], E. KLANN[3], H. A. HOFMANN[1,2], A. A. FENTON[3,2,5];
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Abstract: How do animals suppress a learned behavior in favor of producing a new, more context-appropriate behavioral output when the environment changes? Why do some animals learn, remember and adapt better or worse than others? To gain a more comprehensive understanding of variation and variability in hippocampal learning and memory, our research investigates and integrates information across levels of biological organization including behavioral, synaptic, genetic, and transcriptomic data. We tested the hypothesis that memory is based on long-term changes in hippocampal circuits via modulation of specific molecular pathways that regulate synaptic function and plasticity. Using a hippocampus-dependent active place avoidance task, we examined the transcriptional regulation of synaptic function underlying spatial memory within defined regions of the hippocampal circuit in C57BL/6 wild type and Frm1 knockout (KO) mice lacking the Fragile X mental retardation protein (FMRP). One group of mice received three 10-min place avoidance training trials per day for 2 days and were then

tested for memory retention 24 h later. Another group of animals received identical training on the first day, but after a memory-retention test on the second day, training continued with the shock zone shifted 180°, to create a conflict between memory for the initial and new locations of shock in an otherwise unchanged environment. Yoked control animals were exposed to the identical physical conditions but they were shocked when the trained animals were shocked and thus never conditioned. Behavior was quantified using automated video tracking. After the memory retention test, we prepared dorsal hippocampal slices to electrophysiologically analyze the impact of training on synaptic function at the Schaffer collateral CA1 synapse. Alternate slices were used to obtain tissue punches from CA1, CA2, CA3, CA4, and dentate gyrus for transcriptomic analyses using RNA-seq. In addition to group level differences, our analyses identified patterns of co-variation that span levels of biological organization from molecular networks, to neurophysiology, to animal behavior. This integrative approach dissects the molecular networks within neural circuits that either affect memory persistence or change as a consequence of behavior. The results demonstrate how integrating quantitative behavioral assays, *ex vivo* slice physiology, gene expression analysis, and multivariate statistics provides novel insights into the multilevel processes of hippocampal learning and memory.

Disclosures: **R.M. Harris:** None. **H. Kao:** None. **A. Chung:** None. **J. Alarcon:** None. **E. Klann:** None. **H.A. Hofmann:** None. **A.A. Fenton:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 356.11/JJJ9

Topic: H.01. Animal Cognition and Behavior

Support: DECODE Grant Program

Title: Long-term imaging of neural ensembles in a mouse model of intellectual disability.

Authors: ***E. H. SCHUT**[1], N. NADIF KASRI[1], F. P. BATTAGLIA[2];
[1]Donders Inst. for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands;
[2]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Euchromatic histone methyltransferase 1 heterozygous knockout mice (*Ehmt*$^{+/-}$), a model for Kleefstra syndrome, a genetically inherited intellectual disability syndrome, display hippocampal-dependent learning deficits. Preliminary data suggests that these mice show hippocampal hyperexcitability *ex vivo* (Selten et al, in prep). However, the functional phenotypes of these mice have not yet been extensively described at the neural circuit level. Moreover, *in*

vivo investigation of neural circuits is necessary to link our *ex vivo* results with behavior. We are addressing this by investigating spontaneous activity in *Ehmt1*$^{+/-}$ mice and matching controls using *in vivo* calcium imaging and by examining spatial maps formed by hippocampal place cells in these mice, which are essential for spatial memory. Mice were injected with AAV5-Syn-GCaMP6f into CA1. Ensemble activity was imaged via a lens probe and a microendoscope (Inscopix Inc) during running on a linear track. In our preliminary results, raw signals of calcium activity were observed in wildtype CA1 (n=200 cells) during running on a linear track. We will compare the information processing properties of CA1 neurons in *Ehmt1*$^{+/-}$ mice vs. controls, similar to what we have previously reported *ex vivo*. We are currently analyzing spontaneous activity of CA1 and the information content and long-term stability of hippocampal place cells in intellectual disability mouse models, to understand the mechanisms of this neural disorder at the circuit level.

Disclosures: E.H. Schut: None. **N. Nadif Kasri:** None. **F.P. Battaglia:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 356.12/JJJ10

Topic: H.01. Animal Cognition and Behavior

Support: R01MH106552

Simons Foundation (273886)

Title: Cross-activation of hippocampal place cell patterns by social subjects

Authors: *X. MOU, D. JI;
BCM, Houston, TX

Abstract: Humans and animals frequently learn through observing or interacting with others. How the neural circuits in the brain mediate this behavior of social learning is enigmatic. The local enhancement theory of social learning proposes that presence of social subjects in a spatial environment facilitates other subjects' understanding of the same environment. Because spatial environments are thought to be encoded by hippocampal place cells that are active in specific locations of a given space, to explore the neural basis of the local enhancement theory, here we studied hippocampal place cells in an observational task. We recorded place cells in rats as they stayed in a small box while a demonstrator rat running on a nearby linear track and as they ran on the same track themselves. Our analysis shows that place cell firing sequences during self-

running on the track also appeared in the box. This cross-activation in the box occurred even prior to any self-running experience on the track and was absent without a demonstrator. Our data thus demonstrate that spatial code of an environment can be activated by the presence of a social subject. This finding may contribute to the neural mechanism of social learning.

Disclosures: **X. Mou:** None. **D. Ji:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Program#/Poster#: 356.13/JJJ11

Topic: H.01. Animal Cognition and Behavior

Support: ICM P-09-015-F

ICM P-10-001-F

National PhD Grant - Conicyt

Title: Role of the hippocampus during observational learning of a spatial memory task

Authors: *Y. FUENTEALBA[1], J. VALDÉS G.[2];
[1]Univ. of Chile, Independencia, Chile; [2]Unviersity of Chile, Santiago, Chile

Abstract: Observational learning is defined as the ability to imitate goal-directed behaviors through the observation of a congener acting as a demonstrator or expert. The mirror neuron system could be one of the mechanisms responsible for this phenomenon, at least during motor actions. This system has been described in humans and non-human primates, even though many other species show similar capabilities. Other cognitive capacities may also be improved by observation, thus suggesting that other neurophysiologic mechanisms could also generate the same phenomenon.
The hippocampus is an essential brain region involved in spatial navigation and learning. Both animals and humans with hippocampal damage have shown impaired learning during spatial navigation tasks. There is no data available regarding the participation of the hippocampus in social learning of a spatial navigation tasks. We hypothesized that the hippocampus do participates in the observational learning of a spatial navigation tasks.
To evaluate this, we generated a paradigm of observational learning in which an untrained rat observes how a pre-trained rat solves a spatial maze. After the observation period, the untrained rat is challenged to solve the maze. In order to evaluate the role of hippocampus in this learning process, we injected bupivacaine a voltage-gated sodium channel blocker, bilaterally into the

CA1 region of hippocampus, of the untrained observer animals during the observation period. Our results show that untrained animals presented a better performance in the spatial maze after observation compared to those who did not observe. Interestingly, this effect was sensitive to pharmacological inactivation of the hippocampus.
These results suggest that rats can learn from observation of congeners and that this learning is hippocampus-dependent.

Disclosures: Y. Fuentealba: None. **J. Valdés G.:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 356.14/JJJ12

Topic: H.01. Animal Cognition and Behavior

Support: NSERC grants to S. Wright, D.M. Skinner and G.M Martin

Title: Do distance cues support memory retrieval in response discriminations and reversal learning?

Authors: *S. WRIGHT[1], D. M. SKINNER[2], M. L. INGRAM[2], G. M. MARTIN[2];
[1]Grenfell Campus, Mem. Univ. of Newfoundland, Corner Brook, NL, Canada; [2]St. John's Campus, Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Response reversal learning is facilitated by changes in contextual cues in many species. Subjects are rewarded for one response (e.g., a right turn) to a set criterion. Once criterion is reached they are required to learn the opposite response (i.e., a left turn) to the same criterion. Subjects require fewer trials to reach criterion when acquisition and reversal training occur in different rooms. We have previously shown that the facilitation in response reversal learning after room changes is not based on cues such as changes in lighting or noise. We speculate that it is changes in the metric cue of direction that underlies the effect of room changes. Consistent with this speculation is our demonstration that subtle manipulations of maze orientation, in the absence of other cue changes will facilitate response reversal learning in rats. The current experiments extend our analysis to the metric cue of distance where we show that distance cues support discrimination learning, but unlike direction cues, may not support response reversal learning.

Disclosures: S. Wright: None. **D.M. Skinner:** None. **M.L. Ingram:** None. **G.M. Martin:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Program#/Poster#: 356.15/JJJ13

Topic: H.01. Animal Cognition and Behavior

Support: Medical Research Service of the Department of Veteran's Affairs

NSF Temporal Dynamics of Learning Center Grant

Title: Comparing the effects of dorsoventral CA1 lesions and full hippocampal lesions on anterograde tests of spatial memory in rats

Authors: *A. OCAMPO[1,2,5], S. VINCENT[2], A. HASHI[2], M. GRAVES[2], L. R. SQUIRE[2,5,3,4], R. E. CLARK[2,5];
[1]UCSD, LA Jolla, CA; [2]Psychiatry, [3]Psychology, [4]Neurosciences, UCSD, La Jolla, CA; [5]VA Med. Ctr., San Diego, CA

Abstract: The hippocampus plays an important role in memory. Hippocampal subfield CA1 receives information from the dentate gyrus and subfield CA3 and serves as the sole output of the hippocampus to neocortex. Based on these connections, a lesion targeting the entire CA1 region should block hippocampal output to neocortex. Supporting this idea, targeted CA1 lesions in rats impaired retrograde memory at both recent and remote time points, similar to what is observed following full hippocampal lesions (Ocampo et al., 2015). However, the retrograde impairments observed with both lesions were always severe, demonstrating floor effects. To broaden the comparison between the two lesions, we investigated the effects of CA1 and full hippocampal lesions on anterograde memory. Rats received excitotoxic CA1 lesions (n=5), full hippocampal lesions (n=5), or sham surgeries (n=5) before undergoing behavioral training in two different tasks: the watermaze task and delayed matching-to-position (DMP). The DMP task is a variant of the standard watermaze task involving one-trial learning. In DMP, the platform location is changed for each training session. Rats were tested on DMP first, followed by watermaze training. In both tasks, rats with CA1 lesions performed normally while rats with full hippocampal lesions were impaired. In contrast to the retrograde findings, these results suggest that full hippocampal lesions impair anterograde memory while CA1 lesions do not. Our earlier work, however, showed that CA1 lesions impaired anterograde watermaze performance similarly to full hippocampal lesions when the rats were naïve to both watermaze tasks (Ocampo et al., 2015). Therefore, we will next test the effects of the lesions on anterograde performance in the watermaze task first, followed by DMP. Together, these findings will help to characterize important differences in the way CA1 lesions and full hippocampal lesions affect memory.

Disclosures: **A. Ocampo:** None. **S. Vincent:** None. **A. Hashi:** None. **M. Graves:** None. **L.R. Squire:** None. **R.E. Clark:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant IOS-1146822

Title: Internally organized spatial firing of MEC cells during navigation: If space was time

Authors: ***E. PARK**, S. KEELEY, A. A. FENTON;
New York Univ., New York, NY

Abstract: The spatial discharge of medial entorhinal cortex (MEC) and hippocampus cells can be classified into distinct, functionally dedicated cell classes, thought to constitute a unitary representation of space. But how spatial discharge relationships are maintained within and between these functional classes is unknown. The "externally-structured" hypotheses assert that constant functional relationships amongst the cells are inherited from environmental associations and are reinforced by activity-dependent synaptic plasticity. Alternative, "internally-structured" hypotheses assert that development and experience-driven synaptic plasticity create a neural infrastructure that imposes attractor-like, temporally-structured dynamics on ensemble neural discharge.

To evaluate these two alternatives, we recorded rat MEC and hippocampus principal cells during a hippocampus-dependent 2-frame active place avoidance task on a circular arena that was stationary or rotating. Cognitive map theory predicts the spatial tuning of cells in the two conditions guides spatial behavior in both spatial frames. Because rotation dissociates information into separate stationary and rotating spatial frames, externally-structured hypotheses predict that spatially-tuned cells will discharge in response to the altered environmental spatial features, disturbing the organization of temporal discharge amongst cell pairs. Internally-structured hypotheses predict maintained temporal discharge relations during rotation but disrupted spatial tuning.

Basic features like the firing rates of all cells were stable across the stationary and rotating conditions. During 2-frame avoidance behavior, spatial tuning diminished during rotation and partially reverted when rotation stopped. Using the discharge of single head direction cells as an internal reference, directional tuning was maintained, but was unstably coupled to the environment. At time scales from 40 ms to 5 s, internally structured discharge correlations

between pairs of cells were completely maintained across the stable and rotating conditions if both cells were within either MEC or hippocampus. The maintenance of temporal correlations approached chance for MEC-hippocampus cell pairs. The findings strongly support internally-structured hypotheses, reject externally-structured hypotheses, indicate that strong/weak attractor dynamics organize discharge in MEC/hippocampus, and demonstrate the representations of space in MEC and hippocampus can uncouple during hippocampus-dependent spatial navigation.

Disclosures: E. Park: None. **S. Keeley:** None. **A.A. Fenton:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Program#/Poster#: 356.17/JJJ15

Topic: H.01. Animal Cognition and Behavior

Support: NSF IIS-1429937

Title: Neurophysiological correlates of spatial navigation optimization in the rodent

Authors: *T. PELC[1], M. LLOFRIU[2], N. CAZIN[3], P. SCLEIDOROVICH CHIODI[2], P. DOMINEY[3], A. WEITZENFELD[2], J.-M. FELLOUS[1];
[1]Dept. of Psychology, Arizona Univ., Tucson, AZ; [2]Dept. of Computer Sci. and Engin., Univ. of South Florida,, Tampa, FL; [3]Human and Robot Cognitive Systems, INSERM, Bron, France

Abstract: Significant progress has been made in understanding the neural substrate of goal-oriented spatial navigation in mammals. However, our understanding is still limited to simple tasks, which usually consist of one or two goal locations and limited number of navigation choices. Moreover, since most data are collected from well-trained subjects, our knowledge of the neural dynamics of spatial memory acquisition and optimization across trials in a multi-goal navigational task remains limited. We study the role of the hippocampus (a major brain area involved in spatial navigation) in a challenging spatial task called 'the Traveling Salesperson Problem' (TSP). In this task, subjects have to find the shortest route between a set of rewarded spatial locations. We previously showed that rats were capable of converging to a near optimal path after a few trials. Using different spatial configurations of multiple reward locations, we created navigational tasks of various measurable complexity. In a first series of experiments we show that temporary inactivation (bupivacaine 2.5% solution 1 µl/side) of the dorsal hippocampus produces significant impairments in the task. In a separate group of animals, we recorded the activity of CA1 place cells during the task and during the inter-trial periods when place cells are known to replay in conjunction with sharp wave ripple events. Our hypothesis is

that series of short replay events during spatial learning eventually lead to assembling an entire sequence. We predict that the rate and spatial distribution of replay events within and between trials will be correlated with the convergence onto a preferential path. We compare the cellular content of the replay events during learning, in cases when the rat converges or fails to converge on the final path. We analyze various aspects of the rat path trajectories and establish the extent to which the convergence to the optimal path depends on the target-to-target segments visited on the previous trials. We use a computational model of the hippocampus in which the decision to choose the next target in the TSP task is a function of place cell population firing. We assess the effect of dorsal and ventral hippocampus inactivation on the ability of the model to converge on an optimal path. These experiments and analyses give insights into the complex dialog between the navigational system of the hippocampus and the planning system of the medial prefrontal cortex in a multi-goal spatial optimization task.

Disclosures: **T. Pelc:** None. **M. Llofriu:** None. **N. Cazin:** None. **P. Scleidorovich Chiodi:** None. **P. Dominey:** None. **A. Weitzenfeld:** None. **J. Fellous:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Support: Brain Canada

Alzheimer's Association

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Title: Interactions between brain-behavior state and stimulation frequency determine responses to fornix stimulation in the macaque hippocampus

Authors: *A. GÓMEZ PALACIO SCHJETNAN, T. K. LEONARD, O. TALAKOUB, K. L. HOFFMAN;
Dept. of Psychology, Ctr. for Vision Res., York Univ., North York, ON, Canada

Abstract: Fornix stimulation is currently under investigation as a treatment for memory impairments attributed to hippocampal dysfunction. The physiological interactions between the fornix and hippocampal formation in the primate brain are unclear, and in addition, the effects of stimulation may be influenced by behavioral state. We sought to determine the effects of different fornix stimulation frequencies on hippocampal responses as function of behavioral state. We recorded neuronal responses in multiple sites of the hippocampal formation of two macaques following fornix stimulation. Each macaque received bilateral, bipolar fornix stimulation in trains of 50 bursts, where each burst contained 8 biphasic pulses of 100μs duration every 2-ms delivered at several intervals from 1.75 - 13 Hz. Responses to single pulses of 40 and 100 Hz were also evaluated. Chronically-implanted multi-electrode/tetrode arrays sampled local field potentials from different sites in the hippocampus and subicular complex. The animals were stimulated during a goal-direct memory task and during quiescent periods, including sleep. Evoked responses to fornix stimulation varied with hippocampal recording site, and responses at extra-hippocampal locations were minimal. Responses evoked with low inter-burst intervals (1.75 - 4Hz) during quiescent periods were characterized by longer peak latencies and duration compared to responses evoked by the same frequencies during more active behavioral states. This response variability during quiescence was reduced with higher stimulation frequencies (e.g. 11 Hz), but not with individual high-frequency pulses (40 Hz and 100 Hz) which showed minimal evoked responses. These results suggest that responses to stimulation protocols vary as a function of behavioral state and that response variation can also be controlled by using faster stimulation burst intervals. Depending on the corresponding changes in cognitive function, these findings may guide potential therapeutic strategies to address memory decline, particularly those that consider behavioral state.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NSF 1422438

Houston Bioinformatics Endowment

Title: Robust spatial memories encoded by transient neuronal networks: a topological model

Authors: *Y. A. DABAGHIAN;
Neurol. and pediatrics, Jan and Dan Duncan Neurolog. Res. Institute, Baylor Col. of Med., Houston, TX

Abstract: It is widely accepted that the principal cells in mammalian hippocampus encode an internalized representation of the environment—the hippocampal cognitive map, that underlies spatial memory and spatial awareness. However, hippocampal network, as any other network in the brain is transient: the connections between neurons constantly change due to various forms of synaptic plasticity. In particular, the functionally interconnected groups of hippocampal neurons—the place cell assemblies—emerge as a result of place cell coactivity, via "fire together wire together" plasticity mechanisms. On the other hand, cell assemblies may disband due to cell loss or depression of synapses, caused by reduction or cessation of spiking activity over a sufficiently long times. Some of the disbanded cell assemblies may later reappear during a subsequent period of coactivity, then disappear again, and so forth: electrophysiological studies in rats in mice suggest that the lifetime of the cell assemblies ranges between minutes to tens of milliseconds. In contrast, spatial memories in rodents can last for months, i.e., there is a clear gap between the "cognitive" and the network plasticity timescales, which raises an immediate question: how can the large-scale spatial representation of the environment be stable if the neuronal stratum that computes this representation changes on a much faster timescale? We propose a computational approach to answering this question based on a couple of insights. First, we propose that hippocampal cognitive map is fundamentally topological, and hence it is amenable to analysis by topological methods. We then apply Algebraic Topology techniques to understand how transience of synaptic connections may affect the speed and reliability of spatial learning. By simulating place cell spiking activity in transient cell assembly network during the rat's exploratory movements through different environments, we study the emerging large-scale neuronal representations of space in these environments. We find that despite transient connectivity the cell assembly network, the population of place cells produces a stable representation of spatial connectivity.

Disclosures: Y.A. Dabaghian: None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH099128

NIH Grant R21NS091830

Title: Long-lasting input-specific place learning-induced changes of the hippocampal circuit measured in the freely-behaving mouse

Authors: *A. CHUNG, A. A. FENTON;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Learning is thought to change synaptic function of the neural circuits that store the acquired information in memory, but persistent learning-induced changes in synaptic function have been elusive. We reported strengthening of the evoked response, and altered LTP and LTD at the hippocampal Schaffer collateral synapse in ex vivo mouse dorsal hippocampus slices a month after forming active place avoidance memory, but the changes persisted only if memory persisted. We also previously observed in urethane-anesthetized mice, synaptic and excitability changes at the dentate gyrus (DG) perforant path synapse 1-2 days after forming the memory. The present study investigated, in freely-behaving mice, whether learning causes synaptic circuit function changes. Adult male mice were implanted with sets of stimulating electrodes in the angular bundle and 16- or 32-site recording electrodes that spanned the somatodendritic axis of dorsal hippocampus. Evoked potential responses were localized to DG in response to 0-300 μA test stimulations. Recordings were made during theta, while mice ran on a wheel in a separate environment before and 2 h after each place avoidance training session. Excitability measured by the population spike amplitude (PS) decreased acutely comparing before and 2 h after each training session, whereas the fEPSP slope changed minimally, indicating reduced DG synaptic circuit sensitivity. This acute post-training change was attenuated 24 h later; the excitability had increased, but not to the pretraining level, and the sigmoid relationship between the fEPSP and PS responses had become more linear. Functional changes maintained at least 50 days without further training. Repeating the memory training in a novel environment caused similar acute changes and a day later, the E-S curves became more non-linear and left-shifted, indicating greater sensitivity and excitability. Conflict training to avoid the opposite location in the initial training environment caused changes that reversed the changes caused by learning in the novel environment. Current source density analyses confirmed the effects, indicating that initial training increases the dendritic sink (excitation) and perisomatic source (inhibition), that the changes are partly attenuated 24 h after each training, and further attenuated by learning in a novel environment. Despite maintaining at least 50 days, the changes strongly attenuated after forming a conflicting memory. These data indicate that memory storage is accompanied by large hippocampal circuit function changes that can control memory expression and may dwarf the changes that are presumed to store the information in memory.

Disclosures: **A. Chung:** None. **A.A. Fenton:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Branco Weiss Society In Science Fellowship

ERC Neuroschema Grant

Title: Sharp-wave-ripple disruption after one session learning erases memory

Authors: *L. GENZEL[1], F. BATTAGLIA[2], R. MORRIS[1];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Donders Inst., Nijmegen, Netherlands

Abstract: Many studies have shown that sleep benefits memory. We are starting to understand the mechanisms occurring during sleep, with sleep-related memory replay leading to systems consolidation and strengthening cortical memory networks. Memory replay in the hippocampus and prefrontal cortex occurs during sharp-wave-ripples, a very fast oscillation measurable in the hippocampus. In the prefrontal cortex the sharp-wave-ripple is preceded by a slow-oscillation and followed by spindles, and it has been proposed that the coupling of these oscillations becomes stronger after learning experiences. Correlative evidence for these processes is mounting, but interventional, causative studies are still lacking. Girardeau et al (2009) as well as Ego-Stengel et al (2010) interrupted sharp-wave-ripples in rats every day for one hour after encoding of a reference memory task, and while memory performance was disturbed in comparison to control animals, disrupted animals still achieved above chance performance most likely due to the intensive training paradigm of 1-2 weeks and left-over replay occurring after the daily hour of disruption. By using a one-session learning paradigm in the watermaze and in a dry-land plus-maze task, we attempted to completely erase the spatial memory via a 4-5h sharp-wave-ripple disruption condition in comparison to a control-disruption and undisturbed baseline. Our results show that while the disruption did erase the spatial memory in the plus-maze task, it only weakened the strong memory created in the watermaze. This confirms the importance of replay for memory consolidation, but also shows that strong memories can persist even without memory replay occurring after learning.

Disclosures: **L. Genzel:** None. **F. Battaglia:** None. **R. Morris:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: Department of Biotechnology, Ministry of Science and Technology
BT/PR12531/BRB/10/747/2009

National Centre for Biological Sciences

Title: Mapping time to hippocampus CA1 sequences

Authors: *A. G. KAMBADUR, S. PALCHAUDHURI, D. SINGH, U. S. BHALLA;
Natl. Ctr. For Biol. Sci. (NCBS), Bangalore, India

Abstract: The mammalian hippocampus is implicated in Trace Eye-Blink Conditioning (TEC) wherein a neutral Conditioned Stimulus (CS+), e.g.- a Tone, is associated with an Eye-Blink eliciting Unconditioned Stimulus (US), e.g.- an air-puff, despite an intervening, stimulus free Inter-stimulus interval (ISI). The hippocampus is thought to maintain a "Trace" of the CS+ through the ISI, in specific, reliable cellular activity sequences, triggered by the CS+. Small populations of Hippocampal CA1 cells are known to showcase such time-locked firing (speculatively following upstream CA3 sequences), and can be imaged by 2-photon microscopy, *in vivo*.
We are interested in the following questions:
- How stable are the sequences over time?
- Can behaviourally irrelevant, unpaired stimuli (CS-) also trigger sequences?
- Once a particular ISI is learnt, does changing the ISI affect the structure of the CA1 sequence, in the same animal?
To start answering these questions, we first train head-fixed mice to TEC,
- In multiple sessions, over many days,
- With behaviourally relevant (CS+) and irrelevant (CS-) stimuli, and
- With changes to the ISI, post learning, in the same animal.
We program Arduinos to synchronize the timing of various stimuli and eye-blink measurements. Eye-Blinks are recorded by amplifying the changes in IR reflectance off the eye, as the animals are subjected to auditory tones and LED light flashes with only the CS+ being paired with the US. Our preliminary behaviour-only studies suggest that the animals ignore the CS- only when it is of a different modality than the CS+, consistent with previous studies.
To record activity from CA1 cells over a long period of time, we have begun standardization of a chronic imaging preparation (based on Dombeck et al., 2010). We have been able to acutely (single day) image 110 x 110 μm^2 capturing GCaMP6f reported Ca^{2+} activity from ~100 CA1

cell bodies, using 2-photon microscopy, at a frame rate of ~10-15 Hz, *in vivo*. In naïve animals, we observe cellular responses to the air-puff, and only weak responses to tones. Many CA1 cells show high correlation in activity during pre-stimulus periods. Cells organized based on these spontaneous activity correlations are also spatially clustered, as previously observed (Modi et al., 2014).
The behaviour coupled imaging data will help provide an understanding of how CA1 sequences correlate with the temporal requirement of a task.

Disclosures: **A.G. Kambadur:** None. **S. Palchaudhuri:** None. **D. Singh:** None. **U.S. Bhalla:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Support: NIMH 5R01MH09959402

Title: CA1 hippocampal ensemble neural activity reveals associative representations in mice learning a bi-conditional learning task

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Abstract: It is well established that the rodent hippocampus has a key role in spatial cognition, whereas its role in associative memory is less well understood. Prior studies have identified hippocampal neurons that encode conjunctions of spatial and sensory stimuli. However, it remains largely unknown how these conjunctive representations develop with learning and to what extent they are activated in contexts in which there is conflicting information. To examine the role of hippocampal ensemble neural coding in associative memory, we used a head-mounted miniature microscope to image the somatic calcium dynamics of hundreds of individual CA1 hippocampal pyramidal cells in freely behaving mice as they learned a task requiring mastery of a bi-conditional rule. This behavioral task involved two visuo-tactile stimuli that were independently presented in each of two contextually distinct running tracks. Towards identifying cells that encode either location-specific or location-invariant information, we randomized the locations in each context at which the two different stimuli appeared. In each context, only one of the two stimuli was associated with a reward; thus the mouse had to learn the associations

between stimuli and contexts. Mice readily learned to perform this task, even when they rapidly alternated between the two contexts. We identified CA1 neurons that represented various task-related parameters, including the mouse's spatial location, stimulus identities, and the context. Importantly, we also found neurons that encoded both location-specific and location-invariant associations between stimuli and contexts. We are studying the dynamics of how these associative representations develop during learning and how stable these representations are over extended time periods. Overall, an improved understanding of how hippocampal neural ensembles develop associative representations over the course of learning may lay the groundwork for attaining a more mechanistic understanding of declarative memory.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: HHMI

Title: Circuit architectures for the encoding and processing of 3D orientation

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Abstract: The representation of three-dimensional (3D) orientation of objects or self is an essential function for species ranging from insects to mammals. A seminal psychophysics study (Shepard & Metzler, 1971) has shown that humans are able to easily identify pairs of rotated versions of the same 3D object (mental rotations), with reaction times independent of the axis and growing linearly with the angle of rotation between the objects. More recently, electrophysiological studies in crawling and flying bats uncovered the existence of head direction (HD) cells representing the orientation of the bat in 3D. Both findings strongly suggest that dedicated neural circuits can encode information about orientations of objects/self in 3D. However, little is known about the mechanisms underlying these functions. Extensions of existing models of 2D orientation coding to 3D are hindered by several complications; for

instance, the rotation of objects in 3D is a non-commutative operation: the same rotations applied in a different order would result in different outcomes. Overall, the space described by all possible orientations of an object has a non-trivial structure.Here we propose a continuous attractor neural network model that exhibits patterns of persistent activity of neurons mapped continuously to the group of 3D rotations. The choice of a family of synaptic couplings between the neurons allows us to obtain exact expressions for the stationary solutions of the network dynamics in the form of persistent localized activity. We test possible model applications in two scenarios. First, we extend models of 2D HD cells by studying the network dynamics in the presence of angular velocity inputs along the three Euler angles (yaw, pitch, and roll), inspired by the presence of pure cells in the bat HD system. Second, we analyze the network dynamics in a simplified version of the mental rotation experiment, in which information about the orientations of objects pairs is explicitly provided to the network. Both numerical and analytical approaches show that the time it takes to identify objects in a pair is a linear function of the rotation angle separating the objects and does not depend on the axis of rotation. This accounts for the classical psychophysics observation for mental rotations. Finally, we examine plausible scenarios in which the synaptic structure can be learned via conservative Hebbian plasticity mechanisms. To our knowledge this is the first example of a neural network model of a non-commutative Lie group, specifically the group of 3D rotations.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Title: Neural firing correlates of visual scene memory performance in the subiculum and CA1

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Abstract: The subiculum is located at the critical junction between the hippocampus and its associated cortical areas. We examined the firing properties of single units in the subiculum in a hippocampal-dependent scene memory task in which rats were required to choose either left or right arm in a T-maze in association with a visual scene presented on 3 adjacent LCD monitors. Once the rat reached criterion, a recording drive with 24 tetrodes was implanted to simultaneously target both CA1 and subiculum. Single-unit spiking activities and LFPs were recorded while the rat performed the task with 4 familiar scenes (standard scene memory task, or STD). In an ambiguous scene memory task (AMB), two scenes were picked from the standard 4 scenes and the blurred versions (0%, 30%, 50% Gaussian blur) of those were presented intermixed with the original scenes during a recording session. Rats performed successfully in the standard scene memory task. In the ambiguous scene memory task, however, rat's performance significantly decreased with 50% blurred scenes. Complex spiking cells were recorded simultaneously from CA1 and subiculum in 4 rats. Mean firing rate was significantly higher in subiculum (5.7Hz) than in CA1 (1.3Hz), whereas spatial information content in spiking was significantly higher in CA1 (1.1 bit/spike) than in subiculum (0.2 bit/spike). We further classified the subicular complex spiking cells into three subgroups according to the type of firing-rate modulation observed at the time of sharp wave ripple (SWR) events in the subiculum: (i) SWR-enhanced group (42%), (ii) SWR-inhibited group (45%), and (iii) SWR-unmodulated group (13%). In STD, SWR-inhibited cells conveyed more scene-specific information than SWR-enhanced cells in subiculum (and CA1 cells). By contrast, spiking activity of CA1 cells and SWR-enhanced neurons in subiculum conveyed more choice response information than SWR-inhibited subicular cells. However, when the rat was required to perform using blurred scene stimuli in AMB, CA1 cells showed stronger scene-specific information in spiking activity compared to the subicular neurons. These findings suggest that, in a well-learned scene memory task, the subicular network may convey information on both scene background (or context) and appropriate behavior associated with that context. Our preliminary findings show that there may be separate classes of neurons in the subiculum, one conveying scene memory and the other representing choice response information. These neuronal classes may be modulated differentially by SWR. Furthermore, the hippocampal neuronal activity may become critical as ambiguous visual scenes need to be recognized.

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Poster

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Title: Place cells in the septohippocampal nucleus of freely behaving rats

Authors: *A. G. HOWE[1], R. M. DEGUZMAN[3], G. J. BLAIR[2], H. T. BLAIR[2];
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Abstract: The extreme septal pole of the hippocampus narrows to form the septohippocampal nucleus (SHi) and indusium griseum, together known as the *hippocampal continuation* (Wyss & Sripanidkulchi, J Comp Neurol 219:251, 1983), which comprise 10-15% of hippocampal volume in some species (including humans). Little is known about neural firing properties in this region. Here, we obtained single-unit recordings from >500 neurons throughout medial and lateral septum in freely behaving rats (n=10) foraging for food pellets during 1-3 h recording sessions in an 80 cm circular arena. Many septal neurons were modulated by theta rhythm; a subset of these neurons (n=35 cells from 4/10 rats) were *septal place cells* that exhibited spatial tuning for locations in the arena, in agreement with prior septal recording studies (Takamura et al., Hippocampus 16:635, 2006). Here, septal place cells were localized to SHi and adjacent lateral dorsal septal (LSD) nucleus; place cells were not found below the ventral border of LSD. Comparing firing properties of septal place cells to dorsal hippocampal place cells in the same group of animals (n=34 cells from 4/10 rats), we found hippocampal and septal place cells did not differ significantly in spatial information (.76±.32 vs. .74±.25 bits per spike), peak firing rate (5.9±5.0 vs. 8.0±8.0 Hz), or slope of phase precession against the hippocampal local field potential (-11.7±5.5 vs. -11.6±4.1 degrees per theta cycle), but septal place cells had a higher mean background firing rate (.43±.41 vs. .97±.88 Hz; p=.002) and larger place field size (117±51 vs. 166±64 cm^2). SHi is continuous with the hippocampus, so similar spatial tuning in both regions could indicate that septal and hippocampal place cells are a single contiguous population of neurons, which derive their spatial tuning properties via similar mechanisms. If so, then what functional connectivity is preserved across SHi and hippocampal neurons, allowing both to exhibit similar spatial tuning properties even though SHi lacks the structured canonical circuitry of the hippocampus proper? Alternatively, septal place cells may not derive their own spatial firing fields, but instead inherit spatial tuning through afferent inputs from upstream place cells the hippocampus, or grid cells in entorhinal cortex. Further study of how SHi neurons derive spatial tuning might shed like on circuit mechanisms of place cell formation. Since the septal

region is a major relay for bidirectional connections between hippocampus and subcortical structures, further study of septal place cells may also help to elucidate what information is exchanged between hippocampal and subcortical networks.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Title: Intra ripple features are constant, but post ripple features vary across behavioral state in macaques

Authors: ***A. T. HUSSIN**[1,2,3,4], T. K. LEONARD[1,3,4,5], K. L. HOFFMAN[1,2,3,4,5];
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Abstract: The sharp-wave ripple (SWR) is a local field potential (LFP) event generated in the hippocampus that is characterized by a short-lived, fast oscillation (110-200 Hz), embedded within a slow 'sharp wave', and corresponds to elevated principal cell activity. SWRs are thought to facilitate hippocampus-dependent memory consolidation during rest, but they are also seen during goal-directed task performance. We reasoned that during aroused, attentive states such as during visual search, the characteristics of SWRs might differ from those seen during rest. We therefore examined features of SWR events (ripple magnitude and duration) to investigate whether these features vary predictably with different states (quiescence or active exploration) and with varying goal-directed memory behaviors during visual search. Two female rhesus macaques performed a memory-guided visual search task while we recorded LFPs from a multiple-tetrode array with electrodes positioned to record hippocampal SWRs. LFPs were filtered (100-250 Hz), transformed to z-scores, rectified, bandpass filtered (1-20 Hz) and set to detect events with a minimum duration of 50 ms and at 3 SD, with duration defined as the closest

time points to the peak that crossed 1 SD. The post-ripple LFP deflection seen in these recordings was measured as the peak magnitude of the 200-ms window (filtered between 1-6 Hz) following the ripple mid-point. The peak magnitude and duration of the ripple component of SWR events did not vary across behavioral states (quiescence vs. visual exploration) or memory-related behaviors. The post-ripple deflection was more variable, and in some cases appeared to vary as a function of behavioral state. Our findings suggest that the ripple *per se* appears to be well-conserved across changing states and memory demands in macaques; however activity in the wake of ripples may signify differences in the local or long-range responses that are partly determined by vigilance or cognitive state.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Title: Control of recollection by competition between slow and fast gamma in hippocampus CA1

Authors: *A. A. FENTON, B. RADWAN, F. SPARKS, D. DVORAK;
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Abstract: Behavior is used to assess memory and identify memory deficits in animal models of mental dysfunction, but behavior is a proxy for the neural events that define cognitive variables like recollection. We sought to identify an electrophysiological signature of recollection in mouse dorsal CA1 hippocampus, using the active place avoidance task. We first identified when mice initiated escapes from the vicinity of the shock zone because moments before this are likely when the mice recollect being at the shock zone. The rates of slow (30-60 Hz) and fast (60-90 Hz) gamma oscillations in the dorsal hippocampus CA1 local field potential decreased before mice initiated avoidance movements, but slow dominated fast gamma 2-3 seconds before avoidance. These SG/FG maxima could indicate recollection events as they were not observed if the mouse failed to avoid shock, and increased with learning in wild-type and Fmr1 mutant mice that model intellectual disability in Fragile X Syndrome. Wild-type but not mutant mice quickly

adapted to relocating the shock zone as well as turning it off. This flexibility coincided with decreased SG/FG maxima in the wild types, but the maxima persisted in the mutants, consistent with their cognitive inflexibility. SG/FG maxima occurred throughout the apparatus, recurring with an interval of ~9 seconds, , whether or not the mice were actively avoiding the shock zone, still or actively moving. The hypothesis that SG/FG maxima indicate recollection predicts non-local place representations in the concurrent discharge of CA1 place cells. As predicted, place cell discharge signaled distant places. These findings indicate that neural activity associated with slow and fast gamma compete for control of CA1 spiking and that dominance by the activity associated with slow gamma indicates activation of hippocampal representations of remote places, consistent with recollection of spatial memory.

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Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Support: R01 DA037255

F30-AA020381

Title: Hippocampal CA1 activity encodes space (response key) and time (order)

Authors: *T. G. WEYAND[1], M. KETCHUM[2], P. WINSAUER[2];
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Abstract: We analyzed activity in the CA1 area of the hippocampus in rats required to complete two tasks in a single session. The first task required the acquisition of a different 3-response sequence each session, whereas the second task required the completion of the same, well-rehearsed 3-response sequence (performance). Furthermore, both sequences had to be completed 3 times (9 responses) to obtain food. In performance, averaging activity immediately before a response allowed us to predict position (left, center, right) and often order: the activity encoded space and time. At the most discriminating site, all 9 responses could be discriminated at $p < 0.02$ or better. Other sites generated a clear signal discriminating each of the three keys but not order, or signals selective for a single key but not order. These signals are peri-motor, but not related to kinematics because the rats were trained to nose poke each key, and the observed

activity could consist of a peak or a trough at identical latencies depending on the key poked. Whereas we could correctly predict > 75% of the time some key/order single response (chance = 11%), the remarkable discriminability reported here required averaging over time (typically 40 responses per key/order in a 360 response block). However, this level of discriminability could also be achieved in single responses by downstream neurons pooling across clusters of neurons within CA1 (population averaging). Initial responses in the learning task were marked by high variability (and low prediction) that were soon replaced (within ~50 responses in a 360 response block) by decreased variability, and conformity with averaged responses (and high predictability). Thus, the signals immediately preceding the response are highly fluid; circuits adjust 'on the fly' to encode the new sequence. Interestingly, when the rat made an error, it was often possible to identify not only that it would be an error, but also the identity of the incorrect key. (Supported by NIH: R01 DA037255, F30-AA020381)

Disclosures: T.G. Weyand: None. **M. Ketchum:** None. **P. Winsauer:** None.

Poster

356. Encoding Spatial Memories: Neural Circuits and Ensembles

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Topic: H.01. Animal Cognition and Behavior

Title: A generic model for the generation of theta and replay sequences in the hippocampus

Authors: *A. AZIZI[1], K. DIBA[2], S. CHENG[1];
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Abstract: Hippocampal place cells provide a spatial map of the environment when an animal moves along a trajectory. The sequential activation of these place cells can provide an episode memory trace of spatial locations. Such sequential activity occurs during different stages of a rat's behavior and sleep. During spatial exploration, an ensemble of cells with place fields around the current location of the animal activate. This activity then propagates to cells encoding future nearby locations of the trajectory. Such propagation occurs during the trough of the hippocampal theta oscillation and is assumed to encode goal locations (Wikenheiser & Redish, 2015).During resting states, sequential activity reappears in a different form in conjunction with sharp-wave ripples (SWRs). These "offline" sequences (1) are compressed in time, (2) cover longer decoded trajectories, (3) are biased by the current location of the animal, and (4) can correspond to trajectories that the animal has or has not previously travelled, and in both the same or opposite directions (Diba & Buzsáki, 2007; Foster & Wilson, 2006).In this work, we propose a neural network model of the CA3 region in the hippocampus that can generate both of online theta and

offline SWR classes of the sequential activities. This network has a generic structure, comprising of an intermingled network of excitatory and inhibitory cells, and operates in theta or sharp-wave ripple (SWR) oscillatory states. During the exploration of the virtual animal, the synaptic weights between excitatory cells undergo spike timing-dependent synaptic plasticity. Following exploration, spontaneous activity reoccurs along experienced trajectories, in both forward and reverse, and is sensitive to the immediate behavior of the animal in the preceding online state. This activity dependency of the offline sequential activity fits well with the experimental data. In conclusion we showed that a continuous attractor models of the hippocampus can simultaneously account for the generation of both theta and replay sequential activity.Diba, K., & Buzsáki, G. (2007). Forward and reverse hippocampal place-cell sequences during ripples. Nature Neuroscience, 10(10), 1241-1242.
Foster, D. J., & Wilson, M. a. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. Nature, 440(7084), 680-683.Wikenheiser, A. M., & Redish, A. D. (2015). Hippocampal theta sequences reflect current goals. Nature Neuroscience, 18(2), 289-94.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Title: Distinct lateral septal interneurons broadcast instructive and permissive hippocampal signals to calibrate fear responses

Authors: *A. BESNARD[1,2,3], T. LANGBERG[1,2,3], D. CHU[1,2,3], W. FENG[1,3,2], D. SAUR[4], X. XU[5], A. SAHAY[1,2,3];
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Abstract: The generation of adaptive fear responses to ambiguous threats in the environment is thought to depend on how contextual information is encoded in the hippocampus and then relayed to brain regions that mediate fear and stress responses. Although circuit mechanisms supporting pattern separation in DG-CA3 may facilitate the resolution of interference and discrimination of threats, the neural pathways that link these computations with limbic brain regions subserving fear are poorly understood. To begin to delineate these neural pathways, we performed a brain-wide analysis of co-activated ensembles under conditions of high and low contextual fear discrimination. We identified a non-canonical DG-CA3-Dorsolateral septal (DLS) circuit whose activity was most highly correlated to discrimination performance. Using pathway specific optogenetic inhibition approaches, we uncovered specialized, instructive and permissive, roles for DG-CA3-CA1 and DG-CA3-DLS circuits along the septo-temporal axis in mediating fear responses to certain and uncertain threats. By combining rabies virus based monosynaptic tracing, optogenetic interrogation and most recently, in vivo optical imaging, we have begun to implicate distinct populations of inhibitory DLS interneurons responsible for broadcasting CA3 outputs to limbic circuits subserving fear. Further, genetically enhancing adult hippocampal neurogenesis improved contextual discrimination with preferential recruitment of the CA3-DLS pathway. Together, these studies begin to edify the neural pathways by which DG-CA3 (and neurogenesis) dependent computations underlying disambiguation of threats are rapidly and directly relayed out of the hippocampus to govern adaptive activation of fear circuits.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: I.04. Physiological Methods

Support: NSFC grant #81371305

Title: Optogenetic inhibition of striatal GABAergic neurons promotes functional recovery after ischemic stroke in mice

Authors: L. JIANG, W. LI, Y. LU, Y. MA, Z. ZHANG, G.-Y. YANG, *Y. WANG; Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Introduction: GABAergic neurons play an important part in the neurogenesis process under normal physiological conditions. However, the effect of striatal GABAergic neuronal activities on the neurogenesis under pathological conditions such as ischemic stroke remains unclear. In this work we used *GAD2-Arch-GFP* and *GAD2-ChR2-tdTomato* transgenic mice to investigate how the striatal GABAergic neuronal activity affects the neurogenesis in the SVZ after ischemic stroke.
Methods: Twenty-one 16-week male mice underwent a 60-minute transient Middle Cerebral Occlusion (tMCAo) surgery. In the inhibition group, 15-minute 530-nm green constant laser stimulation was delivered twice a day to inhibit the striatal GABAergic neurons in *GAD2-Arch-GFP* transgenic mice from the 7^{th} to the 13^{th} day after stroke. In the activation group, 15-minute 473-nm blue pulse laser stimulation was delivered twice a day in *GAD2-ChR2-tdTomato* transgenic mice to activate the striatal GABAergic neurons. Homemade optrode was used to simultaneously record the neuronal activity under laser stimulation to validate the inhibition and activation efficiency. Control groups have the identical genetic background and optrode implant, but without laser delivery. Double blind neurological severity score (NSS) analysis was carried out on days 1, 3, 7 and 14 after tMCAo. Mice were sacrificed at day 14. Nestin and Doublecortin (DCX) staining was used to identify neural stem cells (NSCs) and neural precursor cells (NPCs). The fluorescent density of Immunostaining was used to measure the amount of NSCs and NPCs.
Results: Inhibition of striatal GABAergic neurons significantly promoted functional recovery of *GAD2-Arch-GFP* mice compared to the non-inhibition control group (NSS score 4.80.37 vs. 6.50.29, $p < 0.05$). The number of nestin positive NSCs in the SVZ increased in the inhibition group ($p < 0.05$). The numbers of doublecortin (DCX) positive NPCs in the SVZ and peri-infarct area were both decreased in the inhibition group after ischemic stroke ($p < 0.05$). Very few newly generated mature neurons were detected in both groups at day 14. In contrast, in the activation group of *GAD2-ChR2-tdTomato* mice, decreased number of NSCs was found in the SVZ ($p < 0.05$) while more DCX positive cells were found in the SVZ and in the peri-infarct area compared to the non-activation control group ($p < 0.05$).
Conclusions: Inhibition of striatal GABAergic neurons promoted functional behavior recovery at 14 days after tMCAo in mice, while activation of striatal GABAergic neurons impeded functional recovery. These improvements of behavior could potentially related to the proliferation of nestin positive neural stem cells.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: H.01. Animal Cognition and Behavior

Support: NIH intramural research program

Title: Modulation of the hippocampal circuit by oriens lacunosum-moleculare neurons

Authors: *J. HAAM, J. L. YAKEL;
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Abstract: The hippocampus is the interface between memory encoding and consolidation processes, which serves as the brain structure that temporarily stores the encoded memory, but also as the memory module that sends out data for consolidation. Previous studies have suggested that the GABAergic interneurons oriens lacunosum-moleculare (OLM) cells in the hippocampus are activated during memory encoding, but the physiological role of these neurons is not clear. OLM interneurons are major negative regulators of CA1 pyramidal neurons that receive glutamate inputs from the CA1 pyramidal neurons and in return project to the dendrites of the CA1 pyramidal neurons. Here, using electrophysiology, optogenetics, and Cre-expressing mouse lines, we investigated how OLM interneurons regulate the hippocampal output to the entorhinal cortex (EC) circuit. To examine whether OLM interneurons modulate the hippocampal output to the EC, we recorded from the EC layer V (ECV) neurons upon stimulation of CA1 pyramidal neurons. Optogenetic stimulation of OLM interneurons caused a significant decrease in the amplitude of the evoked glutamatergic current in ECV neurons, suggesting that OLM interneurons suppress the CA1-to-EC circuit. Furthermore, using viral expression of diphtheria toxin A and mouse behavioral approaches, we investigated how ablation of OLM interneurons affects memory formation. Oblation of OLM interneurons impaired the object location memory task (OLT), but not the novel object recognition task (NORT), suggesting that the neurons play a critical role in hippocampus-dependent memory formation. In addition, OLM-ablated mice showed reduced spontaneous alternation performance in the Y-maze task, showing that they have impaired spatial working memory. Together, these results demonstrate that OLM interneurons regulate the hippocampal output to EC and that these interneurons are critical in the encoding of hippocampus-dependent memory, which is impaired when the neurons are ablated.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Title: Decreased cholinergic input to hippocampal ca1 olm interneurons in an appps1 mouse model

Authors: *M. MITTAG, L. SCHMID, K. KEPPLER, J. STEFFEN, M. FUHRMANN; German Ctr. of Neurodegenerative Dis. (DZNE), Bonn, Germany

Abstract: The hippocampus, which is important for storage and retrieval of memories as well as spatial navigation, is one of the first regions to be affected by the deposition of amyloid beta plaques during Alzheimer's disease (AD). Since memory deficits have been attributed to hippocampal dysfunction in AD, it has been hypothesized that the occurrence of cognitive deficits in AD is linked to progressive amyloid beta pathology and subsequent imbalance between excitation and inhibition in local circuits of the hippocampal CA1 region. To understand the mechanisms underlying circuit dysfunction, we focus on a specific population of inhibitory interneurons (OLMs) that are present in stratum oriens of CA1 and that provide feedback-inhibition to the distal dendrites of pyramidal neurons. These inhibitory neurons have been shown to be critically involved in associative memory processes. To assess the functional involvement of these neurons in memory dysfunction in AD, we recorded Ca^{2+}-events of OLM interneurons in awake head-fixed wild type and APP/PS1 mice moving on a spherical treadmill. Application of the cholinergic blocker Pirenzipine resulted in a significantly decreased average Ca^{2+}-response to the airpuff in wild type animals. Transgenic animals exhibited a decreased average Ca^{2+}-response compared to wild type that was not further decreased by Pirenzipine application. These findings indicate decreased cholinergic input to OLM interneurons in APP/PS1 transgenic mice, presumably due to decreased synaptic connectivity between cholinergic neurons in the medial septum and OLM interneurons in the hippocampus.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Program#/Poster#: 357.05/JJJ33

Topic: H.01. Animal Cognition and Behavior

Support: NIH P50 MH086403

Title: Neuroligin-3 in hippocampal parvalbumin interneurons facilitates contextual fear extinction by regulating presynaptic Group-III mGluRs

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Abstract: Neuroligins (NLs) are a family of 4 postsynaptic proteins (NL1-4) that interact with presynaptic neurexins. This trans-synaptic interaction between neurexins and NLs has been implicated in the formation, specification, maintenance and activity-dependent strengthening of synapses in various brain regions. Disruption of either the presynaptic neurexins or postsynaptic NLs has been implicated in autism spectrum disorders and schizophrenia. Parvalbumin (PV) interneurons have been implicated in cognition, learning and memory, as well as in the pathophysiology of various psychiatric disorders. However, little is known about the molecular mechanisms that control synaptic transmission onto these cells. To study the putative role of NL3 at synapses onto interneurons, we crossed a conditional knockout mouse for NL3 ($NL3^{fl/fl}$) to a mouse line expressing cre under the parvalbumin (PV-cre) promoter to generate NL3/PV-cre mice. These mice showed reduced reversal learning as assayed by contextual fear extinction, while contextual fear conditioning was unaltered. This reduction in extinction learning was rescued by a region and cell type specific expression of NL3 in hippocampal PV interneurons. To identify the synaptic and circuit deficits underlying this behaviour, we made whole cell recordings from genetically identified PV cells in the hippocampal CA1 region from acute slices. In the NL3/PV-cre mice, a decrease in the paired pulse facilitation of excitatory inputs onto PV cells was observed suggesting an increase in release probability. This suggestion was further supported by an enhanced run down of NMDA receptor (NMDAR)-mediated EPSCs in NL3/PV-cre mice when MK-801 was applied. This increase in release probability at excitatory synapses on PV interneurons lacking NL3 correlated with a specific lack of sensitivity of the EPSC amplitude and paired pulse ratio to agonists or antagonists to Group-III mGluRs. This lack of Group-III mGluR mediated control of release at these synapses makes them function as a low pass filter resulting in the summation of incoming excitatory inputs only at lower frequencies. As a result, NL3 deletion altered hippocampal network activity and reduced sharp wave ripples. These results demonstrate that postsynaptic NL3 plays an important role in regulating synaptic

transmission at excitatory synapses onto PV interneurons by modulating presynaptic glutamate release onto these cells. The synaptic and behavioural deficits observed as a consequence of NL3 deletion in PV cells are likely to play an important role in the pathophysiology underlying the complex neuropsychiatric disorders caused by NL3 mutations.

Disclosures: J.S. Polepalli: None. **T.C. Sudhof:** None. **R.C. Malenka:** None.

Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Challenge Award from Citizens United for Research in Epilepsy

, Connecticut Stem Cell Established Investigator Grant

Title: Viral-mediated overexpression of NLGN2 enhances GABAergic synapses in the hippocampus and alters social behavior and anxiety

Authors: *M. A. VAN ZANDT, J. GUPTA, E. WEISS, S. SHRESTHA, S. MAISEL, J. R. NAEGELE;
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Abstract: Inhibitory interneuron dysfunction plays a major role in many neurological disorders, including autism, schizophrenia and epilepsy. Overexpression or knockdown of the molecules guiding formation and stabilization of inhibitory synapses is a powerful approach for gaining insights into GABAergic interneuron roles in different brain regions and cognition. Neuroligin2 (NLGN2), a scaffolding protein localized post-synaptically at GABAergic synapses, is a key signaling molecule implicated in inhibitory synaptogenesis. Upregulation of NLGN2 induces inhibitory synapse formation *in vitro*. Conversely, reducing NLGN2 or its binding partner, α-neurexin2, decreases inhibitory synapse formation and alters aggressive behavior. Recently, viral-mediated NLGN2 overexpression (OE) in the rat hippocampus was shown to reduce aggression and novelty seeking. Whether levels of NLGN2 in the adult hippocampus modulate inhibitory synaptic function and hippocampal dependent behaviors is unknown. To address this question, we stereotaxically injected an adeno-associated viral vector (AAV/DJ-CMV-mCherry-2a-mNLGN2, titer: $6.4x10^{12}$ GC/mL; Vector BioLabs) to overexpress NLGN2 in the CA1 and dentate gyrus of the adult mouse hippocampus. Control mice received injections of an AAV

vector lacking NLGN2 (AAV/DJ-CMV-mCherry, titer: 1.8 x 10^{13} GC/mL; Vector BioLabs). We then examined the effects on synaptic proteins, synaptic density, electrophysiological properties, and behavior. NLGN2 OE in dentate granule cells dramatically increased pre-synaptic GABAergic synapses and post-synaptic gephyrin clusters. These increases correlated with upregulation of the vesicular GABA transporter (VGAT), without concomitant increases in the vesicular glutamate transporter (vGlut), suggesting that NLGN2 OE alters the balance between synaptic excitation and inhibition. To further address this question, we carried out electrophysiology. Results suggested higher inhibitory post-synaptic current (IPSC) frequency and amplitude in granule cells with NLGN2 OE. In behavioral studies comparing controls versus mice with NLGN2 OE, NLGN2 OE mice showed reduced social interactions, lower social dominance, and less anxiety in the open field and elevated zero maze. However, they showed comparable spatial memory performance in the Morris Water Maze task. These findings suggest that increased NLGN2 expression in the adult CA1 and dentate gyrus selectively promotes the formation of GABAergic synapses and reduces aggression and anxiety.

Disclosures: **M.A. Van Zandt:** None. **J. Gupta:** None. **E. Weiss:** None. **S. Shrestha:** None. **S. Maisel:** None. **J.R. Naegele:** None.

Poster

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Title: Role of hippocampal VIP interneurons in reward-oriented spatial learning

Authors: ***G. F. TURI**[1], Z. LIAO[1], W.-K. LI[1], J. D. ZAREMBA[1], A. GROSMARK[1], X. LUO[2], L. TOPOLNIK[2], A. LOSONCZY[1];
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Abstract: Vasoactive intestinal peptide (VIP) positive interneurons form a morphologically heterogeneous interneuron (IN) population in the hippocampus and the cortex. VIP INs have been shown to provide disinhibitory control over principal neurons' activity via the inhibition of

other IN subclasses. While many functional consequence of this peculiar circuit motif have been revealed in neocortical circuits, there is no data available from the hippocampus on the functional activity of these neurons in the behaving mouse. To determine the activity of VIP INs in hippocampal area CA1, we set out a series of imaging experiments in our head fixed mouse model while the mice were engaged in random foraging or goal-oriented learning (GOL) tasks. We found that a subset of VIP INs located in the CA1 pyramidal layer increased their activity during locomotion while they were not tuned to salient aversive sensory stimuli. Interestingly, a subset of VIP INs strongly responded to the appetitive water rewards. Thus we hypothesized that these cells were involved in goal-directed spatial learning. To test this we used optogenetic methods to manipulate the VIP cells' activity in the reward zone in mice performing the GOL task. We found that silencing VIP INs significantly repressed the learning performance. We then monitored the activity of VIP cells during GOL with two-photon imaging. We found a fraction of cells which activity was gradually increased in the reward location over the time of the consecutive learning sessions. To examine whether the median raphe (MR) projections could potentially drive VIP INs' recruitment in relation to GOL behavior, we used optogenetic stimulation of MR fibers in acute hippocampal slices. We found that while a subset of VIP INs showed no response to light stimulation, others exhibited rapid large-amplitude EPSCs, which were sensitive to 5-HT3 and glutamate receptor antagonists, pointing to the target-specific role of MR projections in modulating VIP INs' activity. There is a high degree of similarity in the functional and physiological responses of cortical and hippocampal VIP INs: the activity of these cells is coupled to the locomotor behavior and strongly modulated by reinforcement signals. Because of their reciprocal connections with other INs these cells are in a key position to regulate the integrative properties of the entire somatodendritic domain of pyramidal cells regulating thereby neuronal plasticity in sensory cortices. Our study provides new information on the functional characterization of hippocampal VIP neurons in the behaving rodent and suggests a specific permissive role of these cells in reward related spatial learning.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: H.01. Animal Cognition and Behavior

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VW I/84 236

Title: Somatostatin-positive interneurons in the dentate gyrus provide local- and long-range septal synaptic inhibition

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Abstract: Temporal coordination of neuronal activity among brain areas is essential for neuronal network computation and behavioral performance. The dentate gyrus (DG) as the main hippocampal input gate processes spatial information transmitted from the entorhinal cortex via the perforant path to the outer molecular layer of the DG. Somatostatin-expressing interneurons (SOMIs) have been proposed to be the synonymous to hilar-perforant path-associated interneurons (HIPPs) representing classical feedback inhibitory cells with axon fibers in the outer molecular layer. HIPPs seem to control the perforant path-mediated information stream to the DG and the size of cell assemblies. Here we provide first evidence that DG-SOMIs fall into at least three types with distinct morphological and physiological characteristics as well as synaptic input and output properties. HIPP and hilar-molecular layer-associated cells (HMLs) are recruited by converging glutamatergic granule cell (GC) inputs and provide feedback dendritic inhibition onto the local DG circuitry. In contrast, hilus-associated interneurons (HILs) are recruited by Mossy Cells and provide strong perisomatic inhibition onto DG interneurons. In addition to their local synaptic interactions, HMLs and HILs form long-range projections to the medial septum (MS) and are connected to glutamatergic, GABAergic and cholinergic cells. DG-SOMIs in turn are the targets of projecting parvalbumin-expressing interneurons originating from the medial septum thereby forming a long-distance bidirectional DG-MS interneuron-interneuron loop. The medial septum is broadly accepted as the center for the generation of theta (6-10 Hz) activity patterns which are forwarded to various cortical and subcortical areas including the hippocampus. Interneuron-interneuron loops between the medial septum and cortical regions in particular have been proposed to be important for the coordination of distant neuronal networks at theta frequencies. Thus, we propose that DG-SOMI types contain two functions: first, they contribute to sparse temporal encoding of spatial perforant path-transmitted information in the DG and second, they temporally coordinate the local activity of DG cell assemblies with theta activity patterns governed by the medial septum.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Title: Feed forward inhibition dictates reactivation of hippocampo-cortical ensembles to maintain remote memory percision

Authors: *N. GUO[1,2,3], M. E. SODEN[4,5], A. BESNARD[1,2,3], L. S. ZWEIFEL[4,5], A. SAHAY[1,2,3]; [1]Ctr. For Regenerative Med., Boston, MA; [2]Harvard Stem Cell Inst., Cambridge, MA; [3]Dept. of Psychiatry, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [4]Dept. of Pharmacology, Univ. of Washington, Seattle, WA; [5]Dept. of Psychiatry and Behavioral Sciences, Univ. of Washington, Seattle, WA

Abstract: Episodic memories become less precise with the passage of time. The loss of details of traumatic memories over time is thought to result in their generalization and consequently, the expression of fear responses to a wide range of neutral cues. This phenomenon of time dependent fear generalization characterizes anxiety disorders such as Post Traumatic Stress Disorder (PTSD) and is captured in numerous animal studies. At a neural level, time dependent fear generalization may be due to degradation of the cortical representations underlying remote memories. We, and others have recently hypothesized that neural circuit mechanisms modulating memory interference in the hippocampus govern the stability of cognate cortical remote memory traces and thereby dictate the precision of remote memories. Feed-forward excitation/inhibition (FFE-FFI) balance in the dentate gyrus (DG)-CA3 circuit may stabilize cortical memory traces by constraining CA3 activation and by promoting hippocampal-cortical communication. Here, we report identification of a molecular factor, Ablim3, with which we can selectively increase the number of excitatory inputs of dentate granule cells (DGCs) onto parvalbumin-expressing stratum lucidum interneurons (PV-IN) without affecting DGC dendritic spine density and DG activity. Viral manipulation of Ablim3 levels in the DG enhanced feed forward inhibition (FFI) in the DG-CA3 circuit as assessed by analysis of neural connectivity, PV-IN activation and electrophysiological whole cell recordings from PV-INs and CA3 pyramidal neurons. Using genetic systems to indelibly tag context-specific neuronal ensembles, we found that selectively

enhancing FFI in DG-CA3 circuit maintains precision of remote fear memories by promoting global remapping in CA3. Importantly, enhancing FFI in DG-CA3 was sufficient to dictate context specific re-activation of CA3 and cortical neuronal ensembles at recent and remote time points. We extended these studies to identify alterations in FFI in DG-CA3 during aging and harnessed Ablim3 to reverse these changes and improve remote memory precision in aged mice. Together, these studies demonstrate how decreasing memory interference in DG-CA3 causally impedes decontextualization of remote cortical memory traces. Furthermore, our work identifies a potential molecular target for constraining time dependent fear generalization and improving memory precision in aging.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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NSERC PhD Fellowship to OC

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Title: Network state dependent recruitment of VIP interneurons in awake mice

Authors: R. FRANCAVILLA, V. VILLETTE, X. LUO, O. CAMIRE, *L. TOPOLNIK; CRCHUQ-CHUL, Laval Univ., Quebec, QC, Canada

Abstract: Hippocampal inhibitory interneurons target different spatial domains of principal cells and release GABA at particular times during behaviorally relevant network oscillations. However, basic information on how specific types of interneurons can be controlled by GABA released in particular behavioral states is still lacking. Hippocampal CA1 interneurons within stratum oriens/alveus (O/A) receive inhibitory inputs from two types of vasoactive intestinal peptide (VIP) expressing interneurons: type III interneuron-specific cells and the long-range GABAergic cells projecting to the subiculum and targeting CA1 interneurons locally. Despite their potentially important role in coordinating rhythmic activity among functionally relevant

brain areas, whether and how different subtypes of VIP cells contribute to network activity during behavior is not known. Using two-photon calcium imaging in awake head-fixed VIP-Cre mice in combination with local field potential recordings and post-hoc neurochemical identification of imaged neurons, we examined the recruitment of hippocampal CA1 VIP interneurons in relation to different brain states and network activity patterns. We found a positive covariance between the theta power and somatic calcium transients indicative of a significant coupling between the theta oscillations and VIP interneuron activity in a subset of VIP interneurons. Moreover, the majority of cells exhibited a plateau-like activity during theta-run epochs associated with locomotion. Furthermore, some VIP interneurons were preferentially recruited during sharp-wave ripple episodes associated with quiet wakefulness. These data indicate that while most VIP interneurons are well positioned to coordinate O/A interneuron activity during theta oscillations associated with locomotion, some may control interneuron firing during sharp-wave ripples. Collectively, this study provides new evidence of VIP interneuron specialization in controlling inhibitory microcircuits during behavior.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: H.01. Animal Cognition and Behavior

Support: NIH-RO1MH084038

Title: Altered metabolic and synaptic functional connectivity in the MAM model of neurodevelopmental insult

Authors: ***K. C. O'REILLY**, E. R. LEVY, M. I. PERICA, A. A. FENTON;
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Abstract: Patients with neuropsychiatric disorders often express deficits in information processing, along with limbic circuit abnormalities. While these disorders appear to have diverse etiology, their common features suggest neurodevelopmental origins. Accordingly, we used the schizophrenia-related gestational day 17 methylazoxymethanol acetate (MAM) model to evaluate the hypothesis that a general gestational insult can alter the adolescent-to-adult maturation of limbic neural circuit function. Systems-level circuit function was evaluated at the levels of behavior, metabolically-assessed inter-regional functional coupling, and synaptic

network function.
A neuropsychological test battery using variants of the two-frame active place avoidance task was conducted to evaluate locomotion, learning, cognitive control, cognitive flexibility, and spatial memory maintenance across 10 minutes or 1 day. Although adult MAM rats are hyperactive, most cognitive abilities are intact once performance measures are corrected for hyperlocomotion. However, MAM rats are impaired in accumulating memory across 10 minutes, but can maintain memory across days indistinguishable from controls.
Circuit function was first evaluated by measuring cytochrome oxidase activity, a metabolic marker of global, steady-state neuronal activity, in adolescent and adult naive rats. We quantified activity in the 1) prefrontal areas, 2) dorsal and 3) ventral parahippocampal regions, and 4) habenular complex. Intraregional coupling becomes more "specialized" from normal adolescence to adulthood in that regions 1-3 increase coupling, but not in MAM rats. Instead, global (inter- and intra-regional) coupling increases, indicating the adult MAM brain is functionally overconnected and therefore poorly specialized.
Importantly, functional coupling between the dorsal hippocampus and entorhinal cortex was consistently higher in adult MAM rats, motivating us to examine their synaptically mediated circuit function. We recorded the evoked response to perforant path stimulation at 16 sites along the somatodentritic axis of dorsal hippocampus in adult, anesthetized rats. While the input-output curves for the field EPSP and population-spike (PS) responses appear left-shifted (more responsive) in MAM rats, the E-S curves describing the coupling between synaptic input and dentate output is right-shifted. This suggests blunted information throughput across the adult MAM dentate gyrus.
These findings indicate that the neurodevelopmental insult induced by MAM alters brain activity across development, resulting in abnormal limbic circuit function.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH084038

Title: Long-lasting input-specific modifications of excitation and inhibition in the hippocampus following spatial training measured in the anesthetized rat

Authors: *E. LEVY, K. C. O'REILLY, A. A. FENTON;
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Abstract: Studies of synaptic plasticity, especially those in brain slices have led to the dogmatic view that memory-inducing experiences cause changes in synaptically-mediated functions of the neural subcircuits that subserve the learning and store the memory. However, synaptically-mediated functional changes that persist on the timescales of memory have been difficult to identify. This has lead some to challenge the dogma and others to assert that any changes must be sparse and difficult to detect in large populations of synapses and neurons, otherwise restricted learning would be sufficient to alter microcircuit function on macroscopic scales. To test whether memory formation changes hippocampus circuit function, 1 week after place memory or control training, we recorded across dorsal hippocampus somato-dendritic compartments of adult male Long-Evans rats, while stimulating Schaffer collateral and perforant path inputs, under urethane anesthesia.
The “Trained” rats learned to avoid a shock zone over 2 days (8, 10-min trials/day). Memory was tested 1 week later. “Untrained” control rats had the identical physical experience but were never shocked. “Naïve” control rats were also studied. Immediately after memory testing, rats were anesthetized, and implanted with a 16-site linear array of recording electrodes and 2 sets of stimulating electrodes. Stimulating electrodes in the angular bundle activated the perforant path and electrodes in the ventral hippocampal commissure activated contralateral CA3 inputs to CA1. Evoked potential responses to stimulations (0-1000 μA) were recorded and field post-synaptic potentials (fPSP) and population spikes (PS) were analyzed along with current-source-density (CSD) analysis to estimate input-specific modifications of synaptic functions.
The most prominent change caused by memory training was in the dentate fPSP response to perforant path stimulation. Relative to the responses in control rats, the CSD source (outward current) at the granule cell layer was increased after the PS, at the time of the polysynaptic response. This difference was only observed when stimulation elicited a PS. These observations demonstrate that synaptically-mediated compartment-specific functional changes can be measured for at least a week with memory persistence. Remarkably, the most salient functional change was the enhancement of local inhibition, demonstrating that memory storage is accompanied by large and wide-spread adjustments in the coupling between excitation and inhibition.

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Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Title: Genetic deletion of Fgf14 disrupts inhibitory connections of the brain hippocampal region in a sex specific manner

Authors: *T. K. ALSHAMMARI[1], M. A. ALSHAMMARI[1], M. N. NENOV,[2], E. HOXHA[3], F. TEMPIA[3], F. LAEZZA[2];
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Abstract: The brain neural circuitry that controls cognition is composed of a complex network of neurons. Among these, γ-amino-butyric acid (GABA) inhibitory interneurons regulate excitability and plasticity of principal neurons balancing the excitatory/inhibitory (E/I) tone of cortical networks. Reduced function of parvalbumin (PV) interneurons and disruption of GABAergic synapses are hallmarks of neuropsychiatric disorders associated with cognitive deficits. Yet, the mechanisms underlying these phenotypes in the male and female brain are still poorly understood. Here we provide new data indicating that genetic deletion of fibroblast growth factor 14 (*Fgf14)*, a regulator of excitability and synaptic transmission and an emerging brain disease-associated factor, leads to loss of PV interneurons in the CA1 hippocampal region, a critical area for cognitive function. This cellular phenotype associates with decreased expression of glutamic acid decarboxylase 67 (GAD67) and vesicular GABA transporter (VGAT), two disease-associated markers of the GABAergic presynaptic terminal. Strikingly, these presynaptic molecular rearrangements are sex-specific with a more widely spread loss of PV neurons across CA1 sub-layers in males and a more pronounced reduction of GAD67 in females compared to corresponding sex-specific controls. Electrophysiological recordings in acute hippocampal slices indicate that the inhibitory tone in CA1 pyramidal neurons is decreased in males, but augmented in *fgf14*$^{-/-}$ female mice compared to corresponding *fgf14*$^{+/+}$ control littermates. This study highlights an unanticipated role of FGF14 in regulating the E/I balance of cortical networks in a sex-specific manner, filling knowledge gaps in the mechanisms underlying psychiatric diseases associated with GABAergic dysfunction and adding FGF14 to the repertoire of potential risk factors for complex brain disorders.

Disclosures: **T.K. Alshammari:** A. Employment/Salary (full or part-time): Department of Pharmacology and Toxicology , College of Pharmacy, King Saud University , Riyadh , Kingdom of Saudi Arabia. **M.A. Alshammari:** None. **M.N. Nenov,:** None. **E. Hoxha:** None. **F. Tempia:** None. **F. Laezza:** None.

Poster

357. Learning and Memory: Role of Hippocampal GABAergic Inhibition

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Topic: G.01. Appetitive and Aversive Learning

Support: MH078064

Title: Role of oxytocin receptor in GABAergic actions

Authors: *A. L. GUEDEA[1], K. A. CORCORAN[1], K. NISHIMORI[2], J. RADULOVIC[1]; [1]Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL; [2]Dept. of Mol. and Cell Biol., Tohoku University-Graduate Sch. of Agr. Sci., Miyagi, Japan

Abstract: There is increasing interest in developing oxytocin-based treatments for patients suffering from schizophrenia, however interactions between oxytocin receptors (Oxtr) and other neurotransmitter systems are not yet fully understood. It has become increasingly recognized that patients who suffer from major psychiatric disorders have compromised inhibitory neurotransmission. In support of this, preclinical findings have shown that the proper functioning of inhibitory neurons (interneurons) is essential for balanced neuronal activity and healthy mental states. As with excitatory neurons, the activity of interneurons is regulated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), which acts through both synaptic and extrasynaptic receptors ($GABA_AR$). Our recent findings suggest that gaboxadol, an extrasynaptic $GABA_AR$ agonist, activates granule cells (GC) in the dentate gyrus of the hippocampus, suggesting disinhibition of local interneuronal networks. At the same time, gaboxadol also induced state-dependent fear conditioning, an effect that was completely abolished by oxytocin receptors (Oxtr) knockout. Together, these experiments suggest that Oxtr, by contributing to the cellular and behavioral functions of extrasynaptic $GABA_AR$, either directly or indirectly control inhibitory neurotransmission in the hippocampus. This study will further our fundamental understanding of inhibitory control in the brain and inform future treatments based on GABAergic and oxytocinergic drugs.

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Poster

358. Learning and Memory: Amygdala Circuits

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Title: A competitive inhibitory circuit for selection of active and passive fear responses

Authors: *J. P. FADOK[1], S. KRABBE[1], J. COURTIN[1], M. MARKOVIC[1,2], C. XU[1], L. MASSI[1], P. BOTTA[1], K. BYLUND[1], C. MUELLER[1], P. TOVOTE[1], A. LÜTHI[1];
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Abstract: In the face of threat, survival of an organism is contingent upon selection of appropriate active or passive behavioral responses. Freezing is an evolutionarily conserved passive fear response which has been used extensively to study the neuronal mechanisms of fear and fear conditioning in rodents. However, under natural conditions, rodents also exhibit active responses such as flight. The central amygdala (CEA) is a forebrain structure vital for the acquisition and expression of conditioned fear responses, and the role of specific CEA neuronal sub-populations in freezing behavior is well-established. Whether the CEA is also involved in flight behavior, and how neuronal circuits for active and passive fear behavior interact in the CEA is unknown. To explore these potential interactions, we developed a Pavlovian conditioning paradigm in which mice switch between conditioned freezing and flight behavior in response to discrete auditory stimuli. To determine the role of defined CEA neuronal subpopulations in conditioned active defensive behavior, three populations of CEA neurons were optically inhibited during the conditioned flight paradigm. These experiments determined that CEA cells expressing corticotropin-releasing factor (CRF+) are necessary for the behavioral expression of conditioned flight. *In vivo* extracellular recordings of identified cell types in behaving animals revealed that CRF+ cells are excited by the auditory stimulus driving flight, whereas somatostatin-positive (SOM+) cells are inhibited during flight and excited during freezing. Using *in vitro* recordings, we determined that CEA CRF+ and SOM+ neurons form reciprocal

inhibitory synaptic connections. Additionally, defensive behavior can be biased towards freezing or flight via optogenetic excitation of SOM+ or CRF+ neurons, respectively. These data suggest that the selection of appropriate behavioral responses to threat is based on competitive interactions between two defined populations of inhibitory neurons, a circuit motif allowing for rapid and flexible action selection.

Disclosures: **J.P. Fadok:** None. **S. Krabbe:** None. **J. Courtin:** None. **M. Markovic:** None. **C. Xu:** None. **L. Massi:** None. **P. Botta:** None. **K. Bylund:** None. **C. Mueller:** None. **P. Tovote:** None. **A. Lüthi:** None.

Poster

358. Learning and Memory: Amygdala Circuits

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 358.02/JJJ44

Topic: G.01. Appetitive and Aversive Learning

Support: Eurospin

Title: Acquired taste valence encoding in cortico-amygdala circuits

Authors: ***K. LAVI**[1,2], G. JACOBSON[1], K. ROSENBLUM[3], A. LUTHI[1];
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Abstract: Conditioned taste aversion (CTA) is a form of associative learning in which pairing of an innately appetitive tastant with gastrointestinal malaise induces a valence switch towards aversion. CTA training modulates neuronal taste responses in the gustatory cortex (GC) and basolateral nucleus of the amygdala (BLA), two structures known to be important for retention of aversive taste associations. The GC and the amygdala are closely inter-connected and CTA results in increased functional connectivity between GC and BLA after conditioning. Using two-photon calcium imaging, we investigated whether CTA induced changes in neuronal taste responses of defined GC projection neurons . Our results provide insight into how acquired stimulus valence is encoded on the neuronal network level in the mammalian brain.

Disclosures: **K. Lavi:** None. **G. Jacobson:** None. **K. Rosenblum:** None. **A. Luthi:** None.

Poster

358. Learning and Memory: Amygdala Circuits

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 358.03/JJJ45

Topic: G.01. Appetitive and Aversive Learning

Title: Central amygdala microcircuit mediating learning and expression of active avoidance

Authors: *M. MARKOVIC, C. XU, S. KRABBE, J. GRUENDEMANN, J. CUSULIN, A. LUTHI;
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Abstract: The amygdala is a site important for the acquisition and expression of conditioned passive and active defensive behaviors in rodents. Numerous studies of classical fear conditioning have yielded a good understanding of the role of central amygdala (CEA) in orchestrating passive defense. Inactivation studies also suggest an involvement of the CEA in modulating conditioned active avoidance responses. However, the neuronal circuitry underlying this phenomenon is largely unknown.
We are interested in understanding how PKCd+ and SOM+ populations of inhibitory neurons in the CEA can influence active conditioned fear responses. We are using a signaled two-way active avoidance (2wAA) paradigm as a basis for studying the dynamics of switching between freezing and conditioned active avoidance. A combination of genetic, viral, and optogenetic tools enables us to bidirectionally manipulate the activity of these neuronal populations during 2wAA training. Deep brain functional Ca2+ imaging in freely behaving mice is used to further explore how CEA modulates learning and expression of 2wAA.

Disclosures: M. Markovic: None. **C. Xu:** None. **S. Krabbe:** None. **J. Gruendemann:** None. **J. Cusulin:** None. **A. Luthi:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Program#/Poster#: 358.04/JJJ46

Topic: G.01. Appetitive and Aversive Learning

Title: Amygdalar PV-interneurons gate prefrontal input to control fear ensemble activity

Authors: *P. DAVIS, S. VIOLA, L. REIJMERS;
Tufts Univ. Sch. of Med., Boston, MA

Abstract: Networks of inhibitory interneurons play critical roles in information processing and memory encoding, including during conditioned fear behavior. How interneuron networks exert selective influence over functionally relevant neuronal ensembles, an attribute critical to their role in producing successful behavioral strategies, however, remains unknown. One methodological approach to studying such neuronal ensembles is the TetTag mouse, which couples the expression of c-Fos, an activity regulated immediate early gene, with the expression of GFP in experimentally defined temporal windows. Previous work in our lab correlated target-specific changes in parvalbumin-positive (PV+) perisomatic synapses around TetTagged "fear" neurons within the basolateral amygdala with the suppression of conditioned fear following extinction. To directly test the role of this interneuron population in the suppression of conditioned fear, we applied a pharmacogenetic approach in the background of TetTag mice to selectively silence PV+ interneurons within the basolateral amygdala (BLA) during the retrieval of an extinguished fear memory and determine the effect on behaviorally relevant neuronal ensembles. We found that pharmacogenetic inhibition of PV+ interneurons following extinction led to the selective disinhibition of TetTagged "fear" neurons in the BLA and a corresponding increase in the conditioned fear response specifically in the conditioned context. We also found that this manipulation caused changes to the activation state of other brain regions known to be involved in the fear circuit, including medial prefrontal cortex (mPFC). Finally, we found that PV-interneurons receive direct input from principal neurons within the mPFC and participate in a reciprocal loop between cortex and amygdala, thereby positioning them to selectively influence descending mPFC control of amygdalar activity. Parvalbumin interneurons may therefore act as a gate for top-down control of sparsely encoded competing neuronal ensembles in the amygdala, thereby providing a potential mechanism for alternating between opposing behavioral strategies.

Disclosures: **P. Davis:** None. **S. Viola:** None. **L. Reijmers:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: NIAAA Intramural Research Program

Title: Intercalated cells of the amygdala in fear and extinction learning

Authors: *O. BUKALO[1], A. LIMOGES[1], M. NONAKA[1], R. PALMITER[2], L. ZWEIFEL[3], A. HOLMES[1];
[1]Lab. of Behavioral and Genomic Neurosci., NIH/NIAAA, Rockville, MD; [2]Dept. of Biochem., [3]Departments of Psychiatry and Behavioral Sci. and Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Persistent anxiety following a psychological trauma is a hallmark of many anxiety disorders. However, the neural circuits mediating the extinction of fear remain incompletely understood. Intercalated cells (ITCs) of amygdala is a network of interconnected GABAergic neurons organized in distinct clusters surrounding the basolateral amygdala (BLA). A role for ITCs in fear extinction has been supported by number of observations and have led a model whereby ITCs reciprocally connected to BLA and central amygdala (CeA) gate cortical and thalamic inputs and amygdala outputs. To test the role of ITCs in mediating fear behavior, we used chemogenetics to target selective ITC clusters and examine their role in fear and extinction behavior. Since ITC neurons can be distinguished from other amygdala neurons by expressing transcriptional factor forkhead box protein2 (FoxP2), we used FoxP2-Cre mice to deliver adeno-associated viruses containing designer receptors exclusively activated by designer drugs (DREADDs). With this approach, we specifically targeted lateral and medial ITC nuclei (lITC and mITC, correspondingly) in different cohorts of mice with a Gi-coupled κ-opioid receptor based DREADD (KORD) and systemically injected the pharmacologically inert ligand salvinorin B (SALB) prior to fear conditioning or extinction acquisition. This approach could yield novel insights into the functional necessity of ITCs in forms of emotional learning known to be impaired in anxiety disorders.

Disclosures: O. Bukalo: None. **A. Limoges:** None. **M. Nonaka:** None. **R. Palmiter:** None. **L. Zweifel:** None. **A. Holmes:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Program#/Poster#: 358.06/JJJ48

Topic: G.01. Appetitive and Aversive Learning

Title: GABAergic and Adrenergic modulation of excitatory inputs to the lateral division of the central amygdala blocks fear conditioning

Authors: *A. DELANEY[1], J. CRANE[1], N. HOLMES[2], F. WESTBROOK[2];
[1]Charles Sturt Univ., Orange, Australia; [2]Univ. of New South Wales, Sydney, Australia

Abstract: The lateral division of the central amygdala (CeAL) receives processed and unprocessed sensory input carrying information about both the conditioned stimulus and unconditioned stimulus in fear conditioning. It is also extensively connected via inhibitory projections to the brainstem- and hypothalamic-projecting output neurons of the medial division of the central amygdala, which activate the behavioural, hormonal and autonomic effects seen in the conditioned response. In this study we have examined how activation of presynaptic $GABA_B$ receptors and presynaptic α2-adrenoceptors regulate synaptic transmission in the CeAL and how these drugs targeting effect context conditioning in rats. Using voltage clamp electrophysiological recordings from CeAL cells in acute brain slices, we show that the $GABA_B$-agonist baclofen inhibits electrically stimulated excitatory input from the basolateral amygdala (BLA) and the parabrachial nucleus (PbN). In contrast, selective activation of presynaptic α2-adrenoceptors using clonidine reduces PbN excitatory input but has no effect on excitatory responses to BLA stimulation. Both baclofen and clonidine also reduced inhibitory input from the BLA, however local CeAL inhibitory connections were selectively inhibited by baclofen. We then tested the effects of these drugs in the CeAL by infusing clonidine and baclofen into the CeAL via cannula immediately prior to context conditioning. Infusion of baclofen and clonidine into the CeAL prior to context conditioning in rats resulted in impaired conditioning. In testing trials 24hrs later, rats infused with either drug across conditioning froze significantly less than sham controls across the drug-free test session. These results indicate that uninhibited excitatory input to the CeAL from the PbN but not the BLA during conditioning is required for the acquisition of contextual conditioning. These findings are consistent with recent findings suggesting that the PbN input to the CeA may be an important pathway for transmission of unconditioned stimulus responses into the amygdala during fear learning.

Disclosures: **A. Delaney:** None. **J. Crane:** None. **N. Holmes:** None. **F. Westbrook:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

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Title: Associative learning is gated by $GABA_B$-GIRK signaling in pyramidal neurons of the basolateral amygdala

Authors: *M. E. TIPPS[1], E. MARRON FERNANDEZ DE VELASCO[1], N. M. MCCALL[2], K. WICKMAN[1];
[1]Pharmacol., [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Introduction: Experience-dependent neuronal plasticity underlies memory formation and long-term changes in behavior. Investigations into the cellular mechanisms of learning and memory have highlighted relevant changes in a number of brain regions and specific cell populations, with most studies focusing on shifts in excitatory neurotransmission. Recent evidence, however, suggests that inhibitory signaling also plays a key role in the development and maintenance of long-term memories. Work in the basolateral amygdala (BLA) has demonstrated that the GABAergic interneurons of this region display highly regulated firing patterns in response to salient stimuli during fear conditioning, suggesting that precise patterns of GABA neuron activity are required for learning to occur. While recent work has advanced our understanding of learning-related GABA neuron activity in the BLA, characterization of the postsynaptic mechanisms that process those GABAergic signals and potential plasticity in these mechanisms is lacking.
Methods and Results: Here, we used neuron-specific manipulations in mice, together with behavioral and electrophysiological assessments, to probe the relationship between inhibitory signaling and fear learning. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we found that chemogenetic inhibition of BLA GABA (but not pyramidal) neurons impaired the acquisition of fear learning. We also observed a striking potentiation of signaling mediated by the $GABA_B$ receptor ($GABA_BR$) in BLA pyramidal neurons following fear conditioning, an adaptation that is largely attributable to an NMDA receptor (NMDAR)-dependent increase in the activity of G protein-gated inwardly-rectifying K^+ (GIRK/Kir3) channels. Finally, we show that DREADD activation of GIRK-dependent signaling in BLA pyramidal neurons is sufficient to generate a learned fear response to an otherwise neutral stimulus, demonstrating that GIRK channel activation and subsequent inhibition of BLA pyramidal neurons can gate associative learning.
Conclusions: Our data demonstrate that GABAergic signaling within BLA pyramidal neurons is dynamically regulated during associative learning. The observed increase in $GABA_B$-GIRK signaling following fear conditioning suggests that the traditional view of acquisition, which is based on pyramidal neuron disinhibition, is too simplistic. Instead, we propose a mechanism in which direct pyramidal inhibition via GIRK signaling is required to gate associative learning in the BLA.

Disclosures: M.E. Tipps: None. **E. Marron Fernandez de Velasco:** None. **N.M. McCall:** None. **K. Wickman:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: MOST 104-2320-B-007-004-

Title: Interactive modulation of the medial prefrontal cortex and orbitofrontal cortex on amygdala neuronal activities.

Authors: *C.-H. CHANG;
Inst. of Systems Neurosci., Natl. Tsing Hua Univ., Hsinchu (city), Taiwan

Abstract: The amygdala receives convergent inputs from the medial prefrontal cortex (mPFC) and the orbitofrontal cortex (OFC). Abnormal connectivity between the mPFC-amygdala and the OFC-amygdala may lead to psychiatric conditions, such as post-traumatic stress disorder (PTSD) and obsessive compulsive disorder (OCD). Because of the convergent pathways onto the amygdala, the amygdala is considered one of the top candidates that attribute to the psychiatric comorbidity. Previously, we have shown that activation of the OFC inserts an inhibitory modulation on the mPFC-amygdala pathway through intra-amygdala feed-forward inhibition, which is abolished by OFC tetanus. In this study, we further examined how mPFC interacts with the OFC-amygdala pathway. *In vivo* extracellular single-unit recordings was used in anesthetized rats to examine how mPFC activation modulates amygdala neurons that fire in response to OFC activation. The majority of the amygdala neurons responded to OFC electrical stimulation also responded to mPFC electrical stimulation. Relative to baseline (BL, ~50% evoked spikes in 50 trials), mPFC stimulation preceding OFC stimulation (50 trials each; mPFC-OFC stimulation delay: 10, 20, 30, 40, 50, and 100 ms) inserted an inhibitory modulatory gating on the OFC-amygdala pathway (changes in evoked probability > 15% relative to baseline). Such inhibitory gating was reversed by blockade of intra-amygdala GABAergic receptors with bicuculline ($GABA_A$ antagonist) and saclofen ($GABA_B$ antagonist) cocktail, but was not affected by mPFC tetanus (200 trials at 20 Hz). Our results suggest that mPFC and OFC impose interactive inhibitory modulation of the amygdala on the other pathway, but have different modulation when the strength of the connectivity is altered.

Disclosures: C. Chang: None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R21MH107970

Whitehall Foundation

Title: Role of the prefrontal-amygdala synapses in the enhancement of Pavlovian conditioning after observational fear

Authors: *W. ITO, A. MOROZOV;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: The observational fear paradigm is a mouse model of emotional trauma. We have shown that a single 4 min exposure of a subject, the observer mouse, to a cagemate demonstrator mouse receiving electrical footshocks (1 mA, 2 sec, every 10 sec) is sufficient 1) to enhance learning of the passive avoidance task (PA) and 2) to generate silent synapses in the dorsomedial prefrontal cortex (dmPFC) inputs to the basolateral amygdala (BLA). Since the causal link between the activity of this inputs and the enhancement of learning was missing, we tested the role of the synaptic transmission in the dmPFC-BLA input by circuit-selective inactivation using DREADD. First, we examined the effectiveness of DREADD in suppressing synaptic transmission in the dmPFC-BLA synapses *ex vivo*. dmPFC neurons were co-transduced with AAV viral vectors expressing hM4Di and channelrhodopsin 2 (ChR2). Excitatory postsynaptic currents (EPSCs) evoked upon blue light stimulation of the dmPFC axons were recorded from BLA principal neurons in slices. As expected, bath application of CNO suppressed the currents by about 80%. The result indicates effective inhibition of glutamatergic transmission in that pathway by DREADD. Second, we performed the pathway-specific inhibition during the observational fear paradigm. To suppress the pathway *in vivo*, the hM4Di was expressed in dmPFC neurons using an AAV viral vector. CNO or saline was infused into BLA via bilateral cannulas 30-60 min before the observational fear procedure. The subsequent PA training and testing revealed that CNO infusion abolished the enhancement of PA learning, whereas saline infusion had no effect. These results indicate that the glutamatergic transmission in the dmPFC input to BLA mediates the effect of observational fear on Pavlovian conditioning at a later time.

Disclosures: W. Ito: None. **A. Morozov:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: Initial Complementary Funds, University of California, Riverside

Title: Synaptic targeting of double-projecting ventral hippocampal neurons to the medial prefrontal cortex and basomedial amygdala

Authors: *J.-H. CHO, W. KIM;
Univ. of California, Riverside, CA

Abstract: The acquisition and retrieval of contextual fear memory require the neural activity in the hippocampus, medial prefrontal cortex (mPFC), and amygdala. Contextual information processed in the hippocampus is conveyed to the mPFC and amygdala for contextual fear conditioning. Previous studies suggest that a neuronal population in the ventral hippocampus (VH) projects to both mPFC and amygdala, and is recruited in context-dependent control of conditioned fear responses. However, it has not been determined previously how double-projecting VH neurons modulate the activity of mPFC and amygdala at the neural circuit level. In this study, we used viral tracing, optogenetic and electrophysiological approaches in mice, and investigated how double-projecting VH neurons are connected synaptically to the mPFC and amygdala. In dual retrograde viral tracing and c-Fos immunostaining experiments, we found that a significant proportion of VH neurons projected to both mPFC and amygdala with axon collaterals and was recruited during the recall of contextual fear memory, suggesting their role in conditioned fear responses. Moreover, optogenetic stimulations of axons of double-projecting VH neurons induced monosynaptic excitation and feed-forward inhibition in both mPFC and basomedial amygdala (BMA), indicating functional neural circuits from these VH neurons to the mPFC and BMA. Activation of double-projecting VH neurons also induced action potential firings in mPFC neurons projecting to the amygdala. These synaptic mechanisms enable double-projecting VH neurons to activate more efficiently both VH-amygdala and VH-mPFC-amygdala pathways, potentially enhancing synchronized neural activity in the mPFC and amygdala for the acquisition and retrieval of contextual fear memory.

Disclosures: **J. Cho:** None. **W. Kim:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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BK21 PLUS BioKAIST Initiative, Biological Sciences, KAIST

Title: Defensive behavior switching mediated by top-down inhibition of freezing cells in the centromedial amygdala

Authors: *J. JHANG, J.-H. HAN;
Dept. of Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Choosing a proper defensive reaction to threat critically affects survival of animals. Freezing is predominant behavioral response to threats, but our brain also requires mechanisms to suppress freezing for discharging active reactions as alternative strategies. We employed optogenetic approaches in innate and learned threat-exposure models in mice, and found that activation of the anterior cingulate cortex (ACC) and its direct projections to the amygdala suppress freezing expression with concurrent induction of risk assessment (RA)-related exploration behaviors. Our results demonstrate that CaMKIIα-positive neurons in ACC have direct projections to centromedial (CeM) and basolateral (BLA) regions of the amygdala, and activation of these pathways may suppress the activity of CeM cells that trigger freezing response. This circuit mechanism may underlie the flexible transition between conflicting defensive strategies, particularly to discharge RA behaviors under ambiguous threat circumstances. Dysregulation of this pathway would result in impaired transition between threat responses, thus involve with various symptoms of anxiety-related disorders.

Disclosures: **J. Jhang:** None. **J. Han:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH DA034010

Title: The retrorubral field is necessary for accurate fear discrimination in Pavlovian conditioning

Authors: *K. M. WRIGHT, M. MCDANNALD;
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Abstract: People with anxiety disorders typically have no difficulty acquiring fear to threatening cues. However, they often show inappropriate levels of fear to cues threatening little or no harm. Thus, the ability to acquire fear may be distinct from the ability to appropriately assign levels of fear to threatening stimuli. An abundance of studies have demonstrated the basolateral (BLA) and central nuclei (CeA) of the amygdala are critical for acquiring fear. Finer discrimination of threat, permitting precise control of acquired fear, almost certainly involves a broader neural network. Here we sought to demonstrate a critical role for the retrorubral field (RRF) in accurate threat estimation, and precise control of fear. The RRF is a midbrain structure positioned just caudal and dorsal to the substantia nigra. With its dense projections to the CeA, it is anatomically well positioned to control fear output. The RRF may be best known for its dopaminergic neurons, but it also contains a large, independent population of glutamatergic neurons. In order to target all neuron types, adult, male Long Evans rats received bilateral, neurotoxic RRF lesions using ibotenic acid or sham lesions. After recovery, rats received fear discrimination in which three cues were associated with different probabilities of foot shock: safety, p=0.00; uncertainty, p=0.25; and danger, p=1.00. Control rats demonstrated excellent fear discrimination: achieving high fear to the danger cue, little or no fear to the safety cue, and intermediate fear to the uncertainty cue. Rats with RRF lesions acquired fear normally, but were impaired in finer discrimination, eventually showing equivalent levels of fear to the safety and uncertainty cues. Neurotoxic lesions result in permanent brain damage, making it difficult to determine if RRF activity is necessary for controlling fear at the time of cue sampling. To overcome this limitation, we are using optogenetic inhibition of the RRF with halorhodopsin under the control of the human synapsin promoter. Preliminary data indicate a causal role for the RRF in generating accurate threat estimates. A full analysis of the optogenetic inhibition experiment will be presented. The ability to appropriately assign fear to a potential threat is vital. The experiments described here will demonstrate a novel and integral role for the RRF in accurately discriminating threatening cues and controlling fear.

Disclosures: **K.M. Wright:** None. **M. McDannald:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Title: Prevention of fear re-appearance by blockage of dopamine signaling

Authors: ***N. HITORA-IMAMURA**[1,2], Y. MIURA[2], C. TESHIROGI[2], Y. IKEGAYA[2], N. MATSUKI[2], H. NOMURA[1,2];
[1]Dept. of Pharmacol., Hokkaido Univ., Sapporo-shi, Japan; [2]Lab. of Chem. Pharmacol., Univ. of Tokyo, Tokyo, Japan

Abstract: Anxiety disorders are often treated with cognitive-behavioral interventions such as exposure therapy. Fear conditioning and extinction are used in animal models of anxiety disorders and their treatment. Prevention of relapses is a major challenge in treating anxiety disorders. About 40% of patients in remission experience a relapse. In experimental animals, fear can be reinstated by a week footshock (reminder shock) even after successful extinction. However, the mechanisms responsible for fear reinstatement are poorly understood. To identify brain regions involved in processing fear reinstatement, we mapped the regional expression of the inducible immediate early gene, c-Fos. c-Fos expression was elevated in the infralimbic cortex (IL) and lowered in the medial subdivision of the central nucleus of the amygdala (CeM). Electrophysiology experiments revealed that reinstatement was accompanied by reduction of synaptic input in the IL. Next we investigated the involvement of prefrontal dopamine signaling in reinstatement. We tested whether a reminder shock induces c-Fos expression in the ventral

tegmental area neurons projecting to the IL. We retrogradely labeled the neurons projecting to the IL by infusing cholera toxin subunit B into the IL. Dopaminergic neurons were identified by tyrosine hydroxylase immunosignals. A reminder shock induced c-Fos expression in the IL-projecting dopaminergic neurons in the VTA. The blocking of IL dopamine D1 receptor signaling prevented reduction of synaptic input, CeM c-Fos expression, and fear reinstatement. These findings demonstrate that a dopamine-dependent inactivation of extinction circuits underlies fear reinstatement and may explain the comorbidity of substance use disorders and anxiety disorders.

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Poster

358. Learning and Memory: Amygdala Circuits

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Program#/Poster#: 358.14/JJJ56

Topic: G.01. Appetitive and Aversive Learning

Title: Differential effects of D1-mediated dopamine signaling in the amygdala on fear, safety, reward cue discrimination learning

Authors: K. NG[1,2], M. POLLOCK[1], P. URBANCZYK[1], E. WOON[1], E. GREINER[1], *S. SANGHA[1,2];
[1]Dept. of Psychological Sci., Purdue Univ., West Lafayette, IN; [2]Purdue Inst. for Integrative Neurosci., West Lafayette, IN

Abstract: Accurate discrimination among cues signifying danger, safety or reward initiates the proper emotional response in order to guide behavior. Since potentially rewarding and dangerous stimuli often occur simultaneously leading to opposing behaviors, reward- and fear-related circuits must interact in order to mediate these antagonistic behaviors. In order to investigate how the fear, safety and reward circuits integrate, we train Long Evans rats to discriminate among a) a fear cue paired with footshock, b) a safety cue in the presence of the fear cue resulting in no footshock, and c) a reward cue paired with sucrose delivery. A selective increase in freezing to the fear cue and reward seeking to the reward cue indicate good fear and reward discrimination, respectively.
Using this task we have previously identified neurons in the basolateral amygdala (BLA) that discriminate among these cues (Sangha et al, 2013). Also within the BLA, dopamine levels increase during learned fear responses (de Oliveira et al, 2011) and D1 receptors are required for fear extinction (Hikind et al, 2008). Dopamine signaling is also implicated in discriminatory

reward learning (Eagle et al, 2015). Based on this, we tested the hypothesis that fear-safety-reward cue discrimination requires D1-mediated dopamine signaling in the BLA. Preliminary data indicate systemic injections of a D1 receptor agonist (10mg/kg SKF-38393; n=8) disrupts both fear and reward discrimination compared to saline controls (n=12). When the D1 receptor agonist is infused directly into the BLA bilaterally (1.0 μg/0.5μL SKF-38393; n=4) 30 min prior to each discrimination session, preliminary results indicate that both reward and fear discrimination are again impaired compared to saline controls (n=7). But, when a D1 receptor antagonist is infused directly into the BLA bilaterally (0.25 μg/0.5μL SCH 23390; n =5), preliminary results indicate that reward discrimination, not fear discrimination, is impaired compared to saline controls (n = 7). Together, this indicates that 1) an optimal level of dopamine activity in the BLA is necessary for reward discrimination, and 2) successful fear discrimination does not require D1 receptors in the BLA but overstimulation of the D1 receptors will impair fear discrimination.

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Poster

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Topic: G.01. Appetitive and Aversive Learning

Support: R01 MH099073

Title: Optogenetic manipulation of the amygdalar pyramidal cells alters fear behavior in foraging rats

Authors: ***M.-S. KONG**[1], E. KIM[1], J. J. KIM[1,2];
[1]Dept. of Psychology, [2]Program in Neurobio. & Behavior, Univ. of Washington, Seattle, WA

Abstract: Amygdalar stimulation has been shown to produce freezing, cardiovascular changes and enhanced startle, indicating that the amygdala can directly activate an ensemble of fear responses. However, these stimulation-evoked responses have all been reported in typical operant chambers that limit the animals' behavior. For example, we have previously shown that the same electrical stimulation that produced freezing and 22 kHz ultrasonic vocalization in an operant chamber elicited fleeing (escape) behavior in rats that had to leave the nesting area to forage for food placed in a large open field (Kim et al., 2013). The present study further examined the role of the amygdala in risky foraging behavior using optogenetics, which allowed

us to target pyramidal cells in the amygdala selectively. Male Long-Evans rats were injected with AAV-CaMKIIα-hChR2-EYFP into the basolateral amygdala to overexpress channelrhodopsin in pyramidal cells. The experiment started 5 weeks after the virus injection to ensure sufficient infection. After postoperative recovery, all rats were maintained ~85% normal body weight to motivate foraging behavior (i.e., procure food pellet and return to the nest) during the baseline session. On the testing day, whenever the animal came near the pellet, the amygdala was stimulated using a blue laser (473 nm) for 2 seconds (10 ms pulse @ 20 Hz). Light activation of the amygdala prevented rats from acquiring the pellet during the 3-minute session. Light stimulation did not damage the amygdala because when animals were tested in the presence of an external threat, all exhibited escape behavior to a surging predatory robot. These results indicate that the activation of amygdala pyramidal cells alone is sufficient to generate a fear response in a dynamic foraging environment.

Disclosures: **M. Kong:** None. **E. Kim:** None. **J.J. Kim:** None.

Poster

358. Learning and Memory: Amygdala Circuits

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 358.16/JJJ58

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH Grant R01 MH107239

Title: Basolateral amygdala nucleus responses to appetitive conditioned stimuli correlate with variations in conditioned behavior

Authors: ***S.-C. LEE**, A. AMIR, D. B. HEADLEY, D. HAUFLER, D. PARE;
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Abstract: In the lateral amygdala (LA), training-induced increases in neuronal responsiveness to conditioned stimuli (CSs) reflect potentiated sensory responses that drive conditioned behaviors (CRs) via LA's targets. The basolateral nucleus of the amygdala (BL) receives LA inputs and projects to various subcortical sites that can drive aversive and appetitive CRs. Consistent with this, BL neurons also develop increased responses to CSs that predict rewarding or aversive outcomes. However, this increased BL activity might not be directly related to the potentiated sensory responses of LA neurons, but instead reflect training-induced modifications in behavioral output. By contrasting the CS-related activity of BL neurons when rats produced the expected CR or not, we found that cells activated by appetitive CSs mainly encode behavioral output, not CS identity. The strong dependence of BL activity on behavior irrespective of CS

identity suggests that feedforward connectivity from LA to BL can be overridden by other BL inputs.

Disclosures: **S. Lee:** None. **A. Amir:** None. **D.B. Headley:** None. **D. Haufler:** None. **D. Pare:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

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Title: Increasing the GluN2A/GluN2B ratio within neurons of the mouse basal and lateral amygdala inhibits the modification of an existing fear memory trace

Authors: ***R. HOLEHONNUR**, A. J. PHENSY, L. J. KIM, M. MILIVOJEVIC, D. T. VUONG, D. K. DAISON, S. ALEX, M. TINER, L. E. JONES, J. E. PLOSKI, S. KROENER; Univ. of Texas at Dallas, Richardson, TX

Abstract: Memory modification utilizing reconsolidation updating is being examined as one of the potential treatment approaches for attenuating traumatic memories in disorders such as PTSD. However, preclinical studies have shown that while weak fear memories can be modified using reconsolidation updating, strong fear memories can be resistant to this approach. Some have speculated that learning induced increases in the NMDA receptor GluN2A/GluN2B ratio might serve as a mechanism to preserve the integrity of a memory due to critical differences between these subunits. However, it has never been directly examined if altering the GluN2A/GluN2B ratio is sufficient to prevent a memory from being modified. Here we report that strong auditory fear memories are resistant to retrieval dependent memory destabilization and are associated with an increase in the synaptic GluN2A/GluN2B ratio within basal and lateral amygdala (BLA) neurons. Next, we tested the hypothesis that increasing the GluN2A/GluN2B ratio within BLA neurons of a weak memory, would be sufficient to render this memory incapable of being destabilized upon retrieval and thus prevent this memory from being modified via reconsolidation updating. To accomplish this, it was necessary to develop the means to specifically overexpress GluN2A within neurons after learning. Therefore we genetically engineered transgenic mice to contain a GFP-GluN2A transgene controlled from a TRE3G promoter. When this line is crossed with a line of mice containing the tTA transcription factor transgene controlled by an αCaMKII promoter, the GFP-GluN2A transgene is specifically

expressed within αCaMKII positive neurons and regulatable using doxycycline. Our findings indicate that increasing the GluN2A/GluN2B ratio within BLA pyramidal neurons, inhibited retrieval dependent memory destabilization and the modification of the existing memory trace. This was associated with a reduction in retrieval dependent AMPA receptor trafficking as measured by a reduction in retrieval dependent phosphorylation of GluR1 at Serine at 845. Additionally, we determined that increasing the GluN2A/GluN2B subunit ratio prior to fear learning significantly impaired long term memory consolidation while short term memory remained unaltered. However, an increase in the GluN2A/GluN2B ratio following fear learning had no influence on fear extinction or expression. Collectively our data indicate that an increase in the GluN2A/GluN2B ratio interferes with retrieval dependent memory destabilization and prevents the initiation of reconsolidation updating.

Disclosures: **R. Holehonnur:** None. **A.J. Phensy:** None. **L.J. Kim:** None. **M. Milivojevic:** None. **D.T. Vuong:** None. **D.K. Daison:** None. **S. Alex:** None. **M. Tiner:** None. **L.E. Jones:** None. **J.E. Ploski:** None. **S. Kroener:** None.

Poster

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NRF 2015M3C7A1027351

Title: LTP induction and maintenance at persistent memory engram

Authors: ***J.-P. OH**, J.-T. KWON, H.-S. KIM, Y. JEONG, H.-Y. CHO, J.-H. HAN;
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Abstract: Memory persistence is one of the major defining features of memory and long-term potentiation (LTP) is one of the candidate mechanism for memory persistence. However, how once acquired memory becomes persistent and its correlation with maintenance of LTP is unknown yet. Previously, we showed optogenetic stimulation of presynaptic auditory input in lateral amygdala (LA) can serve as a conditioned stimulus (CS) to form associative fear memory.

With this behavioral model, we could localize and measure LTP at identical synapse relaying CS information to LA. In the present study, we showed LTP was induced at ChR2-tagged auditory input to LA synapse 24 hours after fear conditioning. However, this LTP was not maintained to 20 days. Surprisingly, optogenetic reactivation 24 hours after fear conditioning maintained LTP to 20 days. Consistent with decay of LTP and its maintenance by reactivation, fear memory expression induced by optogenetic stimulation was maintained to 20 days only when memory was reactivated. Also, c-Fos immunostaining confirmed memory expression is correlated with neuronal activity at LA. With additional behavioral experiment, we showed fear memory was gradually decayed to 20 days and reactivation at each time point maintained fear memory to 20 days without any significant difference from first retention. Also, persistent memory conversion by reactivation was synaptic input specific. Collectively, our results show the first observation of induction and maintenance of LTP at persistent memory engram and further suggest importance of synapse specific reactivation in memory persistence.

Disclosures: **J. Oh:** None. **J. Kwon:** None. **H. Kim:** None. **Y. Jeong:** None. **H. Cho:** None. **J. Han:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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NARSAD Young Investigator Award

Title: Multimodal and site-specific plasticity of amygdala parvalbumin interneurons after fear learning

Authors: ***E. K. LUCAS**[1], A. JEGARL[2], R. L. CLEM[3];
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[3]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Plasticity of discrete neural networks onto excitatory principal neurons in the basolateral amygdala underlies the acquisition and maintenance of emotional memory. Parvalbumin-expressing interneurons (PV-INs) have been observed to gait the acquisition of fear memory through inhibition of principal neurons across multiple brain regions, yet the

contribution of specific inhibitory microcircuits to stimulus gating and experience-dependent plasticity in the fear system remains poorly understood. Here we interrogate synaptic connections between afferent pathways, PV-INs, and principal neurons in the basolateral amygdala. We find that this region is comprised of at least two functionally distinct PV-IN networks based on nucleus location. PV-INs in the lateral (LA), but not the basal (BA), amygdala possess complex dendritic arborizations, receive potent thalamic and cortical drive, and mediate feedforward inhibition onto principal neurons. Fear memory encoding is associated with extensive and target-selective plasticity mainly affecting LA PV-INs, resulting in persistent downregulation of inhibitory function in LA but not BA. These data clarify the role of inhibitory microcircuits in the formation of associative fear memories and reveal previously overlooked specializations of PV-IN function in amygdala subnuclei.

Disclosures: **E.K. Lucas:** None. **A. Jegarl:** None. **R.L. Clem:** None.

Poster

358. Learning and Memory: Amygdala Circuits

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Title: Cortico-amygdalar functional network underlying contextual fear learning in the absence of hippocampus

Authors: ***C. A. COELHO**[1], J. C. K. SOARES[1], T. L. FERREIRA[2], J. R. SATO[2], M. G. M. OLIVEIRA[1];
[1]Univ. Federal De Sao Paulo, Sao Paulo, Brazil; [2]Ctr. de Matemática, Computação e Cognição, Univ. Federal do ABC, São Bernardo do Campo, Brazil

Abstract: Hippocampal lesions result in profound memory deficit in contextual fear conditioning (CFC) in rats when made post-training, whereas pre-training lesions do not affect CFC learning. Other brain regions involved in context learning are hypothesized to compensate hippocampal loss. Here, we asked what are the differences in the functional network (FN) engaged in lesioned rats relative to normal ones during CFC learning. Rats underwent bilateral electrolytic dorsal hippocampus (dHPC) lesions or SHAM surgeries. After recovery, they underwent a single shock CFC protocol. We added a SHAM immediate shock group (S) as a

learning control. Half the sample returned to the homecage and was tested for context memory 48h later. The other half was perfused with paraformaldehyde 3h after training, and their brains were processed for immunohistochemical staining of pCREB. We quantified pCREB expression in 30 regions known to be involved in CFC. Behaviorally, dHPC and SHAM groups showed higher freezing than S, but did not differ from one another. The pCREB activity was not significantly different between dHPC and SHAM in any region. FNs were built as undirected graphs composed by the brain regions as nodes and all the inter-regional Pearson correlations passing a threshold ($p < 0.05$), as edges. FNs of both groups showed a Small World topology, with high Global Efficiency (GE) and high Local Efficiency (LE) relative to random control networks. We computed the weighted degree (wdg) and eigenvector (evc) centralities of each region in both networks. Both FNs showed resilience to random error (sequential removal of random nodes) compared to targeted attack (sequential removal of the highest wdg node). Consistent with behavior, these results show that dHPC lesion did not affect FN architecture. Next, a permutation test comparing the centrality of each region between groups showed that the BLV and LADL had higher wdg and evc values in the SHAM group, whereas RSGd, RSGc, DLE and Cg1 had higher values in dHPC group. Next, we intersected the regions in the upper quartile of wdg, evc GE and LE and identified the network Hubs (regions within at least 3 quartiles). The dCA1, PrL, IL and BLV were hubs in the SHAM network, and the PER_35, PER_36, LAVL and RSGd were hubs in the dHPC network. The different central regions and hubs suggest a functional reorganization in dHPC network. A comparison of connection change between the groups suggest that the differences in dHPC network result from SHAM hubs losing connections, more than the rise of new interactions. This offers new insights on how dHPC rats learn CFC and what they learn in terms of context.

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Poster

358. Learning and Memory: Amygdala Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: RO1MH097085

R25-NS080686

Title: Threat anticipation during encoding impairs visual object recognition memory and pattern separation

Authors: *S. H. BRAREN[1], J. E. DUNSMOOR[1], M. C. W. KROES[1], V. P. MURTY[2], E. A. PHELPS[1];
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Abstract: Recognition memory is typically better for emotional items than for neutral items. Emotional arousal also improves recognition memory for neutral information associated with an emotional event. Whether arousal affects the process by which similar events are separated into distinct memory representations (pattern separation) is unclear. Importantly, it has been proposed that pattern separation may suffer during periods of intense negative arousal. Pattern separation deficits may then promote overgeneralization of fear from known threats to similar but harmless stimuli— exemplified in posttraumatic stress disorder. Across three experiments in healthy adults, we investigated whether memory encoding during anticipation of high-intensity aversive events affects recognition memory and pattern separation. Participants viewed 90 trial-unique objects paired with either a high- or low-intensity electric shock, or no shock. The objects had no predictive value—i.e., participants could not learn to anticipate the outcome based on the object. In Experiment 1, participants made a semantic judgment for each object ("bigger or smaller than a shoebox") prior to the outcome. In Experiments 2 and 3, anticipatory arousal prior to the outcome was generated by presenting each object in the center of a colored border that predicted the outcome. In Experiment 2 participants judged the likelihood and severity of the outcome whereas in Experiment 3 they made a semantic judgment of the object's size. Participants in each group returned 24-hours later for a surprise test of recognition memory and pattern separation between an old image and a highly similar lure. In Experiment 1, neither recognition memory nor pattern separation was affected by whether an object was paired with a high-intensity, low-intensity, or no shock. In contrast, recognition memory performance was linear in Experiments 2 and 3: participants recognized more items that appeared during periods of safety than periods of high-intensity threat, with intermediate performance for items paired with a low-intensity threat. Participants also showed impaired pattern separation for recognized items that appeared during anticipation of a high-intensity threat. Results show that encoding neutral objects during anticipation of high-intensity threat impairs recognition memory. Further, impaired pattern separation for these objects accords with pattern separation deficit models of PTSD, which propose overgeneralization as arising from the inability to discriminate between stimuli associated with a traumatic event from similar but harmless stimuli.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS053907

NIH Grant F31NS096888

Title: Projections from the nucleus prepositus hypoglossi to the head direction circuit and an extraocular motor nucleus are separate but overlapping

Authors: *M. L. MEHLMAN, S. S. WINTER, J. S. TAUBE;
Dartmouth Col., Hanover, NH

Abstract: The nucleus prepositus hypoglossi (NPH) is involved in generating head direction (HD) cell activity and stabilizing gaze. Both processes require information about head movements to function properly. During head rotations, the activity of HD cells (neurons encoding the animal's HD) is updated to represent new orientations. To maintain gaze fixation during head rotations, extraocular motor nuclei generate compensatory eye movements. The NPH contains neurons encoding the animal's angular head velocity (AHV) and is believed to act as a neural integrator of this information, generating an oculomotor signal for eye displacement (Robinson, 1989). The HD circuit (the interconnected structures containing HD cells) and extraocular motor nuclei receive direct projections from the NPH, and lesions of the NPH disrupt HD cell activity (Butler & Taube, 2015) and produce gaze instability (Kaneko, 1999). Thus, AHV and oculomotor signals are likely projected from the NPH to the HD circuit and extraocular motor nuclei, respectively. We examined the anatomical organization of these two projections to determine if a single population of NPH cells projects to both the HD circuit and an extraocular motor nucleus, or if two separate populations exist, each projecting to a different target.

Individual rats (n = 17) received a pair of unilateral retrograde tracer injections (0.2 µl of cholera toxin subunit B, CTB); one color of CTB was injected into the dorsal tegmental nucleus and supragenual nucleus (two adjacent structures and components of the HD circuit), while a different color of CTB was injected into the abducens nucleus (ABN, an extraocular motor nucleus). Only animals with injections confined to their respective targets were analyzed (n = 2). Retrograde labeling was observed in a subset of NPH cells, revealing three distinct populations. One population (~35% of labeled cells) projected to the HD circuit and was distributed throughout the NPH. A second population (~35% of labeled cells) projected to the ABN and was observed primarily in lateral portions of the NPH. The third population (~30% of labeled cells) projected to both the HD circuit and the ABN and was largely confined to ventral portions of the

NPH. All three populations projected primarily to the contralateral hemisphere. These results were consistent across the two rats and indicate that projections from the NPH to the HD circuit and the ABN arise primarily from two separate populations, with an additional population of cells projecting to both targets. The functional correlates of these three populations remain to be determined by future electrophysiological studies.

Disclosures: M.L. Mehlman: None. **S.S. Winter:** None. **J.S. Taube:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NS053907

Title: Contributions of self-generated movements and vestibular inputs to spatial correlates within the dorsal tegmental nucleus of Gudden

Authors: *J. R. DUMONT, M. L. MEHLMAN, M. E. SHINDER, J. S. TAUBE;
Psychological Brain Sci., Dartmouth Col., Hanover, NH

Abstract: An animal's perceived sense of orientation depends upon the head direction (HD) system, involving structures primarily along Papez' circuit. Previous studies have demonstrated the importance of the dorsal tegmental nucleus (DTN) for downstream HD activity. The DTN contains cells whose firing correlates with changes in the rat's angular head velocity (AHV), and are thought to be involved in the generation of HD cell activity. Indeed, lesions of DTN disrupt downstream HD cell activity within the anterior dorsal thalamic nucleus. In addition to AHV cells, DTN also contains HD cells and neurons sensitive to both AHV and HD. The DTN receives both self-generated movement (proprioceptive and motor efference copy) and vestibular signals from several brainstem nuclei, and it remains unknown whether spatially modulated DTN cells utilize both these signals equally.
To investigate the contribution of vestibular and self-generated movement information to HD and AHV cells within the DTN, single units were recorded in the DTN while rats freely foraged in a cylindrical environment. HD cells in DTN, like other regions of the HD system, were able to maintain their preferred firing direction in the dark, and also shifted in register with rotations of a visual landmark, suggesting that visual landmark information may be conveyed to the DTN via the lateral mammillary nuclei, which receive visual information from the postsubiculum. Once AHV or HD cells were identified, rats either received intratympanic injections of tetrodotoxin

(TTX) to disrupt the vestibular system, or the rats were passively rotated using a head-fixed restraint apparatus to eliminate the use of self-generated movement cues. TTX injections disrupted the AHV sensitivity of AHV cells; however AHV signals were still identified within DTN during the period of vestibular inactivation. Consistent with these results, TTX also abolished HD cell neural firing in DTN, and sometimes caused HD cells, which originally did not carry an AHV signal, to now become sensitive to AHV. Similarly, responses to passive rotation were also variable. For example, the AHV signal in some units was abolished, whereas in other units it remained intact. These results suggest that DTN HD and AHV signals utilize both vestibular and self-generated movement information while rats navigate in their environment.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Support: NINDS Grant NS053907

Title: Interthalamic oscillatory coordination in the head direction cell network.

Authors: *W. N. BUTLER, J. S. TAUBE;
Dartmouth Col., Hanover, NH

Abstract: The head direction (HD) circuit is a complex, interconnected network of brain regions ranging from the brainstem to the cortex. Recent work (Peyrache et al., 2015) found that HD cells with similar directional tuning recorded ipsilaterally to one another in the anterodorsal nucleus (ADN) of the thalamus display a striking pattern in their inter-spike interval cross-correlograms. They found that the firing of these overlapping HD cell pairs was related by showing that a high frequency oscillation pattern (130-160 Hz) was visible when these cross-correlograms were examined on a very short (-20 to 20 ms) timescale. Spectral analysis further found that the power of this oscillation was greatest at 0 ms and decreased at greater lags, showing that there was synchrony between HD cells with similar tunings. Here, we first supported those results by observing the same relationship in our own recordings of ipsilateral ADN cell pairs. Further, we demonstrated that the same high frequency coordination exists in cell pairs recorded contralaterally from one another in the opposite half of the thalamus. When

we examined the cross-correlograms of HD cells that were recorded contralaterally from one another and that had similar directional tuning, we observed the same high frequency (~150-200 Hz) oscillatory relationship. The strength of this contralateral synchrony was similar to the synchrony strength seen in ipsilateral HD cell pairs. As the difference in tuning of the contralateral HD cell pairs increased, the degree of synchrony in the cross-correlogram decreased, a relationship that was also seen in Peyrache et al.'s work. Additionally, the frequency rate of this oscillation appeared to be independent of the firing rates of the two cross-correlated cells. These results indicate that co-recorded HD cells in the ADN are equivalently synchronous no matter whether they were recorded ipsi- or contralateral from one another. As a whole, these results imply that the left and right sides of the thalamic HD network are functionally related, despite an absence of direct interthalamic projections between the two ADN. However, anatomical tracing has found that each of the lateral mammillary nuclei (LMN) project bilaterally to both of the ADN. We believe that this parallel, cross-thalamic projection pattern from the LMN is responsible for the functional connectivity we observed between HD cells in the two ADN. These data also further support the hypothesis that the LMN is involved in producing the ring attractor organization of the HD signal.

Disclosures: W.N. Butler: None. **J.S. Taube:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NINDS-NS053907

Title: Grid cell representation across a multi-level maze.

Authors: *S. S. WINTER, M. L. MEHLMAN, J. S. TAUBE;
Psychology and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Real world navigation includes movement in three dimensions, but a vast majority of navigation research is restricted to the horizontal plane. Head direction (HD) cells represent the instantaneous orientation of the head. Grid cells represent multiple locations that form a repeating hexagonal grid pattern spanning the environment. In bats, HD cells represent both the horizontal and vertical planes with firing rates being modulated by preferred direction in the azimuth, pitch, and roll of the head. In rats, HD cells represent 3D space by translating their two-dimensional representation from the horizontal to vertical plane. Grid cell studies in 3D have

used apparati that produce continuous vertical displacement, and thus were not able to assess grid patterns within a single horizontal plane displaced along the vertical axis. The current study used female rats to assess grid cell firing across two horizontal planes occupying the same location in the environment, but offset in the vertical plane. Once a grid cell was isolated, rats were recorded in a grey compartment (lower: 1 x 1 x 0.5 m), followed by a recording on a clear plastic sheet placed over the top of the grey compartment (upper: 1 x 1 m, no walls), and finally again in the grey compartment. Rats were either passively placed in the lower and upper portions, or they walked between the two levels using a ramp (0.7 x 0.1 m) along one wall. Grid cells appeared to maintain the size, spacing, and orientation of nodes across the lower and upper compartments. However, the grid phase was offset between compartments so that there was no overlap between lower and upper level grid nodes, independent of passive or active movement between the compartments. These findings indicate that rats may be calculating their vertical displacement resulting in the two horizontal planes transecting different levels of a 3D grid pattern. Alternatively, the rats could perceive the upper compartment as a novel context resulting in the observed shift in grid patterns.

Disclosures: **S.S. Winter:** None. **M.L. Mehlman:** None. **J.S. Taube:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Rappaport Institute grant

Allen and Jewel Prince Center for Neurode- generative Disorders of the Brain

Title: Performance in a spatial reorientation task is correlated with orientation of grid and head direction cells

Authors: *S. WEISS[1,2], G. TELHAMI[1], X. GOFMAN[1], D. EILAM[2], D. DERDIKMAN[1]; [1]Technion – Israel Inst. of Technol., Haifa, Israel; [2]Zoology, Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: Grid cells and head-direction cells have been hypothesized to guide spatial navigation in rodents. While it is true these cells exhibit stable spatial tuning, the link to behavior has not

been demonstrated in freely moving animals yet. To test if such a link exists, we recorded such cells in a spatial re-orientation task, in which rats were trained to reach a rewarded corner in a rectangular arena (Cheng, 1986). During recording sessions, rats were disoriented on-occasion by being spun on a rotating plate in the center of the arena, and corner choices were logged during recording sessions. We compared the spatial tuning in the random foraging sessions to those of the disorientation task. We found that grid cell and head-direction cell responses to correct choices were significantly correlated with random-foraging responses. In contrast, when the rat made behavioral errors (specifically by running to the opposite "rotational" corner) orientation of grid cells and head-direction cells showed a bi-modal distribution, such that sometimes the cells were aligned to the random-foraging map, and sometimes the cells were aligned to the 180° degrees- rotated version of the same map. These results suggest that when the rat performs the task correctly, its behavior is influenced by the grid-head-direction system, while such an influence is less apparent when the rat is disoriented.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Title: Quantification of head direction drift in rat pups

Authors: *G. TOCKER[1,2], E. BORODACH[2], D. DERDIKMAN[2];
[1]Gonda Multidisciplinary Brain Res. Ctr., Bar- Ilan Univ., Ramat- Gan, Israel; [2]Neurobio. dept., Rappaport Fac. of Med., Technion - Israel Inst. of Technol., Haifa, Israel

Abstract: Mammalian navigation is thought to depend on an internal map of space, consisting of spatially modulated cells in the hippocampus and surrounding regions. One type of such cells are head-direction cells. Studies showed that the response of these cells depends on multiple inputs,

such as vestibular and visual. However, the mechanisms in which the vestibular and visual inputs are integrated into a head-direction signal are unknown. Bjerknes et al. (2014) found that head-direction cells exist without visual input in rat pups before eye opening already at P13, and that their directionality is coherent between cells but unstable. The aim of this study was to quantify head direction drift in these cells. We analyzed previously recorded cells from rat pups before and after eye opening (data from Bjerknes et al., 2014). We first divided each trial into overlapping time-slices and measured the head directionality in each slice. Then, we measured the difference in head direction angle between pairs of slices to measure the accumulation of error over time. We found that the head direction error accumulated according to the following power law: err $\sim t^{08}$, both before eye-opening (P13-14) and after eye-opening (P15-16). The accumulation of drift before eye opening was larger than after eye opening. In adult rats there was almost no drift. We further asked if the environment shape affects the drift. The drift in the square environment was smaller than in the circular environment. These findings suggest that an angular path-integration mechanism, whose error accumulates over time, is combined with a visual input signal, including environmental shape, in order to generate the head-direction cell response.

Disclosures: G. Tocker: None. **E. Borodach:** None. **D. Derdikman:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 359.07/KKK9

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Hippocampal lesions impair place and direction learning in a water plus maze

Authors: *D. M. SKINNER;
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Abstract: We have previously shown that hippocampal lesions disrupt direction as well as place learning on an elevated plus maze (Stringer et al., 2005). However, more recent evidence suggests that directional responding in water maze tasks may persist after hippocampal lesions (Rice et al., 2015) or inactivation (Stackman et al., 2012). To determine whether this discrepancy is due to differences in motivational demands, stress levels, or navigation strategies across water versus dry-land tasks, we assessed the acquisition of direction and place learning in a water plus maze. Hippocampal lesioned and sham control rats were trained to locate a hidden platform in a

water maze located at two positions. Direction rats were required to go in a consistent direction (East or West) to find the platform. Place rats were required to go to a consistent location, relative to room cues, to find the platform. As we previously reported using a dry-land version of the tasks, rats with hippocampal lesions were significantly impaired on both the direction and place problems.

Disclosures: D.M. Skinner: None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 359.08/KKK10

Topic: H.01. Animal Cognition and Behavior

Title: Interactions between vestibular and proximal-distal allothetic frames of reference in an object-place paired associate task

Authors: L. M. SANCHEZ, S. M. THOMPSON, *B. J. CLARK;
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Abstract: Several behavioral and neurobiological investigations have demonstrated that the elements of past experiences, such as distinct representations of "what" and "where", and associations between these elements, can be evaluated in rodent behavioral tasks in which items (what) and places (where) are paired using a bi-conditional association rule. In this procedure, rats are specifically rewarded when selecting object A only when it appears in location 1, but not in location 2. In contrast, object B is rewarded only when it is encountered in location 2, but not in location 1. Thus, rats are required to select a particular object on the basis of where it is encountered in the environment, often referred to as an object-place paired associate. The acquisition and retrieval of object-place paired associates is thought to require a distributed network of brain regions including hippocampal, parahippocampal, limbic thalamus, and prefrontal cortical regions, but the dynamic interactions between these regions during learning and retrieval are poorly understood. Resolving this issue requires an understanding of the precise stimulus sources that contribute to the acquisition and expression of object-place associations. This is further complicated by the fact that animals can determine their place, or where they are in an environment, on the basis of a diverse set of idiothetic (e.g., motor, proprioceptive, vestibular) and allothetic (e.g., vision, tactile, olfaction) stimuli that can operate in parallel or sequentially during behavior. In the present study, we investigated the interactions between vestibular and proximal-distal allothetic stimuli on performance of an object-place paired-associate task by Long-Evans rats (n = 16). The paired-associate task was composed of learning

to discriminate between an identical pair of objects presented in 180° opposite arms of a radial arm maze. Thus, rats were required to select a particular object on the basis of spatial location (i.e., maze arm). After the animals acquired the object-place rule, a series of probe tests determined that rats utilize self-generated vestibular cues to discriminate between the two maze arms. Further, when available, animals showed a strong preference for local proximal cues associated with the maze. Together, the work presented here supports the establishment of an object-place paradigm that requires both idiothetic and allothetic stimulus sources to guide choice behavior. This paradigm will be used in subsequent work to investigate the dynamic interactions between neural systems involved in pairing sensory information with a sense of spatial orientation.

Disclosures: L.M. Sanchez: None. **S.M. Thompson:** None. **B.J. Clark:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

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Program#/Poster#: 359.09/KKK11

Topic: H.01. Animal Cognition and Behavior

Support: NIGMS Grant P30GM103400

Title: Longitudinal assessment of Papez circuit structural change and spatial disorientation in the TgF344-AD transgenic rat model of Alzheimer's disease

Authors: *L. E. BERKOWITZ[1], Y. YANG[2], S. M. THOMPSON[1], E. N. DRAKE[1], E. A. SNEDDON[1], L. O. SILLERUD[2], B. J. CLARK[1];
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia in the United States and is characterized by progressive cognitive decline and the presence of aggregates of amyloid beta and hyperphosphorylated tau. Early diagnosis through cognitive testing is difficult due to comorbidity of symptoms between AD and other types of dementia. As a result, there is a need to identify the unique problems apparent in AD at early stages of the disease. Deficits in spatial orientation are frequently reported in prodromal and early AD, but the precise neural mechanisms underlying these symptoms is poorly understood. In recent work, declines in gray matter volume and white matter integrity have been reported at various stages of AD in Papez circuit regions such as the retrosplenial cortex and presubiculum, both of which are known to be central to spatial processing (i.e., head direction cell activity). Whether impairments in Papez circuitry is predictive of the onset of spatial disorientation in AD is currently unknown and was

examined in the present study. Here we used TgF344-AD rats (n = 16), a comprehensive animal model of AD, and Fischer 344 controls (n = 12) in a longitudinal study that identifies whether white matter integrity and gray matter volume of spatial navigation centers can predict spatial impairments across three time points (4mo, 7mo & 10mo of age). At each age, we obtain T2 weighted and DTI MR images in a 4.7T magnet (Bruker BioSpec) with ROIs centered over key Papez circuit regions involved in spatial and head direction cell processing (retrosplenial cortex, presubiculum, cingulum bundle, and fornix). Further, spatial memory was assessed at each age using Morris Water Maze procedures (hidden platform, matching-to-place, and directional navigation) known to be sensitive to disruptions in Papez circuit head direction cell circuitry. To control for carryover effects, environmental context and spatial test parameters were changed between time points. Our data suggests that at early age points, white matter integrity was similar between groups and no significant spatial memory differences were detected. Ensuing studies will utilize in vivo electrophysiology recordings from Papez circuit head direction cells to determine whether alteration in these neural representations of space are predictive of spatial disorientation and whether treatment interventions can restore deficits in spatial processing. The results of this study will provide significant insight into early circuit-level changes in AD as well as translational methods for identifying these patterns in humans at risk for AD.

Disclosures: L.E. Berkowitz: None. **Y. Yang:** None. **S.M. Thompson:** None. **E.N. Drake:** None. **E.A. Sneddon:** None. **L.O. Sillerud:** None. **B.J. Clark:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIGMS (P30GM103400 and Grice Faculty Research Enhancement Award

Title: Impaired retrieval of spatial memory and response perseveration in a radial arm maze after muscimol inactivation of the anterodorsal thalamus

Authors: *R. E. HARVEY[1], S. M. THOMPSON[1], L. M. SANCHEZ[1], E. A. SNEDDON[1], R. M. YODER[2], B. J. CLARK[1];
[1]Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM; [2]Dept. of Psychology, indiana university, purdue university fort wayne, fort wayne, IN

Abstract: The ability to navigate depends on neural systems involved in tracking an animal's moment-to-moment changes in directional orientation and spatial location when moving from

place to another. Previous studies have shown that the anterodorsal thalamus (AD) contains a large population of head direction cells, which fire as a function of an animal's directional orientation in an environment, thereby providing a neuron-like compass guiding navigation. Recent work has demonstrated that when the AD is damaged animals display poor performance on spatial reference/working memory tasks. However, while studies have confirmed that AD lesions impair the acquisition of new spatial information, few have attempted to dissociate the unique contributions of the AD to the acquisition vs. retrieval of allocentric spatial information. Here, we trained rats in a radial arm maze (RAM) procedure that requires the acquisition of directional trajectories to obtain a reward. Animals were trained to asymptotic levels (mean = 67.9% correct $\pm$ 3.2% SEM; chance performance = 25%). Twenty-four hours after training, animals were administered muscimol inactivation of the AD before a 4 trial probe test. Specific measures across training and probe testing included: latency to complete the task, amount of errors (working and reference memory), and percent of correct arm choices per trial. We found that when the AD was inactivated, RAM latency and reference memory were significantly higher, and the percent correct was significantly lower than control animals. In addition, a large number of working memory and perseverative errors were observed in AD inactivated animals throughout testing, suggesting a general absence of improved navigation across training trials. In contrast to retrieval and working memory deficits, it was observed that inactivated animals could express a non-spatial "cued" behavior, indicating that the impairments were specific to spatial processing. Taken together, the results above suggest that the AD modulates the retrieval of previously acquired allocentric spatial information in a RAM procedure, but also suggests a critical role in the online guidance of accurate spatial behavior. The results are discussed in relation to the anterodorsal thalamo-cortical circuits involved in spatial information processing.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 359.11/KKK13

Topic: H.01. Animal Cognition and Behavior

Title: Directional discrimination in an object-place paired associate memory is impaired after muscimol inactivation of the anterior thalamus

Authors: *S. M. THOMPSON[1], S. S. WINTERS[3], B. J. CLARK[2];
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Abstract: The recollection of previous experiences and events, which include representations of what and where and associations between these elements, has been extensively evaluated in paired procedures such as object-place paired associate (OPPA) tasks. Simply stated, this procedure rewards rodents when selecting an object encountered in a specific location, but not in other locations. Preliminary evidence from our laboratory has shown that representations of directional orientation, based on vestibular cues, facilitate the disambiguation of spatial locations in paired-place associate learning. The precise neural mechanisms underlying directional discrimination in the OPPA is poorly understood, but it is well known that the anterior thalamus contains large populations of head direction cells, which fire as a function of an animals directional orientation. In the present study, we addressed the hypothesis that the anterior thalamus contributes to directional discrimination by inactivating the anterior thalamus with muscimol, a GABA agonist, and measuring subsequent performance in a previously trained OPPA task. Briefly, the task requires that rats learn to discriminate between an identical pair of objects presented in 180° opposite arms of a radial arm maze. Thus, animals were required to select a particular object on the basis of its directional orientation or place (i.e., maze arm) in the room. Twenty-four hours after the rats reached asymptotic performance in the OPPA over the course of 14 days (32 trials per day), animals received either muscimol or saline infusion in the anterior thalamus and were tested in a 32 trial session. Our preliminary results indicate that muscimol inactivation of the anterior thalamus significantly reduced object choice accuracy relative to saline treated rats, but spared performance in a simple object discrimination procedure. These preliminary observations therefore provide confirmatory evidence that the anterior thalamus contributes to directional discrimination in OPPA tasks and supports the notion that subcortical limbic circuits have a fundamental role in the recollection of previous events. This will allow us to investigate the subcortical underpinnings of Alzheimer's disease and increase the early detection of dementia in clinical behavioral assessments.

Disclosures: S.M. Thompson: None. **S.S. Winters:** None. **B.J. Clark:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC BB/L000040/1

Title: Repetition of place cell fields depends on the head direction system

Authors: E. R. WOOD[1], B. HARLAND[2], R. GRIEVES[2], R. STENTIFORD[1], D. BETT[2], *P. A. DUDCHENKO[2];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Univ. Stirling, Stirling, United Kingdom

Abstract: Previous work by Spiers et al. (2015) has shown the place cells in the hippocampus exhibit similar fields within each chamber of a multicompartment environment. Our work demonstrated that this effect occurs when the chambers face the same direction, but does not occur (at least to the same extent) when the chambers face different directions (Grieves et al., 2016). This finding implies that place cells use a directional input to disambiguate the chambers. To test whether the head direction cell system is the source of this input, we produced ibotenic acid lesions of the lateral mammillary nuclei (LMN) in nine Lister Hooded rats. The LMN is likely a critical node in the head direction circuit for upstream structures. The lesioned animals were compared to control animals (n = 6) who underwent a sham surgery. Both groups were equipped with tetrodes in the dorsal CA1 cell layer of the hippocampus. Upon recovery from surgery, all rats were screened for place cells. When such cells were encountered, recordings were conducted in an environment with four identical compartments, connected to one another with a hallway. These compartments could be arranged such that they were parallel to one another, or such that they faced different directions from one another (radial arrangement). In the control animals, we observed robust repetition of place fields across parallel compartments, but not across radially arranged compartments, as previously reported. Animals with LMN lesions exhibited clear place fields, and these showed robust repetition in both the parallel and radial configurations of the compartments. This suggests that the head direction cell system underlies the ability of place cells to disambiguate local environments facing different directions. In a related experiment, we also assessed the effects of LMN lesions on grid cell firing in the medial entorhinal cortex. Our preliminary results indicate that grid fields are no longer observed following removal of the head direction cell input, consistent with the findings of Winter et al. (2015).

Disclosures: E.R. Wood: None. **B. Harland:** None. **R. Grieves:** None. **R. Stentiford:** None. **D. Bett:** None. **P.A. Dudchenko:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01 MH60013

NIMH R01 MH61492

Title: Representation of environmental boundaries within an egocentric reference frame

Authors: *J. R. HINMAN, G. W. CHAPMAN, IV, M. E. HASSELMO;
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Abstract: The hippocampal formation maintains an allocentric representation of space composed of grid, head direction and border cells within medial entorhinal cortex and place and boundary vector cells within the hippocampus. This world-centered spatial map has been proposed to be critical for the ability to navigate locally within an environment, as well as across longer distances beyond the scale of even the largest spaced grid cells (Bush et al., 2015). Yet it has been suggested that the allocentric translation vectors generated in the hippocampal formation for the purpose of navigating to a distant goal need to be transformed into an egocentric reference frame for action selection (Byrne et al., 2007). Consistent with this functional requirement, we have identified a new spatial representation in the striatum that operates within an egocentric reference frame. Rats were implanted with hyperdrives targeting the striatum and recordings were obtained while rats foraged within an open field. Cells were identified that coded for the animals' heading orientation and distance relative to the boundaries of the environment. We have termed these cells egocentric boundary cells (EBCs). Egocentric boundary cells fire when the animal is heading with a specific orientation and distance to any of the boundaries of the environment. For example, a specific cell responds during movement parallel to any boundary on the animal's right at a distance of 0 - 10 cm. This contrasts with border and boundary vector cells in the hippocampal formation that are allocentric in response and usually fire along one or two walls of an environment. Across the population of EBCs all orientations relative to the animal are represented, as well as at a variety of distances. In order to confirm that EBCs respond to boundaries in general, recordings were obtained while animals ran on an elevated table top where EBCs respond similarly to the drop off as they do to the walls of an open field. When recorded in a novel open field EBCs respond immediately and identically to their response in a familiar open field, suggesting that EBCs maintain a stable egocentric representation of environmental boundaries across different environments. Overall, EBCs may be part of an egocentric spatial representation necessary for implementing allocentric navigational translation vectors provided by the hippocampal formation. References Bush D, Barry C, Manson D, Burgess N (2015) Using grid cells for navigation. *Neuron* 87(3): 507-20. Byrne P, Becker S, Burgess N (2007) Remembering the past and imagining the future: a neural model of spatial memory and imagery. *Psychol Rev* 114(2): 340-75.

Disclosures: J.R. Hinman: None. **G.W. Chapman:** None. **M.E. Hasselmo:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Interdisciplinary Biosciences PhD Consortium

Title: Involvement of the head direction system in discrimination of visually ambiguous spaces

Authors: *D. W.-U. OVERINGTON, P.-Y. JACOB, K. JEFFERY;
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Abstract: Place cells (PCs) are hippocampal neurons that fire when an animal is in a particular place in the environment, producing focal activity known as a place field. Head direction cells (HDs) fire when an animal faces a particular direction in the environment, providing the 'sense of direction' complementary to the positional coding of PCs. Together, these spatially modulated neurons are thought to form part of the brain's internal cognitive map, allowing flexible and efficient navigation through the external world. These cells use both self-motion and visual landmark information to update their spatial activity, and form an accurate representation of space. Sometimes, the spatial meaning of a landmark can be ambiguous *e.g.* when it can be approached from different directions (for example, a tree on the border of two fields). In such cases, context information (such as odour, colour or texture) can provide clues to separate one environment from another and thus enable use of the landmark. The present study investigated whether PCs and HDs can use odour-context information to resolve visual landmark ambiguity, in this case in order to solve a spatial task across multi-compartment space.
We performed experiments in a 'context box' - two visually identical but oppositely oriented rectangular compartments connected by a doorway. One compartment had a lemon odour and a white cue card placed on the 'North' wall, while the other had a vanilla odour and an identical white cue card on the 'South' wall.
We first tested whether rodents can behaviourally encode relative position of identical objects in each compartment. We observed increased exploration in response to spatial displacement of one object, reflecting an ability not just to recognise object location within a compartment, but also to discriminate the two compartments based on odour information. Electrophysiological recordings of hippocampal PCs and anterior thalamus HDs confirmed that both cell types can use odour-context information to discriminate these visually ambiguous spaces; therefore, we tested potential involvement of the HD system in the spatial displacement task by temporarily inactivating the anterior thalamus with an awake muscimol infusion. Preliminary data shows an impairment in task performance in infusion animals compared to control and sham, suggesting that the formation of an 'object-location-odour' representation uses the head direction system.

Overall, these results indicate that rodents can use odour-context information to inform landmark use in otherwise identical multi-compartment environments, and suggest that the head direction system is involved in this process.

Disclosures: **D.W. Overington:** None. **P. Jacob:** None. **K. Jeffery:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

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Program#/Poster#: 359.15/KKK17

Topic: H.01. Animal Cognition and Behavior

Title: A hypothesis for path integration of orientation via oscillatory inhibition

Authors: ***C. KIRST**, J. GREEN, G. MAIMON;
Rockefeller Univ., New York, NY

Abstract: Navigating in complex environments is crucial for many species. Signals coding for the animals' movements have to be integrated in a reliable way via a discrete set of neurons, if the animal is to build an internal representation of its position and orientation in space. Attractor models based on a large number, or even a continuum, of neurons have been shown to effectively solve such tasks.
However, neuronal circuits, for example in the fly, have been identified that integrate the animal's orientation even with a small number of neurons. For circuits with few elements classical ring attractor models will require tuning and strong signals to steer the activity patterns encoding orientation.
Here we describe a hypothetical mechanism for the integration of orientation based on global oscillatory inhibition. In each oscillation within this model, when inhibition is strong the system selects highly robust and localized activity patterns, whereas when inhibition is low, a series of bifurcations allows the activity pattern to be actively moved. These two modes act akin to ratchet dynamics to integrate orientation. More generically, these dynamics provide a basic mechanism for integration on discrete topologies. Future work should test this model in integration-related circuits across animals.

Disclosures: **C. Kirst:** None. **J. Green:** None. **G. Maimon:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: NRF Grant 2013-R1A1A2053280

Title: Learning head directional information by spike timing-dependent plasticity enhances the boundary avoidance in neuromorphic navigational model

Authors: *S. KIM, J. KWAG;
Korea Univ., Seoul, Korea, Korea, Republic of

Abstract: Hippocampal neural circuit is known to contain neurons specialized in processing boundaries in an environment and an animal's head direction called boundary vector cell (BVC) and head direction cell (HDC), respectively. Previous neuromorphic navigational models based on BVC and HDC found that including spike timing-dependent plasticity (STDP) in the neural network enhanced boundary avoidance. However, it is unclear which specific synaptic connections undergoing STDP involving BVC and/or HDC are crucial for boundary avoidance. Therefore, we set out to investigate the effect of STDP on different synaptic connections on the boundary avoidance.
Our neuromorphic navigational model consisted of four BVCs, six HDCs and two motor neurons (MNs) as Hodgkin-Huxley neurons. The BVCs received excitatory inputs from HDCs, and MNs received excitatory inputs from BVCs and HDCs. Each BVC was modeled to spike at boundary of each cardinal direction and each HDC was modeled to spike at heading directions separated by 60°. The synaptic strength was modeled to be changed through STDP where presynaptic spike followed by postsynaptic spike increased the synaptic weight while postsynaptic spike followed by presynaptic spike decreased the synaptic weight. A virtual rat was controlled by navigational model for 10 minutes (repeated 10 times) and boundary avoidance efficiency was quantified as the number of collision in early phase (NC_{early}, 0-2 min) and late phase (NC_{late}, 8-10 min) during simulation. Statistical significance was tested using Student's *t*-test.
First, when we performed the simulation without STDP as a control condition, there was no significant difference between NC_{early} and NC_{late} ($NC_{early} = 129 \pm 14$, $NC_{late} = 133 \pm 11$, $p = 0.33$). In the presence of STDP at all three BVC-MN, HDC-MN and HDC-BVC synapses, NC_{late} was significantly decreased compare to NC_{early} ($NC_{early} = 169 \pm 38$, $NC_{late} = 116 \pm 38$, $p < 0.01$), suggesting that STDP enhanced boundary avoidance. To investigate which synaptic connection was mostly involved in boundary avoidance, we applied STDP either at BVC-MN or HDC-MN synapse. When STDP was only applied at BVC-MN synapse, boundary avoidance efficiency was not significantly different between NC_{early} and NC_{late} ($NC_{early} = 133 \pm 25$, $NC_{late} = 130 \pm 22$,

$p = 0.70$). In contrast, when STDP was only applied to HDC-MN synapse, NC_{late} significantly decreased compared to NC_{early} ($NC_{early} = 139 \pm 22$, $NC_{late} = 113 \pm 11$, $p < 0.001$). These results suggest that STDP at HDC-MN synapse is crucial in the boundary avoidance efficiency, indicating that learning of head directional information may play a major role in boundary avoidance, which will be an interesting hypotheses to test *in vivo*.

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

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 359.17/KKK19

Topic: H.01. Animal Cognition and Behavior

Title: Gravity tuning in mice head direction cells

Authors: D. ANGELAKI, H. CHAM, M. SHINDER, *J. DICKMAN, J. LAURENS;
Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Head Direction (HD) cells are thought to form a "neuronal compass" that encodes head orientation in the animal's plane of locomotion. A recent study in bats has shown that HD cells in these flying mammals are tuned in three-dimensional (3D) space and signal the angular orientation of the head in pitch and roll, in addition to horizontal, planes. Furthermore, we recently identified pitch- and roll-sensitive cells in the Anterior Thalamus of Macaque monkeys that are tuned to gravity. However, we were unable to asses conclusively whether these primate neurons were HD cells since our responses were obtained in head and body restrained conditions.
Whether 3D tuning also exists in the HD system of rodents remains unknown.
Here, we recorded HD cells in the anterior thalamus of mice. 27 HD cells were identified when animals walked freely in a circular enclosure. Animals were then installed, head-fixed, in a three-axis motorized rotator within a visual environment and were rotated along the yaw, pitch and roll axes (30°/s velocity), in light or in darkness. In a subset of cells, we systematically measured the gravity tuning field by tilting the animal along 200 combinations of pitch and roll covering the whole range of possible head tilt uniformly.
Of the 27 HD cells 9 exhibited significant tuning ($p<0.01$, bootstrap) during passive yaw rotation, and these responses were weak (mean resultant vector $R = 0.13$). In contrast, 21 and 20 cells exhibited significant response to pitch and roll rotation (mean $R = 0.32$ and 0.2 respectively). In agreement with previous findings, neuronal responses were significantly higher ($p = 0.0004$) during pitch than roll. A fraction of cells presented different responses depending

on rotation direction, indicating that they may respond to the derivative of gravity.
Systematic recordings of the gravity tuning field in 15 cells revealed that most (12 cells) presented hill-shaped tuning, the firing rate being maximal at one preferred head orientation relative to gravity and decreasing smoothly away from this orientation. Three cells presented two distinct response peaks. Altogether, preferred directions were distributed across the entire range of head tilts.
These findings indicate that mice HD cells present pitch and roll tuning similar to the responses described in bats and monkeys. HD responses were suppressed in restrained mice, whereas gravity responses could be measured Using the full range of possible tilts, we were able to characterize the complete gravity tuning curves of HD cells.

Disclosures: D. Angelaki: None. **H. Cham:** None. **M. Shinder:** None. **J. Dickman:** None. **J. Laurens:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Ring attractor dynamics in the *Drosophila* central complex

Authors: *S. KIM[1], H. ROUAULT[1], J. D. SEELIG[1,2], S. DRUCKMANN[1], V. JAYARAMAN[1]; [1]Janelia Res. Campus / HHMI, Ashburn, VA; [2]research center caesar / Max Planck Society, Bonn, Germany

Abstract: A recent study used two-photon calcium imaging in the central brain of head-fixed behaving flies to study the activity of a set of neurons innervating the ellipsoid body (EB), a torus-shaped brain structure. These neurons, called wedge neurons because their dendrites each innervate a single wedge of the EB, collectively display bump-like localized activity whose position on the torus follows the fly's orientation in the presence of landmarks or in darkness, reminiscent of the population dynamics postulated for mammalian head direction (HD) cells. Models of compass-like HD cell activity typically use networks called ring attractors, which assume specific patterns of recurrent connectivity between HD cells based on their tuning preferences. Selectively perturbing the activity of HD cells with specific tuning and assessing the consequences on the population would allow key features of such models to be tested. However, the lack of obvious tuning-based topography in the HD cell population, and fact that mammalian

HD experiments can typically sample only a small fraction of neurons makes such tests impractical. The tiling of *Drosophila* wedge neuron dendrites in the EB, in which orientation preferences vary continuously around the torus, provides an opportunity to overcome such limitations. We used genetically targeted two-photon calcium imaging and optogenetics to simultaneously observe and manipulate the activity of the entire wedge neuron population. This approach allowed us to directly test predictions of theoretical ring attractor models. We first perturbed selected subsets of wedge neurons using optogenetics to show that recurrent interactions, but not simple feedforward dynamics, can account for bump-like activity. We then tested two classes of ring attractor models with distinct connectivity, each predicting a different population response to a visual stimulus that abruptly changes its position. We found that a classical ring attractor model, in which all synaptic weights are broadly tuned according to the angular distance between neurons in the ring, cannot explain the dynamics of the bump, which jumped to the matching position on the torus in response to the visual position change. On the other hand, a model with local recurrent excitation and global uniform inhibition can. Finally, we theoretically analyzed the space of models to further constrain the locality of excitatory recurrent connections. This approach, which combines genetic manipulation, physiological recording, and theoretical analysis, shows how flies can contribute to uncovering the mechanistic basis of network dynamics that are thought to underlie cognitive computations.

Disclosures: **S. Kim:** None. **H. Rouault:** None. **J.D. Seelig:** None. **S. Druckmann:** None. **V. Jayaraman:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 359.19/KKK21

Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: How to move a compass needle: angular velocity integration in the *Drosophila* central complex

Authors: D. B. TURNER-EVANS[1], J. D. SEELIG[1,2], S. WEGENER[1], H. ROUAULT[1], S. KIM[1], R. FRANCONVILLE[1], C. DAN[1], H. HABERKERN[1], Y. SUN[1], T. WOLFF[1], S. DRUCKMANN[1], *V. JAYARAMAN[1];
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Abstract: A recent study used two-photon calcium imaging to identify a neural population in the *Drosophila* central complex (CX) that exhibits compass-like dynamics reminiscent of mammalian head direction (HD) cells. The dendritic activity of the population of wedge neurons —so named because their dendrites innervate single wedges arranged around a donut-shaped CX substructure called the ellipsoid body (EB)— is localized in a bump that moves around the EB and tracks the fly's heading in darkness using self-motion cues (Seelig & Jayaraman, 2015). Here we explored how the fly's turning velocity is integrated to update this heading representation.

We used anatomical information to identify tile neurons, whose axons project to single EB tiles (spanning about two wedges), as the likeliest source of turning velocity input to the wedge neurons (Wolff et al., 2015). Both wedge and tile neurons link sectors of the EB to matching columns on one or the other side of a different, bilaterally symmetric CX substructure —the handlebar-shaped protocerebral bridge (PB)— but are opposite in polarity. Tile neurons whose dendrites share a PB column with axons from specific wedge neurons send their own axons to EB tiles neighboring the wedges innervated by their PB partners. This loop-with-a-shift bears a remarkable resemblance to early theoretical models of angular velocity integration in HD cells (Skaggs et al., 1995; Xie et al., 2002): Tile neurons from one side of the PB shift from their wedge neuron partners in the clockwise direction in the EB and those from the other side shift counterclockwise, providing a potential mechanism for converting asymmetric activation of the PB into movement of the EB compass needle —the bump. Calcium imaging and whole-cell patch clamp recordings showed asymmetric activation of tile neurons in the PB during turning. Functional connectivity experiments and dual color imaging provided further evidence that this PB asymmetry drives EB wedge neuron bump movement. Optogenetic activation of tile neurons produced deviations in walking trajectories, consistent with disruptions of the internal compass. Finally, we propose a firing rate model that accounts for these activity dynamics. We used the model to explore circuit properties that would allow angular velocity to be linearly integrated, a feature important for faithful heading computation in the absence of external cues, but lacking in some classical models. Analytical treatment and numerical simulation show that these properties hold for our anatomically constrained models. Overall, these results represent a remarkable example of structure predicting function for a broadly relevant navigational computation.

Disclosures: D.B. Turner-Evans: None. **J.D. Seelig:** None. **S. Wegener:** None. **H. Rouault:** None. **S. Kim:** None. **R. Franconville:** None. **C. Dan:** None. **H. Haberkern:** None. **Y. Sun:** None. **T. Wolff:** None. **S. Druckmann:** None. **V. Jayaraman:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Visual inputs to a neural representation of heading in *Drosophila*

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Abstract: Animals integrate external sensory cues with self-motion and internal state information to maintain their bearings in their surroundings. In *Drosophila,* such sensorimotor integration has been linked to a region of the brain termed the central complex (CX). This region, consisting of a few thousand neurons, is associated with visuomotor behaviors including spatial orientation memory and visual place learning (reviewed in Turner-Evans and Jayaraman, 2016). In recent experiments performed in tethered flies walking in a virtual reality arena on an air-supported ball (Seelig & Jayaraman, 2015), two-photon imaging was used to monitor the calcium activity of so-called wedge neurons whose dendrites tile a toroidal shaped substructure of the CX, the ellipsoid body (EB). In experiments in which the fly's rotation on the ball controlled the angular rotation of its visual environment (i.e., in closed loop), imaging revealed a spatially localized rotating bump of activity in the EB. This bump moved in a clockwise or counterclockwise direction around the EB, tracking the tethered fly's heading in darkness and in visual surroundings.

Here, we explored the relevance of different visual landmark and motion cues to the maintenance and updating of this abstract internal representation of heading. We began by testing the effects of optic flow alone on the bump motion by presenting the fly with sinusoidal gratings and correlated white noise. Although these optic flow cues surrounded the fly and moved in concert with the fly's rotations on the ball, the internal heading representation was not reliable, building up error over time. In contrast, the bump of activity faithfully tracked the fly's heading when the visual environment only contained a single vertical stripe. When we used thermogenetics to selectively block optic flow responsive neurons in the visual system, the fly's ability to respond to flow cues was diminished, but the heading representation still reliably tracked the vertical bar. Thus, the EB representation of heading appears to rely on visual features in the environment rather than integrating visual motion information.

In ongoing experiments, we are now exploring the competing effects of local and global visual features on the heading representation by putting the fly in a two-dimensional virtual world. By

dissecting the relevant visual inputs for heading representation in the CX, we hope to shed some light on broadly relevant neural computations underlying visually guided navigation.

Disclosures: D. Turner-Evans: None. **V. Jayaraman:** None. **H. Haberkern:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Landmark-guided navigation in a 2D virtual reality environment

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Abstract: Navigating mammals are believed to use abstract internal representations of space, such as head-direction cells, place cells and grid cells. However, we do not yet have a good mechanistic understanding of how these representations are generated, nor of how exactly they are used to guide navigation. In insects, a conserved brain neuropil called the central complex (CX) is involved in various navigational behaviors (for example, Neuser et al., 2008; Ofstad, Zuker & Reiser, 2011). Recent calcium imaging experiments in the CX of behaving *Drosophila* found an internal compass (Seelig & Jayaraman, 2015), which is updated using self-motion cues as well as salient visual landmarks. Our goal is to use the fly's powerful genetic tools to understand, on a behavioral and circuit level, computations performed during visually guided navigation in a two-dimensional environment. We would like to understand how innate preferences and experience shape this process.
Current two-photon calcium imaging and electrophysiological techniques to study neural circuits in behaving flies require the animal to be head-fixed. We therefore developed a virtual reality (VR) setup, which enables us to study visually guided navigation in head-fixed flies walking on an air-supported trackball. Using this VR system we study how flies use visual landmarks in a variety of contexts.
We validated that naïve head-fixed flies track prominent visual landmarks and interact with them in our VR system in much the same way that freely walking flies interact with real objects. We then paired sensory stimuli of other modalities with the visual environment to imbue specific

objects with negative valence. By coupling, for example, the spatially restricted activation of heat sensing neurons to the visual environment, we found that flies avoid virtually hot areas in the VR. We are now in a position to examine whether and how flies alter their naïve preferences for specific visual landmarks in VR based on such virtual negative reinforcement.

Disclosures: **H.J. Haberkern:** None. **C. Bruns:** None. **M. Basnak:** None. **B. Ahanonu:** None. **M. Bolstad:** None. **J. Cohen:** None. **V. Jayaraman:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Exploring the neural circuit basis of visual learning in *Drosophila*

Authors: *C. DAN, T. WOLFF, Y. ASO, G. RUBIN, V. JAYARAMAN;
Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Many animal species, including *Drosophila melanogaster*, can effectively navigate their visual environment to search for food, find conspecifics, and avoid predators. It is also critical to their survival to be able to adjust their navigational strategies based on past experience, for example, by remembering the negative or positive consequences of being in specific visual surroundings. However, the neural circuit mechanisms that enable animals to make such associations and thereafter modify their behavior are not yet fully understood. *Drosophila melanogaster* has been shown to associate visual patterns with a heat punishment in a flight simulator, remember the location of a cool spot in an otherwise hot environment using visual place learning, and associate color or light intensity with a reward or punishment. To investigate the circuit mechanisms of adaptive visual navigation, we developed a system in which a head-fixed flying fruit fly can orient within a virtual visual surround through closed-loop control. We have also established a protocol for the fly to associate visual scenes (conditioned stimulus, CS) with a heat punishment (unconditioned stimulus, US) delivered by an infrared laser pointed at its back, while we monitor the activity of specific neural populations using two-photon calcium imaging. We are now examining the neuromodulatory responses to the US both during open-loop stimulation and closed-loop visual training, as well as responses in identified visually responsive neurons in the central complex before, during and after training. We hope to use this

setup to understand how moment-to-moment correlations between visual, neural and behavioral spaces alter the fly's neural dynamics and its selection of actions.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Dual-color imaging reveals transformation of visual representations along an input pathway to the central complex

Authors: *Y. SUN, A. NERN, H. DANA, J. P. HASSEMAN, G. TSEGAYE, G. HOLT, E. R. SCHREITER, L. L. LOOGER, K. SVOBODA, M. B. REISER, G. M. RUBIN, D. S. KIM, V. JAYARAMAN;
HHMI Janelia Res. Campus, Ashburn, VA

Abstract: A major limitation in using calcium imaging to study the transformation of information across synapses is that neural processes belonging to different cell types often overlap spatially in densely innervated regions of interest. This makes it difficult to assign recorded calcium activity to one or the other of pre- and post-synaptic neural populations. This problem is particularly severe in cases where the regions of interaction are not stereotyped enough in anatomy or function to permit characterization by a comparison of pre- and post-synaptic activity across different animals. Here we demonstrate how selectively expressing green genetically encoded calcium indicators (GECIs) (1) in one population and red GECIs (2) in another can help track the transformation of stimulus information across successive layers of a visual pathway into the central complex in *Drosophila*.
The insect central complex has been implicated in a variety of visually guided behaviors (3). We first used two-photon photo-activation of PA-GFP to map a multi-stage pathway into the central complex from the optic lobe. This pathway brings visual information into the central complex by connecting the medulla (ME) of the optic lobe through the anterior optic tubercle (AOTU) and the bulb (BU, also known as lateral triangle) to the rings of the ellipsoid body (4,5) in the central complex. We used two-photon imaging with GCaMP6 and jRGECO1 to study progressive changes in visual feature representations of different genetically targeted neural populations at

the AOTU and BU. By comparing putative pre- and post-synaptic responses within well-defined neuropil sub-regions (glomeruli) in these structures, we show how each stage of the pathway contributes to increased visual stimulus selectivity that may aid the fly's navigational decisions.
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(4) Homberg (2014). Organization and functional roles of the central complex in the insect brain. Annual Review of Entomology.
(5) Seelig & Jayaraman (2013). Feature detection and orientation tuning in the *Drosophila* central complex. Nature.

Disclosures: **Y. Sun:** None. **A. Nern:** None. **H. Dana:** None. **J.P. Hasseman:** None. **G. Tsegaye:** None. **G. Holt:** None. **E.R. Schreiter:** None. **L.L. Looger:** None. **K. Svoboda:** None. **M.B. Reiser:** None. **G.M. Rubin:** None. **D.S. Kim:** None. **V. Jayaraman:** None.

Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Sparse reconstruction of recurrent networks within the *Drosophila* central complex

Authors: *S. ALI, R. FRANCONVILLE, S. WEGENER, C. PETERSON, S. TRAN, A. SHERIDAN, B. QU, E. NIELSON, T. WOLFF, J. S. LAURITZEN, D. BOCK, V. JAYARAMAN;
Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: The central complex (CX) is a highly conserved brain region implicated in adaptive sensorimotor integration and spatial navigation in insects. The region comprises several stereotypically organized structures, including the protocerebral bridge (PB), fan-shaped body (FB), ellipsoid body (EB), and the paired noduli (No). In the fly, genetic tools allow specific experimental access to dozens of different cell types that connect the different CX structures. Physiological recordings from populations of these neurons have revealed compass-like neural dynamics in the CX, including a localized "bump" of persistent activity in the donut-shaped EB

tracking the fly's orientation in visual surroundings and in darkness. Thus, the CX offers an opportunity to understand the mechanistic basis of broadly relevant navigational computations in the brain of a genetic model organism. A key piece of this puzzle is identifying structural connectivity motifs in the CX that could underlie the generation, maintenance and propagation of compass-like activity. Here, we discuss our results from tracing and reconstruction of CX neurons from high-throughput transmission electron microscopy of serial thin-sections of a complete fly brain. We focused in particular on PB, EB and No neuron types that we believed — based on light-level anatomical work, optogenetic perturbation and functional imaging in behaving flies— to be part of recurrent CX circuits involved in generating compass-like neural dynamics. In particular, multiple types of columnar neurons with opposing polarity appear to recurrently connect specific wedges of the donut-shaped EB and columns of the handlebar-shaped PB. We successfully identified and fully traced several such PB-EB neurons and mapped their synaptic connections in the EB and PB. Although we did find some of the expected connections between these different types of columnar neurons, we also uncovered unexpectedly dense reciprocal connectivity in the EB. Connectivity in the PB was more complex still, with wide-field interneurons connecting different columns within the structure. We will discuss our quantitative analysis of the reconstructed neurons and synaptic connections. Finally, we hope to provide a summary of structural motifs that might provide a concrete anatomical basis for models of how the CX represents the fly's heading.

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Poster

359. Spatial Navigation: Head Direction Cells and Spatial Orientation

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Topic: H.01. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: A functional connectivity atlas of the fly central complex

Authors: *R. FRANCONVILLE, C. BERON, S. NAMIKI, T. WOLFF, V. JAYARAMAN; Janelia Res. Campus, Ashburn, VA

Abstract: The insect central complex offers an excellent opportunity for mechanistic investigations of high-level sensorimotor processing: it has been implicated in a variety of

navigational behaviors (1), contains abstract internal representations of orientation (2), and has stereotypically organized and genetically tractable neuroanatomy (3). Identifying the circuit basis of navigational representations nevertheless requires knowing the nature of the connections between the region's dozens of cell types.
Using the detailed anatomical data available from light-level imaging of central complex neuron classes (4), we identified over a hundred potential synaptic pairs between cell types of the central complex and its accessory structures. We then tested the functional reality of each of those potential connections by activating the presynaptic population with CsChrimson (5) while recording the postsynaptic neurons with GCaMP6m (6) using a standardized two-photon imaging protocol. We also performed experiments to control for the specificity of responses and genetic drivers, and validated putative excitatory or inhibitory functional connections with appropriate pharmacological experiments.
These experiments confirmed a number of connections that were suspected to exist, but also found more sparseness than the light-level anatomy would suggest. We are in the process of comparing our functional connectivity results to ongoing electron-microscopy-based tracing efforts in the same brain region. Together, these data help constrain models of the function of the central complex and will guide future experimental design. Finally, we plan to make this dataset publicly available as an evolving source of information for the central complex community.
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(5) Klapoetke et al. (2014). Independent optical excitation of distinct neural populations. Nature Methods.
(6) Chen et al. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature.

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Poster

360. Perception and Imagery: Visual Processing

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Topic: H.02. Human Cognition and Behavior

Support: the National Nature Science Foundation of China Grants to H. L. (31522027, 31571115)

Title: Concurrent tracking of global and local processing using MEG

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Abstract: Visual scenes contain local and global properties. Numerous behavioral studies demonstrate that the global features are processed more rapidly than local features, namely global precedence effect. Specifically, the holistic structure of visual scene is extracted before fine details are analyzed, known as a coarse-to-fine model in visual perception. However, the neuronal temporal dynamics underlying the global and local processing as well as their interactions remains largely unknown.
To address the issue, we recorded magnetoencephalography (MEG) from 20 human subjects as they viewed 5-sec long glass pattern stimuli, the luminance of which was randomly modulated continuously. Critically, the form coherence of the glass pattern was also randomly and independently modulated simultaneously. Based on the two random sequences (luminance and form coherence) within one trial, we were able to calculate and separate the brain response that specifically tracks changes in local (luminance) and global (global form) property from the same MEG responses, by employing a temporal response function technique (TRF).
The TRF for local and global property processing showed quite distinct spatiotemporal patterns. Specifically, local feature changes elicited activations in early visual area (EVA), including V1, V2, and V3, around 100 msec. Global features, on the other hand, elicited much earlier responses (< 50 msec), which first developed in orbitofrontal area (OFA), then in V3A, V1, and TPJ. We further examined the interactions between global and local processing and found a two-stage course. In particular, within 100-200 msec, the OFA activations in global processing negatively correlated with EVA activities in local processing, suggesting a global-local competition relationship, whereas within 200-300 msec, OFA and TPJ responses in global processing and EVA responses in local processing showed a positive relationship, implicating a global-local integration process.
Our experiments, by employing a TRF technique, successfully enables the dissociation of global and local processing within the same trial response. Commensurate with previous findings, our results support that OFA initiates the coarse-to-fine process in visual perception. Furthermore, the global and local process showed interesting dynamic relationships, undergoing competition (100-200 msec) followed by integration (200-300 msec).

Disclosures: **L. Liu:** None. **H. Luo:** None.

Poster

360. Perception and Imagery: Visual Processing

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Topic: H.02. Human Cognition and Behavior

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The Max Planck Society, Germany

Title: A generic mechanism for Gestalt and high-level stimulus interpretation in the human brain

Authors: *P. R. GRASSI[1,2,3], N. ZARETSKAYA[1,2,3], A. BARTELS[1,2,3];
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Abstract: A common denominator in all vision tasks is scene segmentation: what is fore- and background, which visual components belong to the same or different entities? In prior studies we used a bi-stable stimulus that can either be perceived as separate local components or as a global Gestalt. fMRI and TMS showed that posterior parietal cortex (PPC) was selectively and causally involved in global Gestalt perception (Grassi et al., 2016; Zaretskaya et al., 2013). Here we employed three additional such local vs. global bi-stable stimuli. Importantly, we found that apart from the classification of the two possible percepts into local (ungrouped, component) versus global (grouped, Gestalt), the two possible perceptual interpretations can alternatively be classified according to a second dimension: the complexity or sophistication of the interpretation, i.e. default (simple) versus non-default (complex, high-level, sophisticated). As these two dimensions overlapped differentially across the four stimulus classes (for two stimuli, global coincided with complex, in another two with simple), we were able to identify whether parietal cortex involvement reflected grouping, or the complexity of the perceptual interpretation. We found that the involvement of parietal cortex reflected the level of sophistication of the visual interpretation rather than grouping into a single Gestalt. For all four stimuli, we found activity pattern that was highly similar for the contrast of default (simple) vs. non-default (complex, sophisticated) perceptual interpretations. It consistently and prominently involved posterior parietal cortex. Also consistent with previous findings, we found for all stimuli strong early visual cortex deactivations during *sophisticated* perceptual interpretations. Mid-level regions such as LOC or motion regions were differentially involved with each stimulus class and percept-type. Our results lead us to suggest that PPC is not necessarily involved in mere

grouping toward global Gestalt, but instead more generally it is involved in generating the more complex, high-level, or more sophisticated perceptual interpretation of a given stimulus. The activation of high-level dorsal areas (PPC) and the concurrent deactivation of early visual areas during high-level perceptual interpretations is in line with predictions from generative models of visual perception, also known as predictive coding theory, but not with attention. Our findings suggest a generic mechanism for scene segmentation with the PPC as its anatomical substrate.

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Poster

360. Perception and Imagery: Visual Processing

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Title: Externally applied noise influences state-switching dynamics of binocular rivalry

Authors: *O. L. VAN DER GROEN, N. WENDEROTH;
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Abstract: Random noise can enhance the detectability of weak signals in nonlinear systems, a phenomenon known as stochastic resonance (SR). The concept of stochastic resonance is not only applicable to stable systems but can also be applied to dynamical systems with multiple attractor states. Binocular rivalry can be characterized by three marginally stable attractor states, i.e. three possible percepts: 1. Left-eye image dominance, 2. Right-eye image dominance, 3. Combination of the left and right-eye image. The brain switches between these states in a spontaneous, stochastic manner. The switches are thought to be driven by a combination of neuronal adaptation and noise within the system. In a series of 2 experiments we presented a left tilted visual grating to one eye and a right tilted grating to the other eye using a mirror stereoscope. Subjects continuously reported which image they perceived (left or right tilted) or whether they perceived a mixed percept. We influenced the amount of visual noise either by adding noise (zero-mean Gaussian white noise, individualised contrast) to the visual stimulus (Exp. 1) or by applying transcranial Random Noise Stimulation (tRNS 1mA, 100-640 Hz zero-mean Gaussian white noise) to induce electrical noise directly in visual cortex (Exp. 2) while participants perform the binocular rivalry paradigm. More specifically, the visual or tRNS noise was applied for 5 seconds followed by ± 5 seconds of no noise stimulation. Our results demonstrate a significant reduction in the mean mixed percept duration in Exp. 1 when the noise

is added directly to the visual stimulus (paired t-test: t (9) = 2.712, $p = 0.02$). Interestingly we observed a similar effects when we added noise to the visual cortex with tRNS (paired t-test; t(15) = 2.353, $p = 0.03$). Additional control experiments revealed that this effect was only present for low-contrast but not for high-contrast visual stimuli. Thus it is likely that the effect of noise is small relative to the effect of adaptation and therefore only observable for low contrast gratings. Our results demonstrate that both central and peripheral noise can influence state-switching dynamics of binocular rivalry under specific conditions (e.g .low visual contrast grating).

Disclosures: O.L. Van Der Groen: None. **N. Wenderoth:** None.

Poster

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Support: NSF DGE-1069104

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Title: Changes of alpha oscillations during binocular rivalry reflect neural competition

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Abstract: When two conflicting images are presented separately, one to each eye, perception typically alternates between the two images. This phenomenon is called binocular rivalry. The mechanisms of rivalry are extensively studied, with evidence from a wide range of techniques identifying specific neural computations in occipital-temporal and parietal regions that produce perceptual alternations. Here we investigate the role of naturally occurring alpha oscillations during rivalry, a feature of the electroencephalography (EEG) spectrum in humans. We recorded 64-channel EEG while subjects viewed dichoptically presented, orthogonal, colored (red/green) gratings matched in physical properties. Data were analyzed by an automated algorithm optimized for extracting alpha oscillations; we combined the 5 largest components, from an independent components analysis (ICA), which were identified as having the highest single-sided spectral amplitude in the alpha band (8-12 Hz) compared to the surrounding frequency range (3-6 Hz and 14-140 Hz). We found a prominent decrease in the alpha oscillation amplitude in occipital areas prior to the behavioral report (button press) of switching to a new percept. The alpha amplitude recovered, sometimes to a higher level, around and following the time of the

button press. As expected, there was a decrease of alpha amplitude in the motor cortex at the time of the button press, distinct from the changes observed in the occipital areas. In the replay control condition, in which the two eyes' images were presented singly in alternation without interocular competition, we found that the alpha activity decreased more abruptly, at close to the time of the button press. These results suggest that during binocular rivalry, changes in alpha oscillations reflect the neural competition leading up to a change in perceptual state, while in the replay condition they are more likely to reflect a change in percept subsequent to the physical switch of stimuli.

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Poster

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Title: Transcranial alternating current stimulation modulated steady-state visual evoked potentials and conscious perception in binocular rivalry

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Abstract: When two dissimilar stimuli are presented separately to each eye, we experience perceptual alternations between the two stimuli. This phenomenon is called binocular rivalry. By using two flickering stimuli with different temporal frequencies, previous electroencephalography (EEG) and magnetoencephalography (MEG) studies have shown that the steady-state visual evoked potential (SSVEP) at the flicker frequency of one of the stimuli was suppressed during perception of the other stimulus flickering at another frequency. Some have used the frequency-tagging method to investigate neural correlates of consciousness, however, underlying neural mechanisms of binocular rivalry and a causal link between SSVEP and conscious perception are not understood well enough. To this end, we investigate the causal relationship between the SSVEP and information processing for conscious perception in

binocular rivalry by modulating SSVEP by transcranial alternating current stimulation (tACS), which can modulate rhythmic human brain activity. We delivered tACS over the occipital cortex of healthy human participants and analyzed the SSVEP during a binocular rivalry task before and after 20-min tACS. During the binocular rivalry task, two Gabor patches with different orientations were presented to each eye separately, and participants were required to answer the perceived orientation of the Gabor patch by depressing one of two keys. The Gabor patches flickered at different frequencies: the flicker frequency of one Gabor stimulus was matched to the frequency of tACS and the flicker frequency of the other stimulus was mismatched to that of tACS. We found that perception was biased toward the flickering stimulus whose frequency was matched to the frequency of tACS. We also observed that EEG power of stimulated frequency was increased during the binocular rivalry task after delivering tACS. In contrast, we did not find any modulations of visual perception and EEG power after delivering tACS when tACS was delivered over the frontal cortex. Together, the results indicate that the neural networks associated with visual information processing can be modulated by occipital tACS and modulations of the neural networks can bias visual perception in binocular rivalry. These results suggest that there is a causal link between neural oscillations in the occipital cortex and information processing for conscious perception. We speculate that tACS presumably caused plastic changes of neural circuits which mediate conscious perception of a visual stimulus flicking at the tACS frequency.

Disclosures: **M. Katsurakawa:** None. **K. Kitajo:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 EY02301

Title: A population of neurons that signal interocular conflict signal in human visual cortex

Authors: ***S. KATYAL**, S. HE, M. JI, B. HE, G. PETERSON, S. A. ENGEL;
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Abstract: When incompatible images reach the two eyes, one eye's output is often strongly and temporarily suppressed, resulting in binocular rivalry. What controls this suppression? It could arise from winner-take-all mechanisms, but here we test the alternative that engagement of interocular suppression depends upon neurons sensitive to differences in the two eyes' inputs.

Specifically, we tested for neurons in early visual cortex responsive to interocular conflict by using steady state visually evoked potentials (SSVEP). If those regions contain conflict-detecting neurons, then SSVEP signals should show selective adaptation to interocular conflict. A parallel perceptual experiment demonstrated that these neurons are important for initiation of rivalry. Subjects ($N = 16$) adapted to square-wave gratings (3° radius, 1 cycles/°), which were either in the same (matching) or orthogonal (conflicting) orientations in the two eyes. Following adaptation, subjects viewed two test stimuli, both of which were binocular plaids comprised of orthogonal gratings. In one test, a different component grating was reduced in contrast by 20% in each eye. This generated conflict, that we termed interocular contrast difference (IOCD), of 0.2. In a second, the two eyes viewed identical plaids (IOCD = 0). Neural responses were measured by EEG. Stimuli flickered at a different frequency in each eye (7.2 Hz and 12 Hz) so that the recorded SSVEP could index the signals from the two eyes and their interactions. Importantly, SSVEP signals at the beat frequency, 4.8 Hz, could only arise from neurons that combine input from the two eyes.

Beat frequency amplitudes increased by 15% as IOCD rose from 0 to 0.2, suggesting increasing activity in neurons sensitive to conflict (Katyal et al, 2016). Critically, this signal was substantially reduced following adaptation to conflicting stimuli, but was unaffected by adaptation to matching stimuli; the difference between the two conditions was reliable ($t(15) = 3.3$, $p < .005$).

A parallel experiment ($N = 10$) revealed perceptual consequences of this reduced neural responsiveness to conflict. A staircase procedure measured the amount of IOCD required before subjects started experiencing binocular rivalry. Rivalry
thresholds increased following adaptation to conflict as compared to adaptation to matching patterns ($t(9) = 5$, $p < .001$).

The simplest account of these results is that human cortex contains neurons sensitive to interocular conflict, whose signals are the basis for initiating binocular rivalry. Conflict detecting neurons could potentially be an important part of how vision resolves ambiguous inputs more generally.

Disclosures: **S. Katyal:** None. **S. He:** None. **M. Ji:** None. **B. He:** None. **G. Peterson:** None. **S.A. Engel:** None.

Poster

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Support: KAKEN 26870426

Title: Visual change detection process is relevant to exogenously-driven perceptual alternation of the bistable image

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Abstract: When a bistable image is continuously viewed, its percept alternates from one possible percept to the other. The perceptual alternation of the bistable image was reported to be induced by a flash on the bistable image (Kanai et al., 2005), and such external perturbation has been theoretically interpreted as a generator of neural noise prompting the perceptual alternation between two stable perceptual states (e.g., Moreno-Bote et al., 2007). To date, little is known experimentally about the neural mechanism relevant to the exogenously-driven perceptual alternation. Focusing on the abrupt break in the sequential regularity of visual events, our recent psychological study suggested that the break around the bistable image would destabilize a bistable image's percept as the noise (Urakawa et al., 2016). Since the break is expected to evoke the deviant-related negativity (DRN), a differential VEP (break - no-break) relevant to automatic visual change detection process, we hypothesized that the visual change detection process was closely related to the exogenously-driven perceptual alternation, and attempted to clarify whether an enhancement of the DRN would correlate with facilitation of the perceptual alternation as the noise. Ten healthy subjects participated in the present study. In stimulation, eight bars with identical orientation were symmetrically arranged around the continuously-presented Necker cube and were intermittently presented (duration: 250 ms, inter-stimulus interval: 250 ms). The orientation was manipulated for the last image of a trial. While the orientation abruptly changed by 90º for the last image of the Break trial, the orientation did not change for the last image of the No-Break trial. Participants reported whether perceptual alternation occurred before and after the last image for each trial. Results showed that the mean proportion of perceptual alternation for the Break trials was significantly higher than that for the No-Break trials. Amplitude of the early DRN appearing at a latency of around 150 ms after the Break image's onset negatively correlated with differential proportion of the perceptual alternation (the mean proportion for the Break trials - that for the No-Break trials) across participants. In contrast, there was no such significant correlation for late DRN at around 300 ms. These findings support involvement of automatic visual change detection process in the exogenously-driven perceptual alternation, and showed that progressive emergence of the early visual change detection process at around 150 ms is relevant to facilitating the perceptual alternation as with the noise.

Disclosures: **T. Urakawa:** None. **M. Bunya:** None. **O. Araki:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Sloan Research Fellowship

Title: The relation of oscillatory-phase to visual perception is dependent on attention and location of stimuli

Authors: *T. DONOGHUE[1], W. FOX[4], A. KIM[2], B. VOYTEK[3];
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Abstract: Many recent studies emphasize the importance of alpha (8-13 Hz) oscillations for visual perception. The power of alpha oscillations systematically relates to visual perception, and more recent reports suggest that the phase of alpha oscillations also influences subsequent behavioral performance. For example, when using brief flashes of light in a visual detection task, the phase of posterior and central low frequency oscillations has been shown to correlate with task performance. Additionally, behavioral measures, in the absence of neurophysiological recordings, can also exhibit oscillatory properties, potentially related to underlying neural oscillations. Based on the previous literature, open questions regarding the relationship between oscillations and perception include: 1) The impact of attention on the relationship between oscillatory phase and visual perception, including whether attentional factors can explain inconsistencies in the reported topography of previous studies, and; 2) Whether potential oscillations in behavioral performance relate to physiological measures. Here, we use a novel visual target detection task, in combination with scalp EEG, to address these questions. In the task, brief stimuli can occur at four distinct spatial locations under three distinct attention conditions (for a total of twelve conditions). The attention conditions vary by the specificity to which the trial onset cue indicates the location of the upcoming, difficult to detect, target flash. For each trial type, adaptive methods were used to set the stimulus parameters such that behavioral performance was thresholded to a 50% detection rate. For each participant, individual alpha center frequency was determined in order to extract the phase at the moment of presentation across each trial, to be compared between hit and miss trials. Results of this analysis show that the spatial topography and extent to which alpha phase correlates with behavioral performance depends on the both the attention condition and the location of the stimulus. We find that there is rhythmic variation in behavioral performance, which has similar frequency contents and is partially phase locked to alpha phase from the EEG recordings, supporting the idea that the fine-time of neural oscillations systematically relates to behavioral outcomes. To

conclude, we support previous findings that alpha oscillations are implicated in perception, and suggest that task parameters such as attention condition and stimulus location are important. We provide initial support for the argument that rhythmic variation in behavioral performance relates to underlying neural oscillations.

Disclosures: **T. Donoghue:** None. **W. Fox:** None. **A. Kim:** None. **B. Voytek:** None.

Poster

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Nielsen Corporation

Alexander von Humboldt Foundation Feodor Lynen Fellowship

Title: Coupled delta and alpha oscillations mediate top-down control on visual perception

Authors: *R. F. HELFRICH, M. HUANG, G. WILSON, R. T. KNIGHT;
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Abstract: Conscious visual perception might arise from the dynamic interplay of functionally specialized but widely distributed cortical areas. Previously, it has been suggested that different cortical functions might be implemented in distinct frequency bands to facilitate visual perception and to dynamically implement temporal predications as well as endogenous priors. In particular, it has been debated whether the parieto-occipital alpha oscillation constitutes a passive gate-keeping mechanism to suppress irrelevant information or whether it has an active role to optimize visual perception. Here, we tested how a predictive sequence that was embedded in a rapid visual stream modulates the perception of a near-threshold target. Importantly, we probed visual perception every 34ms over the course of 850ms to study the temporal evolution of visual perception in 18 subjects. Crucially, the rapid stream was presented at either 10 Hz to entrain parieto-occipital alpha oscillations or arrhythmically. In a fully balanced 2x2 (rhythmicity x prediction) design we found that visual perception cycled as a function of the alpha phase. However, during predictive trials, a delta rhythm (2-4 Hz) co-modulated the behavioral alpha oscillations. Simultaneous EEG recordings revealed that this delta activity originated from prefrontal areas. The best behavioral performance was observed during predictive trials, when alpha power was high and the alpha and delta trough coincided in time. Taken together, the

results indicate that top-down processing is associated with long-range delta-alpha phase-amplitude coupling, which might selectively modulate parieto-occipital alpha activity to facilitate visual perception. These results indicate that the rich spatiotemporal structure of cortical activity might constitute the functional architecture for cortical processing and specific multi-site communication.

Disclosures: **R.F. Helfrich:** None. **M. Huang:** None. **G. Wilson:** None. **R.T. Knight:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R25NS070694

Title: Saturation of population activity predicts small phosphene size produced with electrical stimulation of human visual cortex

Authors: ***W. H. BOSKING**[1], P. SUN[1], M. OZKER[2], X. PEI[1], M. S. BEAUCHAMP[1], D. YOSHOR[1];
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Abstract: A major goal of neuroscience is to understand the relationship between spatiotemporal patterns of brain activity and perception. Here we take advantage of the unique access to human visual cortex that is possible in some epilepsy patients to investigate the relationship between electrical stimulation, cortical activity, and perception.
Electrical stimulation of visual cortex results in the perception of small spots of light known as phosphenes. However, a framework for understanding why phosphenes are so small has not been provided. Here we assume that electrical stimulation activates a patch of neurons within the map of visual space, and that the size of the activated patch varies with current. Stimulation of single site in visual cortex should result in the perception of a single phosphene in the corresponding location, with a larger current resulting in a larger phosphene. Predicting the actual size of the phosphene also requires knowledge of the of the local cortical magnification factor (M). Since M is highest in regions that represent the fovea, we would expect stimulation of the foveal region of visual cortex to produce the smallest phosphenes.
To test these predictions, we characterized phosphenes obtained from 103 electrodes from 14 patients. Phosphene size varied over a large range (0.13 to 10.8 deg), and two parameters

accounted for much of this variation. First, phosphene size increased with an increase in the amplitude of the current. Unexpectedly, however, this relationship quickly reached a plateau, with 90% of the maximum phosphene size obtained by a mean current of 1.4 mA. Second, at identical stimulation currents, electrodes located near the representation of the fovea produced smaller phosphenes than those located in the periphery.
We developed a simple model to predict phosphene size. For a given stimulation current, the extent of activated cortex is determined using a sigmoidal activation curve. Then, the inverse cortical magnification factor (1/M) for each electrode is estimated using a published equation for the map of visual space. Finally, the extent of activated cortex is multiplied by 1/M to predict phosphene size. This model accounted for 86% of the observed variance in phosphene size.
The success of this model suggests that our overall framework is correct. In addition, the fact that cortical activity appears to saturate at higher currents suggests that the functional architecture of the cerebral cortex may impose fundamental restrictions on the spread of artificially evoked activity. These findings have important implications both for understanding the visual cortex, and for the development of visual cortical prosthetic devices.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Perception of backward visual masking in a patient with bilateral frontal leucotomy

Authors: H. RIEIRO[1], S. MARTINEZ-CONDE[2], J. CHANOVAS[2], E. GALLEGO[4], F. VALLE-INCLÁN[4], *S. L. MACKNIK[3];
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Abstract: J.R., a patient that had most of the prefrontal cortex disconnected from the rest of the brain after receiving a bilateral frontal leucotomy in his youth, participated in a series of backward masking experiments conducted at the Institute Pere Mata, a psychiatric hospital in Reus, Catalonia, Spain. Visual stimuli were presented on a computer screen and consisted of vertical bars, where the central bar (target) was abutted by two flanking bars (masks). J.R. conducted multiple sessions of a 2-alternative forced choice (2-AFC) task, where he indicated which of two targets, presented on the left and right sides of the screen, was longest, by pointing

at the corresponding side of the screen. Experimental conditions included left vs right presentation on the screen, 2 target durations (34 ms and 100 ms), 3 target and masks lengths (3, 4, and 5 dva), and 6 stimulus onset asynchronies (SOAs) between target and masks (0ms, 34ms, 67ms, 100ms, 134ms, no masks). J.R. also indicated verbally whether he thought that the left and right targets were 'equal' or 'different' in each trial. The 2-AFC results indicated significant masking at 0ms, 34ms and 67 ms SOAs. Yet, J.R.'s complementary verbal reports suggested unawareness of the targets.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: supported by the NIMH-DIRP

Title: Orienting of endogenous spatial attention can impact subjective awareness more than objective performance

Authors: *M. VERNET, S. LOKEY, S. JAPEE, L. G. UNGERLEIDER;
Lab. of Brain and Cognition, NIMH/NIH/DHHS, Bethesda, MD

Abstract: Spatial attention is believed to improve objectively measured visual performance by increasing the signal gain at the attended location and reducing noise at unattended locations. Attention is also believed to influence decision-making and thus subjective awareness: observers assign more weight to information extracted from the attended location. In this experiment, we assessed whether attention differentially modulates objective performance and subjective awareness in the same discrimination task.
Near-threshold Gabor patches were presented to the right or left of a fixation cross 400 ms after a cue indicated, with 80% validity, where the target would appear. These targets were either embedded in white noise (noise experiment) or presented at low-contrast (contrast experiment). Participants (N=12) reported the orientation of the target, either in a 3 alternative choice task (3AC) allowing subjective reports (left, right or unknown) or in a 2 alternative forced-choice task (2AFC) for a strict objective performance evaluation (left, right).
Results showed that, for both experiments, attention decreased reaction time and increased subjective awareness in the 3AC task (i.e., participants used the 'unknown' option less for valid

than invalid trials). In addition, attention increased objective performance in both the 3AC and 2AFC tasks (i.e., the percentage of correct response), but only for the contrast and not the noise experiment.
In the contrast experiment, weak, low-contrast target stimuli competed with the noise arising from the empty location at the opposite site. Thus, attention had opposite effect on those two competing signals (i.e., increased at the attended location and decreased at the unattended one): a clear attention effect was observed on objective performance. On the contrary, in the noise experiment, target stimuli were embedded in noise presented at the same location. Thus, the signal arising from both the target and the noise were similarly modulated by attention (i.e., increased when attended and decreased when unattended): this could explain the absence of an attentional effect on objective performance. In both cases, however, a strong effect on conscious reports was observed.
In conclusion, our results show that attention can have an effect on both subjective awareness and objective visual performance. Nevertheless, it is possible to eliminate the effect on objective performance without changing subjective awareness. Endogenous spatial attention can thus make people think they see better, even if they do not.

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Poster

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Support: NIH grant 1R01EY022355

Title: Attention to object form modulates informational connectivity between dorsal and ventral visual streams.

Authors: ***J. TAYLOR**[1], M. VAZIRI-PASHKAM[2], Y. XU[2];
[1]Psychology Dept., [2]Psychology, Harvard Univ., Cambridge, MA

Abstract: Although the dorsal and ventral visual streams are often studied as separate systems, several recent strands of evidence suggest that they may interact. To probe the nature of this interaction, we used a recently-developed fMRI analysis technique called informational connectivity to examine the hypothesis that the two streams share object category information as needed for the present task. This method extracts timecourses for the strength of multivoxel patterns in different brain regions, and examines the extent to which these timecourses are

correlated across regions. In three different experiments, we examined whether attending to an object's shape increases informational connectivity between dorsal and ventral brain regions. Each of these task involved two conditions, one of which was a one-back repetition detection on an object's shape, the other of which was a one-back repetition detection on the color of either the object itself, the background, or dots spatially superimposed on the object. In this manner, we were able to examine the effects of spatial, object-based, and feature-based attention on the coupling between different brain regions. We identified several cases in which attending to object shape significantly modulated coupling between various IPS subregions and the lateral occipital complex (LOC), suggesting that regions in the two streams may dynamically share information as needed for the present task.
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Disclosures: J. Taylor: None. **M. Vaziri-Pashkam:** None. **Y. Xu:** None.

Poster

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Support: Sofja Kovalevskaja-Grant, Alexander von Humboldt Foundation

Title: Neo's spoon and Newton's apples: Motion of rigid and non-rigid deformations as cues to material properties

Authors: *L. M. ALLEY, A. C. SCHMID, K. DOERSCHNER;
Psychologie und Sportwissenschaft, Justus-Liebig-Universität Gießen, Gießen, Germany

Abstract: Image motion can be a powerful cue in the perception of rigid and nonrigid moving objects. We have recently shown that motion can also be rather diagnostic for several optical and dynamic material properties (e.g. gelatinousness, Schmid & Doerschner 2016 VSS). Here we utilize a 'violation of expectation' paradigm to investigate the neural mechanisms involved in deriving material properties from motion. We discuss results with respect to the role of predictive coding and expectation in the perception of material qualities. We generated 3 categories of objects under realistic lighting (environment maps) using Blender, with 6 exemplars each: 1) familiar objects with very specific functions/affordances, which would generate strong expectations as to the material 'behavior' or properties (e.g. a wooden chair, velvet cloth, clear wine glass, drop of honey, gelatin dessert) 2) novel, unfamiliar objects: globally convex, three-dimensional forms with systematically generated features varying

in amplitude and frequency ("Glavens", see Phillips, 2004) and 3) recognizable 3D-shapes with neutral utility (e.g. sphere, cube, cylinder). Shapes in the "Glavens" and neutral shape categories were rendered with the same set of optical material properties as the familiar objects, i.e., wood, velvet, glass, and translucent materials for honey and gelatin. We then produced movies of these objects as they were falling and colliding with a virtual surface. There were 2 motion conditions, defined with respect to the familiar object category: 1) congruent, where the material of the object behaves as expected (i.e. a falling soft cloth would wrinkle) and 2) incongruent, where the dynamics of the material would violate expectations (i.e. a falling soft cloth would shatter). On each trial, the static image of the object would appear for 3 seconds, followed by a congruent or incongruent motion sequence. Ten observers (6 female; *M*= 30.9, *SD*= 5.70) were asked to rate each movie on a visual rating scale (minimum to maximum) with respect to 4 adjectives: 'Hard', 'Gelatinous', 'Heavy', and 'Liquidy/Fluid' – as quickly and accurately as they could. The experiment was blocked by object category. Results show that object familiarity and concomitant expectations play a role when judging material properties in dynamic sequences. We found an interaction between motion and object type. Reaction times were significantly higher for incongruent versus congruent motion for the familiar objects, but as expected, not for "Glavens" or neutral objects.

Disclosures: L.M. Alley: None. **A.C. Schmid:** None. **K. Doerschner:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Title: Investigating the Reversed Letter Effect for stimuli with and without basic features by means of event-related brain potentials

Authors: *L. BECKER[1], T. SCHENK[2];
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Abstract: *Background*: The Reversed Letter Effect (RLE, Frith, 1974) describes the phenomenon that it is easier to find a reversed letter in a background of the same, normally oriented letters than vice versa. By means of event-related brain potentials (ERPs), it was investigated at which time point the RLE occurs and whether the time point is different for target letters with basic features (e.g., orientation differences) versus target letters without basic features. It was assumed that according to the two-stage model (e.g., Treisman, 1985), the RLE

occurs later for stimuli that consist of target and context elements differing in one basic feature. In two experiments, three ERPs representing different phases in the processing of visual stimuli (the N1, the N2p and the P3) were examined.
Methods: In Experiment 1, the stimuli consisted of lines with 21 elements of either the letter A or its upside-down rotated version. In 50% of the trials, a target letter was embedded into the stimuli. Stimuli were presented for less than 100 ms to prevent eye movements. Participants had to decide whether the target element was present. Besides the three ERP components, behavioral data (hit rates, false alarm rates and reaction times) were collected. In Experiment 2, the set-up was almost the same as in Experiment 1. However, this time N's and mirror-reversed N's (И's) were used.
Results: For the behavioral data, the RLE was found in both experiments. For the ERP data, the RLE had a significant effect on all three ERP components in Experiment 1, whereas in Experiment 2 only the two later ERPs (the N2p and the P3) were significantly affected by the RLE.
Conclusions: The findings suggest that the RLE is not purely based on top-down processing. The results of Experiment 1 show that even early (bottom-up) components can be affected by this effect. However, early effects are cancelled out and the RLE modulates only the late (top-down) components of the visual processing if the target and the distractor elements differ in one basic feature (orientation).

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Intramural Research Program: NIH

Title: Examining the segregation of number, letter, and word form selectivity in human ventral visual cortex

Authors: *D. JANINI, C. BAKER;
NIH, Washington, DC

Abstract: Many daily tasks, from dialing a phone number to completing simple arithmetic, require recognition of visual symbols. Recent studies using ECoG and fMRI have reported regions of ventral visual cortex recruited during the processing of written numbers. However, it

is unclear to what extent these regions are specifically selective for number compared to other types of written stimuli. Imaging these regions with fMRI of conventional resolution has proved difficult because of signal dropout caused by the interface of air and petrous bone. Here, we used ultra high-resolution (7T) fMRI to investigate whether ventral visual cortex contains an anatomically distinct region specifically selective for number stimuli. Two block-design experiments using a one-back task were completed during a single scanning session. Experiment 1 was drawn from Grotheer et al. (2016) and included blocks of numbers (one and two character length), letters (one character length only), false numbers, false letters, Fourier randomized numbers, Fourier randomized letters, and objects. Experiment 2 included blocks of single letters, single numbers, letter strings, and number strings. Using data from Experiment 1 as a localizer, we replicated the findings of Grotheer and colleagues by contrasting number against false number, and number against the average of all other stimuli. These contrasts revealed bilateral regions in ventral temporal cortex. However, this candidate number form area showed high overlap with ROIs defined by completing analogous contrasts with letter stimuli. Moreover, a direct contrast of number against letter did not yield a reproducible ROI. Similarly in Experiment 2, no reliable selective regions were found by directly contrasting single numbers over single letters. Percent signal change within the candidate number form area did not differ between single letters and numbers or between strings of letters and numbers. However, percent signal change for strings of characters exceeded that of single characters. Defining ROIs by contrasting digit strings versus letter strings implicated larger regions of cortex, highlighting the importance of controlling for stimulus length when localizing number and letter selective regions. Collectively, these results suggest that univariate fMRI methods do not readily reveal regions of visual cortex specifically selective for number stimuli. Number processing may be distributed across regions with more general selectivity for orthographic stimuli including both letters and numbers. Future experiments will employ multivoxel pattern analyses to elucidate mechanisms of number processing within these areas.

Disclosures: **D. Janini:** None. **C. Baker:** None.

Poster

360. Perception and Imagery: Visual Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 360.17/KKK44

Topic: H.02. Human Cognition and Behavior

Title: Predicting stimulus and response category in a multisensory simulated real world environment with fMRI and EEG.

Authors: *J. C. ELLIOTT[1], W. WANG[1], D. KRNAVEK[2], A. ASTURIAS[1], V. BABENKO[1], A. SHAPIRO[1], P. CONNOLLY[2], S. T. GRAFTON[1];
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[2]Teledyne, Durham, NC

Abstract: The spatial and temporal characteristics of stimulus processing and categorization have been well characterized in the laboratory environment, though this typically includes unrealistic restrictions on stimulus presentation and isolation of stimulus characteristics. Yet, real world applications require that neural detection algorithms function under more enriched and diverse conditions. In the current simultaneous fMRI/EEG experiment, 8 participants engaged in a simulated real-world environment that required the identification of visual and auditory stimuli. Specifically, participants monitored a continuously moving desert scene and on visual trials categorized a vehicle as either a target, friendly, or neutral. On auditory trials, participants categorized the magnitude of a spoken number representing the distance of a target. The magnitude was categorized as near, in range, or out of range. Importantly, in both the visual and auditory trials the mapping between the three categories and response was randomly assigned on each trial in order to prevent participants from preparing a response prior to the presentation of the response mapping display. The response mapping display occurred between 2-5 seconds after the presentation of either an auditory or visual stimulus. Overall behavioral performance was high (visual accuracy: .9 - .98; auditory accuracy: .85 - .99). Eye tracking was used to determine the duration of fixations prior to the response mapping display. Only trials where fixation occurred 1 second prior to the response mapping display were included in the EEG analysis. After removing MRI and BCG artifacts, a classifier was trained to discriminate between the three response categories based on EEG frequency power over 62 electrodes and between 4 and 30 Hz. Mean classifier performance was .54, which was significantly higher than a permutation test with mean performance of .37 ($p = .014$). Whole brain fMRI classification also accurately predicted whether a given trial was a visual or auditory trial (10 fold accuracy = .95, permutation test accuracy = .5). These results demonstrate that whole brain classification can determine stimulus modality in a dynamic multisensory environment. Furthermore, EEG activity recorded during simulated real-world environment can accurately predict subsequent response selections independent of preparatory motor response activity and visual information. Therefore, these results indicate that it is feasible to use these neural signals to assess and predict categorization in a real world environment.

Disclosures: J.C. Elliott: None. **W. Wang:** None. **D. Krnavek:** None. **A. Asturias:** None. **V. Babenko:** None. **A. Shapiro:** None. **P. Connolly:** None. **S.T. Grafton:** None.

Poster

360. Perception and Imagery: Visual Processing

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Program#/Poster#: 360.18/KKK45

Topic: H.02. Human Cognition and Behavior

Support: Intramural Research Program NIH

Title: Differential impact of stimulus format on representational spaces

Authors: *B. B. BANKSON, C. BAKER;
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Abstract: Conceptual knowledge is thought to be organized in a hierarchical, categorical structure. Recently, representational similarity analysis (RSA) has been used to elucidate object concept categories, based on the underlying representational geometry of behavioral, fMRI, and M/EEG data. The majority of these studies have used pre-defined categories of objects presented as isolated pictures to measure the representational space; however, other RSA work has shown differences in semantic representation based on stimulus modality. Here, we use a previously developed behavioral object arrangement task to systematically address the effects of stimulus format on the measured representational space. The same 84 concrete object concepts were presented in word, cropped image, or context-embedded image format; this allowed us to modulate the amount of perceptual and semantic information conveyed by each item. To avoid any categorical bias in the sampled items, we selected them based on lexical frequency counts from the Corpus of Contemporary American English. In addition we extracted word co-occurrence statistics in a vector space from the GloVe algorithm (Global Vectors for Word Representation). Separate groups of participants completed the arrangement task in one of the three formats. Using k-means and hierarchical clustering on pair-wise distance data between all items in each set, we compared the resulting representational spaces. Across the three stimulus formats certain object groupings were consistent: people, body parts, animals, clothes, food, and transportation. These groupings occurred in spite of relatively low correlations between the rank group-level representational dissimilarity matrices (RDM) for each condition, and high variance among individuals. The cropped and context embedded images demonstrated more similar representational structure with each other than with the word condition, indicating differences in task-relevant information between the pictorial and linguistic formats. Correlations with the GloVe representational space were weaker than between the different image formats and there was much less clustering and apparent categorical effects. These differences suggest that behavioral pairwise distances and co-occurrence statistics reflect different aspects of object semantics. Taken together, these results show that stimulus format does indeed affect conceptual

representation for concrete object concepts, while highlighting the salience of certain basic categories that are centered, for the most part, around humans.

Disclosures: **B.B. Bankson:** None. **C. Baker:** None.

Poster

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Title: Category boundaries and typicality warp the neural representation space of real-world objects

Authors: ***M. IORDAN**[1], M. R. GREENE[1], D. M. BECK[2], L. FEI-FEI[1];
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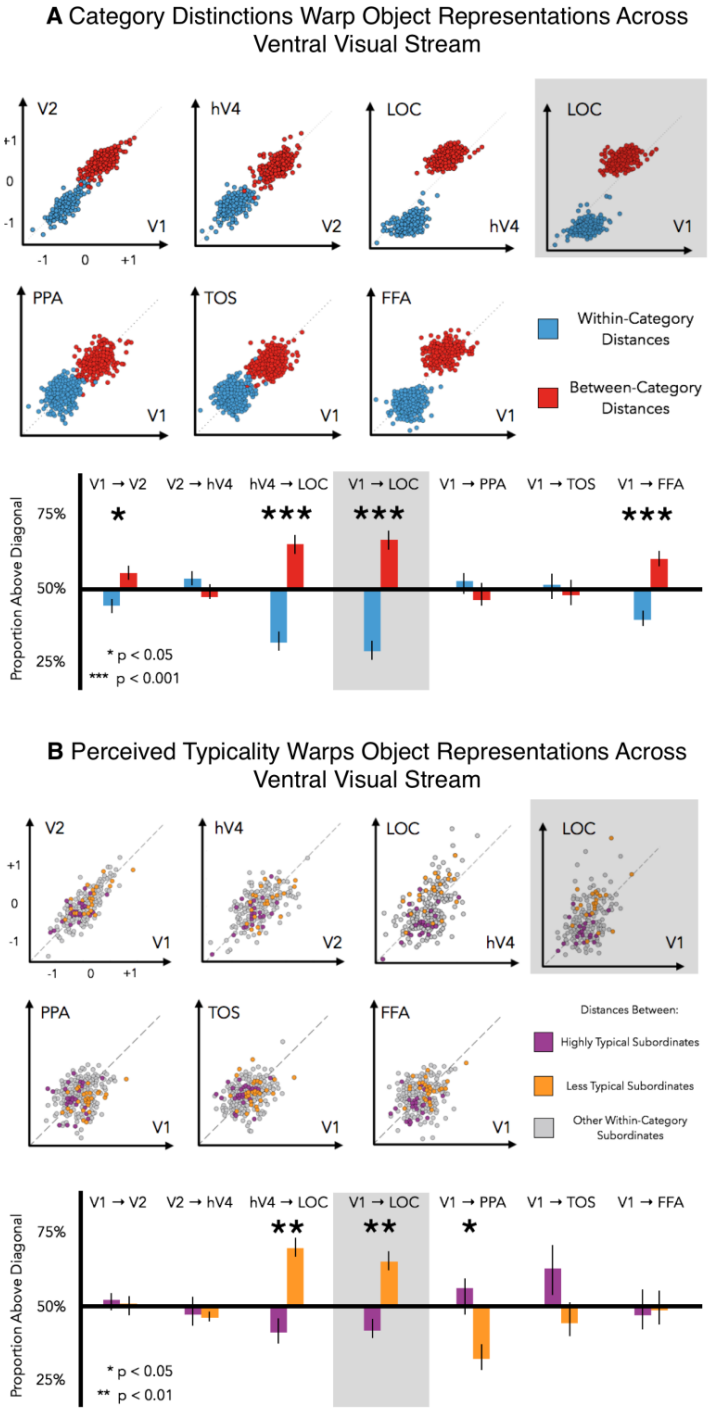
Abstract: Our interaction with the world is largely mediated through high-level categories, which represent cognitively useful collections of self-similar entities that share features, affordances, and meaning. Yet, the trillions of objects that make up our varied visual experience are initially represented by our visual system as highly overlapping collections of low-level features. To bridge this representational disconnect, sequential computations across the ventral visual stream effectively untangle these initially overlapping manifolds into invariant, linearly separable categories. It is unclear, however, whether this process occurs stepwise in visual cortex and, if so, to what extent at each step. In the present study, we hypothesize that the eventual emergence of our behaviorally relevant learned category structure guides the step-by-step transformations occurring along the ventral visual stream hierarchy. Using two fMRI experiments employing ninety-four object categories, we found strong evidence that between-category distinctions and within-category typicality structure both warped neural representations sequentially across the ventral stream: category distinctions slowly pushed representations apart between early and mid-level visual areas and, simultaneously, perceived typicality of category members modulated the neural intra-category space so that in later processing stages highly typical items became more similar to one another and less typical items were pushed away from

the category central tendency. This suggests that behavioral category structure strongly influences processing in visual cortex, markedly reorganizing the representation space of both object-selective cortex, as well as that of earlier stages of the visual hierarchy. Taken together, our findings imply a more complex interaction between human category space and its early representation than hitherto assumed.



Disclosures: **M. Iordan:** None. **M.R. Greene:** None. **D.M. Beck:** None. **L. Fei-Fei:** None.

Poster

360. Perception and Imagery: Visual Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 360.20/KKK47

Topic: H.02. Human Cognition and Behavior

Title: You gain some you lose some: changes in synesthetic perception overtime

Authors: *A. S. HOCHMAN[1,2], J. BUENROSTRO[2], J. F. AWAD[2], A. ILNICKI[2], R. MOSHER[2], S. A. DREW[2];
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Abstract: Introduction: Synesthesia is a condition in which a single stimulus elicits multi-sensory experiences. Our current study focuses on a grapheme-color synesthete (Subject 1): an individual who experiences colors elicited by achromatic (black) numbers and letters and a subject in a pilot study focusing on bidirectionality in synesthesia. Subject 1 returned three years later to participate in the resultant study. **Methods:** As a preliminary step for both the pilot and subsequent experiment, Subject 1 completed the online 'Synesthesia Battery' designed by Eagleman et al. (2007a), a series of tests developed to assess individuals with synesthesia. **Results:** Upon review of the subject's responses, we noted a difference between Subject 1's reported synesthetic perceptions in the two different assessment sessions. This observation is unanticipated as the literature typically suggests that synesthetic percepts remain consistent over time. However, some researchers have reported changes in synesthetes' concurrents. For example, Eagleman et al. (2007b) found that a particular synesthetes grapheme-colors had changed in less than a year. **Discussion:** We report here a synesthete that not only experienced changing of color percepts, but the loss of some grapheme's ability to induce synestheteic colors, while other graphemes gained the ability to induce colors. Cognizance of the nature of transitioning synesthetic perceptions and the potential for gaining and losing percepts is critical to the study of synesthesia, as well as to the understanding of synesthesia and the neurological mechanisms underlying synesthesia as a whole.

Disclosures: A.S. Hochman: None. **J. Buenrostro:** None. **J.F. Awad:** None. **A. Ilnicki:** None. **R. Mosher:** None. **S.A. Drew:** None.

Poster

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Program#/Poster#: 360.21/KKK48

Topic: H.02. Human Cognition and Behavior

Title: Synesthetic grapheme-color associations are processed early in time and can guide attention during visual search.

Authors: *O. F. CHESLEY, C. GRAULTY, E. CANSECO-GONZALEZ, M. PITTS; Psychology, Reed Col., Portland, OR

Abstract: Grapheme-color synesthesia is a condition in which affected individuals experience consistent associations between graphemes (letters or numbers) and colors. The current study addressed two questions: (1) What is the timing of neural events related to synesthetic color processing? (2) Are synesthetic color associations processed pre-attentively, or is focused attention required? Electrophysiological and behavioral data were collected from ten grapheme-color synesthetes and ten matched controls in two separate experiments. All participants completed the Eagleman synesthesia battery to confirm their status as synesthetes (or not) and to allow for individual tailoring of the stimuli. Identical stimuli were presented to each synesthete-control pair, but stimuli differed across pairs and were selected according to strength, consistency, and colors associated with each letter.

In experiment 1, participants performed a 1-back task while stimuli from 4 categories were presented in random order: 1) physically uncolored letters with synesthetic color associations, 2) physically uncolored letters without synesthetic color associations, 3) physically colored letters, and 4) physically uncolored false fonts. An early (110-150 ms) occipitally-focused event-related potential (ERP) component was linked with the neural processing of synesthetic grapheme-color associations. This ERP difference was isolated by comparing stimulus categories 1 vs. 2, and 1 vs. 4. The spatio-temporal characteristics of this synesthetic-color ERP were distinct from those elicited by the physically colored letters (category 2 vs. 3).

In experiment 2, participants performed a visual search task to find a target letter amongst 7 heterogeneous distracter letters. For each synesthete, 2 letters that shared the same color association (e.g. both red) were designated as potential targets while the distracter letters were each associated with a different (e.g. non-red) color. Hence, although the stimuli (physically uncolored letters) and task (find a target letter and report it) were identical for synesthetes and controls, synesthetes were likely to utilize color associations to guide them to the target letter. On each trial, 8 letters were randomly positioned in a circular array with an equal number on each side. ERPs were compared between electrode sites contralateral vs. ipsilateral to the target allowing measurements of the “N2pc” component (200-300 ms), a well-studied marker of

selective attention. The results showed faster reaction times, along with larger and earlier N2pc components for synesthetes vs. controls.

Disclosures: O.F. Chesley: None. **C. Graulty:** None. **E. Canseco-Gonzalez:** None. **M. Pitts:** None.

Poster

360. Perception and Imagery: Visual Processing

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Program#/Poster#: 360.22/KKK49

Topic: D.06. Vision

Title: Consistently Incorrect: Potential implicit numerical activation in non-synesthetes

Authors: *J. F. AWAD[1], N. URENDA[2], D. LARRANAGA[2], B. C. HACKNEY[2], S. A. DREW[2];
[1]California State University, Northridge, Reseda, CA; [2]Psychology, California State University, Northridge, Northridge, CA

Abstract: A synesthete is a person who has multisensory experiences when presented with a single stimulus and a nonsynesthete is a person that lacks these experiences. Our research compares the experiences of synesthetes who have colors elicited by achromatic (black) numbers and letters ("grapheme-color" synesthetes) with the experiences of nonsynesthetes. Traditionally, research relating to synesthesia has reported synesthetic experiences to be unidirectional: numbers elicit colors, but colors do not elicit numbers. More recently however, reports have been made suggesting the notion of bidirectionality in synesthetic experiences. To examine bidirectionality of synesthetic percepts, we have utilized a task in which synesthetes were presented with sequences of color patches on either side of a crosshair and asked to choose which sequence was higher in magnitude, under both fast (< 150 ms) and slow presentation times (unlimited presentation time for stimuli). Synesthetic responses were compared to those of age and gender matched nonsynesthetes. While our data supported the notion of synesthetic bidirectionality for the numbers paradigm, we observed an additional intriguing outcome in which several of the nonsynesthetes performed significantly different than chance. This observation led us to inquire about possible alternative explanations for this pattern of results. One possibility could be implicit numerical associations formed due to higher vividness of mental imagery in synesthetes that performed significantly different from chance. The purpose of our current study was to investigate whether nonsynesthete vividness is associated with performance on the bidirectionality tasks. Results revealed no significant correlations between performance and vividness scores, for the slow condition (r = .020, p= .954) nor for the fast

condition (r = .184, p=.587) suggesting an alternative mechanism is responsible for the observed pattern of results in nonsynesthetes.

Disclosures: **J.F. Awad:** None. **N. Urenda:** None. **D. Larranaga:** None. **B.C. Hackney:** None. **S.A. Drew:** None.

Poster

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Program#/Poster#: 360.23/KKK50

Topic: H.02. Human Cognition and Behavior

Title: On harnessing eye movements to read the mind and to alter it

Authors: *B. R. SHETH[1], A. R. TIJIBOY[2];
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Abstract: Eye movements are a window into cognition. Here, we extend this idea: i) Can the eye movements of a subject (S) prior to their response reliably predict it? Our goal is to ask Ss to think of a number from a limited number of choices and then anticipate what (s)he will think of from their pattern of eye movements prior to their response. Ss (n=51) were shown a number line (with no numbers) for 300 ms followed by a blank screen and an oral prompt: "Think of number 1, 2, or 3 and say it out loud". Gaze locations and saccade amplitudes at various times prior to response were features in a pattern classifier trained to predict the S's choice of number. Based on information gain ratios, the two best features were the eye position and saccade amplitude after the number line (> 75% correct for each) is extinguished and before the prompt is played, indicating these features predict S choice even before (s)he has had a chance to begin thinking of a number. To reduce the dimensionality of the feature set, principal components analysis (PCA) was performed on the selected features. The first three PCs accounted for 96.3% of the variance and were used in the classification. Test performance was 94.1% (=48/51) correct. Thus, an individual's eye movements reliably anticipate their response in an abstract number choice task. ii) We further asked: Can we drive subject eye position and/or spatial attention so that it reliably alters their thought process and eventually, their response? Our goal is to yoke the S's eye with an attention-grabbing cue to a part of space and see if this systematically influences the number the S thinks of later. Control Ss (n=21) had to choose between two numbers: 1 or 2. A strong bias was observed in favor of "1" (16/21). New experimental Ss (n=21) faced the same binary choice as controls but with one key difference: Prior to the number line and prompt, they viewed a video shown on the screen's right. Experimental Ss showed a strong bias in favor of "2" (18/21) - in the direction opposite to control. The dramatic reversal in number choice is not

attributable to a non-directional, non-specific effect of attention cueing, as a third group of Ss (n=21), who viewed the same video, but on the screen's left, continued to be biased in favor of "1" (17/21), similar to control. Thus, having an external stimulus shift the eye in a particular direction alters systematically the number one will think of later. Our findings show how eye movements both inform and guide subsequent thought. We are currently exploring if our findings generalize to other dimensions that naturally map onto space as number and ones that do not map onto space naturally but rather ones that is imposed on them by the experimenter.

Disclosures: B.R. Sheth: None. **A.R. Tijiboy:** None.

Poster

360. Perception and Imagery: Visual Processing

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Program#/Poster#: 360.24/KKK51

Topic: H.02. Human Cognition and Behavior

Title: EEG oscillations during visual processing task are different in expert and non expert subjects

Authors: *D. NOURI[1], M. JAVIDJAM[3], A. BONYADINAEINI[3], R. LASHGARI[2];
[2]Neural Engin. Res. Lab., [1]Inst. For Res. In Fundamental Sci., Tehran, Iran, Islamic Republic of;
[3]Dept. of Industrial Engin., Iran Univ. of Sci. and Technol. (IUST), Tehran, Iran, Islamic Republic of

Abstract: There is mounting evidence that cortical fluctuations in specific frequency bands reflect specific functional states of neural networks. Cortical activities responding to visual stimuli in occipital lobe play a major role in visual perception and awareness. Here, the EEG signals were obtained with 64 channels to study the brain frequency modulations while expert (Logo designer) and non-expert subjects were viewing the logo of the electronic devices including Apple, Sony, Xvision, Huawei, and LG appearing on the screen. To measure the cortical activity and behavioral response time, twenty healthy subjects (25-56 years old, 10 subjects for each) were participated in our experiment. In the expert subjects, the early modulations of mean power in occipital lobe began around 100 ms at the initial recordings of representing logo stimuli that show an increased power spectrum at low frequency oscillations (theta and alpha bands (4-12 Hz), $p<0.01$). Our primary results also demonstrated that the amplitude of mean power had a negative correlation between occipital and frontal lobes during 50 to 500 ms of stimuli, which importantly was different in experts than non-experts (r_s=-0.91 and r_s=-0.82; $p<0.0001$, respectively, spearman's correlation). We conclude from our primary results that expert and non-expert subjects are differently processing the information, expert

subjects need more time to make decision for choosing the preferred logos than non-experts (M_{expert}=2.8±1.1; $M_{non\text{-}expert}$=1.7±0.6) Keywords: EEG, Brain Oscillations, Expert and Non-Expert Subjects

Disclosures: D. Nouri: None. **M. JavidJam:** None. **A. Bonyadinaeini:** None. **R. Lashgari:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: SNSF Grant P300P1_161178

SNSF Grant PBBEP1_146288

Volkswagen Foundation

German Research Council (DFG)

Title: Human single unit activity during attentional blink

Authors: *T. P. REBER[1], J. FABER[1,2], J. NIEDIEK[1], J. BOSTRÖM[3], V. COENEN[3], C. E. ELGER[1], F. MORMANN[1];
[1]Dept. of Epileptology, Univ. of Bonn, Bonn, Germany; [2]Dept. of Neurology, Univ. of Bonn, Bonn, Germany; [3]Stereotaxy and MR based OR Techniques, Dept. of Neurosurgery, Univ. of Bonn, Bonn, Germany

Abstract: Along the monkey ventral pathway, neuronal firing increasingly follows perception rather than retinal input of ambiguous visual stimuli from posterior to anterior regions. In humans, neuronal firing in the medial temporal lobe has been shown to predominantly follow the contents of perception in fast-masking and binocular rivalry paradigms. It remains unclear, however, whether a posterior-anterior gradient of tuning to perception also exists in humans. We measured single-neuron activity in 21 epilepsy patients implanted with microwires along the posterior-anterior axis of the medial temporal lobe (PHC: parahippocampal cortex, EC: entorhinal cortex, H: Hippocampus, and A: Amygdala). We recorded 40 sessions of an attentional blink paradigm to investigate effects of varying awareness on unit activity. Attentional blink manifests itself as a deficit of reporting the second of two target stimuli (T1/T2) among a series of non-target stimuli presented in rapid succession (6-10 Hz presentation

rate). Stimuli were selected from a previous screening session so as to elicit selective visual responses in individual units.
Firing in response to second targets reported as seen (T2-seen) was higher than firing in response to unseen targets (T2-unseen). This effect was smallest in the most posterior region (PHC), intermediate for more anterior regions (H/EC), and largest in the most anterior region (A). Similarly, classification of trials into T2-seen and T2-unseen using a rate code was worst in PHC and improved for more anterior regions (H/EC, A) as suggested by area under the curve from receiver operating characteristics (ROC) analyses in sliding windows of 200ms width in 1ms steps. Nevertheless, firing exceeding baseline activity following response-eliciting stimuli was reliably observed across all regions also in T2-unseen trials. Furthermore, sliding window ROC analyses suggested that stimulus identity could be reliably classified even from neuronal firing following unseen stimulus presentations. Here, best classification was obtained for PHC neurons. Taken together, our results suggest that there is a posterior-anterior gradient of tuning to the contents of conscious perception within the human medial temporal lobe.

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Poster

360. Perception and Imagery: Visual Processing

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Program#/Poster#: 360.26/KKK53

Topic: H.02. Human Cognition and Behavior

Support: Volkswagen Foundation

German Research Council (DFG)

Title: Probing the causality between neural activation and perception using electrical stimulation in the human medial-temporal lobe

Authors: ***S. KNIELING**[1], T. P. REBER[1], J. BOSTROEM[2], C. E. ELGER[1], F. MORMANN[1]; [1]Dept. of Epileptology, Univ. of Bonn, Bonn, Germany; [2]Dept. of Neurosurgery, Univ. of Bonn, Bonn, Germany

Abstract: Neurons in certain regions of the human medial-temporal lobe (MTL) have been reported to respond preferentially to specific stimulus categories. While cells in the parahippocampal cortex (PHC) show a preference for landscapes, neurons in the right amygdala (RA) preferentially respond to animals. The observed correlation between the presentation of a

stimulus and the activity of these cells, however, allows no conclusion as to whether the cell activity causes the percept or whether it is merely an epiphenomenon. If the cell activity caused the percept, stimulating these cells directly should evoke a percept even in the absence of an external stimulus.
In the present study epilepsy patients implanted with combined macro- and micro-electrodes in RA and PHC performed a forced-choice categorization task in which they were instructed to classify images either as animals or landscapes. Images were superimposed with varying amounts of random noise ranging from 5% to 100%. During stimulus presentation, patients received either no stimulation, PHC-stimulation or RA-stimulation through the implanted macro depth electrodes.
Preliminary results from up to now 2 patients show that for pure noise trials, PHC-stimulation was able to bias patients' perception towards landscapes compared to no stimulation or RA stimulation. RA stimulation showed no significant shift compared to no-stimulation.
These results suggest that neurons in the PHC could indeed be causally involved in perception, while cells in the RA are less likely to be involved. This hypothesis is supported by the finding that RA neurons have a greater response onset latency of about 400 ms, compared to 280 ms in PHC neurons, and are thus more likely to be involved in later mnemonic processing rather than object recognition.

Disclosures: **S. Knieling:** None. **T.P. Reber:** None. **J. Bostroem:** None. **C.E. Elger:** None. **F. Mormann:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

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Title: Neural dynamics of event segmentation: evidence from intracranial recording

Authors: *A. JAFARPOUR[1], J. J. LIN[2], R. T. KNIGHT[1];
[1]Helen Wills Neurosci. Instutite, Berkeley, CA; [2]Univ. of California Irvine Sch. of Med., Dept. of Neurol., Irvine, CA

Abstract: We segment the steam of ongoing events into episodes to make sense of what is happening in our environment. Event segmentation is a process applied to the stream of perception that identifies the temporal context of an episode and its relations to other episodes (e.g. the temporal order of events). The stream of events is segmented based on their temporal relevance; that starts with perception of a salient change in the stream of perception. The intensity of changes in the temporal context depends on the level of the new event saliency or novelty. The human prefrontal (PFC) and the medial temporal cortices (MTL) are critical for detecting new and salient events and establishing their temporal context. To assess the dynamics of event segmentation, we recorded intracranial electroencephalography from epilepsy patients PFC and MTL with electrodes implanted for clinical purposes. The patients watched movies; the degree of event saliency for every movie frame was established by previous behavioral analysis. We used this saliency behavioral regressor to examine the relationship between event saliency and the neural activity. The results revealed that the power of gamma band activity (30-70 Hz) in the Hippocampus and the power of high-gamma band (70-150 Hz) and gamma band (30-70 Hz) activity in the PFC predicted the intensity of event saliency (corrected $P < 0.05$). Moreover, the peak of power intensity in the gamma band in the PFC onset about 80 milliseconds before the peak intensity in the Hippocampus - suggesting that the PFC establishes saliency before the hippocampus. The functional connectivity between the PFC and the MTL in the theta band changed according to the saliency of events (corrected $P < 0.05$). For example, the functional connectivity between the anterior cingulate and the anterior Hippocampus was enhanced when retaining the same temporal context of events, and when the context changed, this functional connectivity diminished. Our findings provide evidence that PFC-MTL interactions coordinate event segmentation in the stream of perception.

Disclosures: **A. Jafarpour:** None. **J.J. Lin:** None. **R.T. Knight:** None.

Poster

360. Perception and Imagery: Visual Processing

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Topic: H.02. Human Cognition and Behavior

Support: Intel Corporation

Title: Representation of real-world event schemas during narrative perception

Authors: ***C. BALDASSANO**, U. HASSON, K. A. NORMAN;
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Abstract: During perception of narrative stimuli, a network of brain regions overlapping with the default mode network (DMN) represents long-timescale semantic information (Lerner et al. 2011). These regions exhibit activity patterns that are specific to particular events, generalize across modalities (audio-visual movies and audio descriptions), and are evoked during free recall of the events (Chen et al. 2016, Zadbood et al. in prep). What is the content of these abstract event representations in long-timescale regions? Classic psychological studies have proposed that as a story unfolds, subjects construct and update a "situation model" that specifies the spatial, temporal, and causal relationships that define specific situations (Zwaan and Radvansky 1998). Situation models are built not only from contextual information accumulated throughout the event, but also from prior knowledge about event schemas, which describe typical event sequences that we have encountered throughout our lifetime (Zacks et al. 2007; Schank and Abelson 1977).

We assessed whether long-timescale regions represent information about real-world event schemas by measuring the fMRI response evoked by narratives occurring in a similar context but differing extensively in terms of characters, timing, plot, and modality. Sixteen 3-minute stories were presented, four from each modality (Hollywood movie clips or audio narration) and each schema (eating at a restaurant or catching a flight at an airport). Whole-brain data from 31 subjects was temporally divided into four subevents for each schema (e.g. entering the airport, going through security, walking to the gate, and boarding the plane), and an average activity pattern was computed for each scene. We then searched for regions having a consistent pattern for a given subevent (e.g. going through security) across all of the different narratives.

We identified long-timescale regions including angular gyrus, retrosplenial cortex, and parahippocampal cortex that showed schema-related event representations. We also propose a new Hidden Markov Model for dynamic brain activity during narrative perception that accounts for scene-specific patterns, and can automatically identify corresponding event representations across narratives without human-supplied event segmentations.

Disclosures: C. Baldassano: None. **U. Hasson:** None. **K.A. Norman:** None.

Poster

360. Perception and Imagery: Visual Processing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 360.29/KKK56

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant DA029149-05

Title: The dorsal and ventral default mode network respond differentially to the valence and vividness of imagined events

Authors: *T. PARTHASARATHI, J. W. KABLE;
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Abstract: The default mode network (DMN) is active when humans imagine the future. However, it is unknown whether different aspects of imagination engage different nodes of the DMN. In particular, prospection entails both a generative component, in which episodic components are combined in novel ways to generate predictions about possible futures, and an evaluative component, in which these predictions about possible futures are evaluated on a positive to negative scale. Here, we test how the vividness and valence of an imagined event differentially affects BOLD activity, hypothesizing that vividness would modulate generative mechanisms and valence would modulate evaluative mechanisms involved in prospection. Twenty-four people were scanned using fMRI while imagining scenarios manipulated for vividness and valence. Subjects rated each scenario for vividness and valence. We analyzed our neuroimaging data using the general linear model; during the imagination period, we included separate regressors for comparing scenarios that were high versus low in vividness or high versus low in valence. A region-of-interest analysis was also conducted using dorsal and ventral DMN masks obtained from a previous study. At the whole brain level, precuneus and hippocampus had increased activity for more vivid scenarios compared to less vivid scenarios, and greater activity was was seen in the ventromedial prefrontal cortex (vmPFC) and ventral striatum (VS) for positive scenarios compared to negative scenarios. ROI results confirmed that valence modulates activity in the dorsal, but not ventral DMN, while vividness modulates activity in the ventral, but not dorsal DMN. These results show that different aspects of imagination differentially modulate separate nodes of the default mode network.

Disclosures: T. Parthasarathi: None. **J.W. Kable:** None.

Poster

360. Perception and Imagery: Visual Processing

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Discovery Award

NSERC CGS M

Title: A gaze into the mind's eye: gaze as an indicator of cortical reinstatement during mental imagery

Authors: *M. BONE, M. ST-LAURENT, C. DANG, D. MCQUIGGAN, J. D. RYAN, B. R. BUCHSBAUM;
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Abstract: When a previously encountered image is visualized, the spatiotemporal pattern of fixations observed during encoding is often reinstated, even in the complete absence of visual input. Modern theories posit that this phenomenon of "looking at nothing" is the consequence of a reciprocal interplay between spatial attention and visual recollection. Specifically, such theories claim that fixation reinstatement facilitates, and is facilitated by, the retrieval of detailed internal memory representations. However, there currently exists no neuroscientific evidence supporting this claim. Using a functional magnetic resonance imaging (fMRI)-based measure of neural reinstatement as a proxy for internal memory representations, we found significant correlations between fixation reinstatement and neural reinstatement during visualization, both between- and within-subjects. Additionally, we found a strong correlation between memory performance and neural reinstatement, which mediated a non-significant correlation between memory performance and fixation reinstatement. These findings provide the first neuroscientific evidence that the reinstatement of overt visuospatial attention facilitates and/or is facilitated by the recollection and visualization of naturalistic scenes in the absence of visual feedback. A secondary goal of our research was the development of a fixation similarity measure optimized for visualization tasks. We discovered that a novel measure based solely on fixation angle outperformed a traditional location measure, which we argue supports a chunk-based theory of fixation reinstatement.

Disclosures: M. Bone: None. **M. St-Laurent:** None. **C. Dang:** None. **D. McQuiggan:** None. **J.D. Ryan:** None. **B.R. Buchsbaum:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant 101759/Z/13/Z

Wellcome Trust Grant 091593/Z/10/Z

Title: Explaining the role of the hippocampus in verbal memory

Authors: *I. A. CLARK, E. A. MAGUIRE;
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Abstract: Patients with bilateral hippocampal damage are impaired on word-pair associate tests. Given the visuospatial bias of many theories of hippocampal function, accounting for a verbal deficit such as this is challenging. Verbal tasks in the extant literature have typically used word stimuli that represent imageable entities. One possibility, as proposed by the scene construction theory of the hippocampus, is that healthy individuals may automatically use scene imagery during the processing of imageable verbal material, for example, constructing a scene to represent a word pair. As such, word pairs that are less imageable, such as those comprising abstract words, should not engage the hippocampus. Here, we explored verbal processing in healthy participants who were asked to encode words, either in pairs or singly, while undergoing functional MRI scanning. Stimuli were scene words (monastery - cove), object words (dragon - crown) or abstract words (kind - neutral). Words were matched on a wide range of variables including word frequency, age of acquisition, valence and length. Following scanning, participants undertook a memory test and were debriefed regarding their encoding strategies during scanning. fMRI analyses showed that scene pairs activated the hippocampus in comparison to either object or abstract pairs, and object pairs activated the hippocampus when compared to abstract pairs. Further analyses disclosed that associative binding alone was not sufficient to account for these effects because abstract pairs compared to single abstract words did not reveal increased hippocampal activity. Of note, all of the fMRI findings persisted when subsequent memory performance was taken into account. Debriefing revealed prominent scene imagery use for scene and object pairs, suggesting hippocampal involvement for the imageable word pairs via scene imagery. Moreover, the hippocampus was specifically engaged by single words when scene imagery was employed. Overall these results accord with the scene construction theory in suggesting that the hippocampus might be involved in processing words when scene imagery is evoked. Patients with bilateral hippocampal damage are reportedly unable to construct scenes in their imagination, which may explain their deficits on some verbal memory tasks.

Disclosures: I.A. Clark: None. **E.A. Maguire:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Support: FRQNT-Team Grant # 181390 (G.W., P.J., V.B)

NSERC Discovery Grant # 436140-2013

FRQNT-Early Researcher Grant # 181515 (G.W.)

Title: The impact of video game experience on hippocampal grey matter integrity

Authors: *G. WEST[1,2], K. KONISHI[3], M. DIARRA[2], J. BENADY-CHORNEY[3], B. DRISDELLE[2], L. DAHMANI[3], D. SODUMS[3], F. LEPORE[2], P. JOLICOEUR[2], V. BOHBOT[3]; [1]Psychology, [2]Univ. of Montreal, Montreal, QC, Canada; [3]Douglas Res. Inst., Montreal, QC, Canada

Abstract: The hippocampus is critical to healthy cognition, yet results in the current study show that action video game players have reduced grey matter within the hippocampus. A subsequent longitudinal training experiment demonstrated that first-person shooting games reduce grey matter within the hippocampus and increase grey matter within the amygdala in participants using compensatory non-spatial memory strategies. Conversely, participants who use hippocampus-dependent spatial strategies showed increased grey matter in the hippocampus after training. A control group that trained on 3D-platform games displayed growth in either the hippocampus or the functionally connected entorhinal cortex. These results show that video games can be beneficial or detrimental to the hippocampal system depending on the individual who is playing and the genre of the game.

Disclosures: G. West: None. **K. Konishi:** None. **M. Diarra:** None. **J. Benady-Chorney:** None. **B. Drisdelle:** None. **L. Dahmani:** None. **D. Sodums:** None. **F. Lepore:** None. **P. Jolicoeur:** None. **V. Bohbot:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

NWO 406-14-114

Title: Neural dynamics underlying the acquisition of wikipedia concepts

Authors: *L. S. SCHURMANN, S. THEVES, N. DE HAAS, A. R. BACKUS, C. F. DOELLER;
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Abstract: How do we represent knowledge about the world? Recent fMRI studies have reported learning-related changes of neural activity in medial temporal and prefrontal structures during concept learning but the vast majority of these experiments used classical lab stimuli and tasks, thus limiting the generalizability of the findings to real-world settings. Here we examined the neural dynamics underlying the acquisition of realistic knowledge by assessing changes of neural representations of pseudo-words in combination with a Wikipedia concept learning task. In a first fMRI experiment, participants learned simple associations between pseudo-words, which were carefully controlled for aspects such as length and transition frequency of their subsyllabic elements, before and after subjects viewed the same words in isolation in separate 'pre-learning' and 'post-learning' scan sessions. We observed increased neural pattern similarity for associated pseudo-words in the hippocampus as a consequence of learning. In a second fMRI study, these pseudo-words were embedded in two different Wikipedia texts of concepts unknown to our participants by replacing key words of the original texts. Participants learned the pseudo-words' meaning by their semantic embedding in either Wikipedia text. Specifically, by embedding probe pseudo-words in the texts, we were able to compare the neural pattern related to these pseudo-words in controlled 'pre-learning' and 'post-learning' scan sessions before and after the exposure to the Wikipedia texts. Behavior was assessed with a knowledge questionnaire about details of learned concepts and a concept assignment task. Preliminary analyses suggest that pseudo-word representations systematically changed as a function of semantic relatedness in the hippocampus, reflected in increased neural pattern similarity for within-concept relative to between-concept comparisons. In sum, our study takes a first step towards closely simulating the acquisition of realistic, everyday knowledge and can shed new light on how the human brain learns new information within a putative neural concept space.

Disclosures: L.S. Schurmann: None. **S. Theves:** None. **N. de Haas:** None. **A.R. Backus:** None. **C.F. Doeller:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Sloan Foundation

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CFI

Title: Goal-directed search in natural scenes improves with explicit, MTL-dependent long term memory

Authors: *S.-A. YOO[1,2], R. S. ROSENBAUM[1,2,6], J. K. TSOTSOS[2,3], M. FALLAH[2,4,5], K. HOFFMAN[1,2];
[1]Psychology, [2]Ctr. for Vision Res., [3]Electrical Engin. and Computer Sci., [4]Kinesiology and Hlth. Sci., [5]Canadian Action and Perception Network, York Univ., North York, ON, Canada; [6]Rotman Res. Institute, Baycrest, Toronto, ON, Canada

Abstract: Visual search in natural scenes changes over time and with scene repetition: fixations last longer and cover fewer regions of the scene, focusing on informative regions. Typically these changes are measured shortly or immediately after initial viewing, and they are often measured during incidental search ('free viewing'). Goal-directed search, e.g. locating an object embedded in the scene context, also leads to search efficiency, measured as time to detect the target object. The detection-time benefit is seen even after lags of days or weeks from initial viewing, but it is not clear if other search repetition effects are preserved. Furthermore, the detection-time benefit is seen when the object and scene are explicitly remembered and only with intact medial temporal lobe function, whereas some of the other repetition effects may be implicit and/or independent of MTL function. To determine which repetition effects of search are preserved in long-term memory and specific to explicit recall, we analyzed the eye movements of 10 young adults viewing novel and repeated scenes, presented across different memory intervals, and grouped by explicit memory (target-and-scene remembered, target forgotten, target and scene forgotten). To determine which effects depend on MTL integrity, we further tested an amnesic with bilateral hippocampal damage and 7 age-matched older-adult controls. In the training session and two separate testing sessions, participants completed flicker change detection trials, identifying an object in a scene that had been manipulated, while their eye movements were monitored with an infrared eye tracker. After each trial, participants reported whether they had seen the scene before and whether they remembered the target (i.e., changing object). When young adults remembered the targets, fixation durations became shorter than when they saw the scenes for the first time or when the targets were forgotten. Gaze was directed closer to the target region on repeated trials even before detection, but only for explicitly remembered targets. Both effects were seen even after a month lag from the initial presentation, suggesting that they were elicited during explicit, long-term memory recall. In contrast, the amnesic did not show any search improvements with repetition, whereas his age-matched controls showed similar patterns to those seen in young adults. The results suggest that several scanning changes in goal-directed search arise from explicit memory, involving the medial temporal lobe.

Disclosures: **S. Yoo:** None. **R.S. Rosenbaum:** None. **J.K. Tsotsos:** None. **M. Fallah:** None. **K. Hoffman:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: NIH F32-EY023162

NIH R01-EY021755

Title: Action-based prediction for known and novel associations between real-world objects

Authors: ***N. C. HINDY**, E. W. AVERY, N. B. TURK-BROWNE;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Every time we open a door, bite an apple, or kick a ball, our actions afford expectations about what we will see next. Such action-based expectations may be generated by the hippocampus: Making an action to a familiar cue stimulus may reactivate a conjunctive representation that includes absent but associated outcome stimuli, allowing these stimuli to be reinstated in sensory areas. Indeed, we have shown in prior studies with novel objects and simple actions that such pattern completion in the hippocampus supports predictive coding in visual cortex (Hindy et al., 2016, *Nature Neurosci*), including through enhanced functional connectivity between the hippocampus and visual cortex for predictive actions (Hindy et al., in prep). Although experimentally convenient to train novel action-outcome associations, pre-existing knowledge accrued over longer timescales can also be leveraged to generate predictions about action outcomes. Such knowledge can be represented outside of the hippocampus (e.g., semantic associations between actions and objects), and thus we hypothesized that the mechanism for prediction based on this knowledge may differ. To test this hypothesis, we collected high-resolution fMRI data while participants made familiar actions involving either Known or Novel associations between real-world objects. For each type of association, actions were either predictive of the outcome (e.g., to “roll” or “fold” a dollar bill in the Known condition) or were non-predictive of the outcome (e.g., to “drop” or “toss” a coin). Stimuli for both conditions were drawn from the same set of object photographs, but the Novel associations were arbitrary (e.g., to “point” or “wave” to transform a dollar bill into a carrot or a newspaper), and learned in a training session three days prior to the scan. After removing confounding variables as well as stimulus-evoked responses, we examined temporal correlations in BOLD activity between visual

cortex, the hippocampus, and medial temporal lobe cortex. Preliminary analyses reveal enhanced background connectivity between the hippocampus and visual cortex for predictive actions regardless of the timescale over which the associations have been known, as well as enhanced connectivity between medial temporal lobe cortex and visual cortex for known associations regardless of predictability of the outcomes. These findings suggest that the hippocampus may continue to mediate prediction even for long-term semantic associations, at the same time that adjacent cortical areas become more involved in other associative aspects of object perception.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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German Research Council (DFG)

Title: Visual responses of single neurons in the human medial temporal lobe are modulated by context

Authors: ***M. BAUSCH**[1], J. NIEDIEK[1], T. P. REBER[1], S. MACKAY[1], J. BOSTRÖM[2], C. E. ELGER[1], F. MORMANN[1];
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Abstract: The medial temporal lobe (MTL) is crucial for the formation, consolidation, modulation and retrieval of declarative memories. A population of neurons within the human MTL has been shown to respond to abstract concepts attributed to particular stimuli. These cells display similarities to the well-characterized place cells in rodents. Just as the firing of place cells is thought to reflect locations on a cognitive navigational map, they are thought to reflect "locations" on a cognitive semantic map, that is, concepts. These concept cells are thought to be building blocks of episodic memories. It has been shown that rodent place cells are modulated by contextual information such as surrounding colors, shapes, odors or even motivational states, but it is unclear whether human concept cells likewise show a modulation by context.
During 31 experimental sessions, we recorded from 2039 units in the amygdala, parahippocampal cortex, entorhinal cortex, and hippocampus of 11 neurosurgical patients to quantify the effect of contextual information on (visually selective) human MTL neurons.

Subjects compared different pairs of sequentially presented images with respect to five different questions. Three of the questions required semantic memory (“More expensive” or instead ”Older?”,”Bigger?”), one required reward/valence processing (“Which do you like better?”), one was perceptual (“Brighter image?”) and one required episodic memory retrieval (“Last seen in real life?”). The images were chosen from a set of 4 stimuli pre-screened to elicit selective visual responses. The five questions were combined with the 12 possible image pairs in pseudo-random order (5 blocks, total of 300 trials).
In a two-way ANOVA with factors stimulus identity and type of question performed with an alpha level of 0.001, we identified 298 units (14.62 %) with a main effect of stimulus identity only, 81 units (3.97%) with a main effect of question type only, 40 units (1.96%) showing both main effects simultaneously, and 34 (1.67%) units showing an interaction. All of these numbers significantly exceed chance levels for this alpha value as confirmed by binomial tests ($p<.00001$ in all cases). The finding of units showing both main effects argues in favor of a contextual modulation of visually selective MTL neurons. This modulation was far more pronounced in the hippocampus (3.07%) and entorhinal cortex (3.07%) than in the amygdala (1%) and parahippocampal cortex (0.6%).
In conclusion, visually responsive neurons are modulated by presentation context, particularly in the hippocampus and entorhinal cortex.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Volkswagen Foundation

Title: Encoding of auditory-visual associations by single neurons in the human medial temporal lobe

Authors: ***M. S. KEHL**[1], A. RACZ[1], J. NIEDIEK[1], T. P. REBER[1], M. BAUSCH[1], B. SAMIMIZAD[1], J. BOSTRÖM[2], C. E. ELGER[1], F. MORMANN[1];
[1]Dept. of Epileptology, Univ. of Bonn Med. Ctr., Bonn, Germany; [2]Dept. of Neurosurgery, Univ. of Bonn Med. Ctr., Bonn, Germany

Abstract: The medial temporal lobe (MTL) plays a crucial role in the learning of new associations. Recordings of single units in humans have provided evidence for rapid changes in the firing of single neurons after learning contextual associations using composite pictures (Ison et al., Neuron 87:220, 2015). Here we investigated whether these findings generalise to cross-modal sensory associations. To this aim we analyzed changes in the firing of single neurons after learning associations between visual and auditory stimuli.
We recorded 894 units in the MTL (hippocampus, amygdala, entorhinal cortex, parahippocampal cortex) of up to now 9 patients. Based on a preceding screening session, we selected 4-8 response-eliciting stimuli and paired each of these with a short (1.5 sec) instrumental melody. After an initial screening for responsiveness to the individual sounds and images (10 trials), the composite stimuli (picture and sound) were presented simultaneously, and patients were asked to learn the associations. In a subsequent phase of the experiment, only sounds were presented and patients had to identify the associated pictures. Finally, sounds and pictures were presented individually once again to assess the effects of the learned associations on neural firing.
We identified 22 visually responsive units based on a Wilcoxon rank-sum test between baseline and response interval ($p<0.005$). Comparison of the firing rates of visually responsive units during presentation of the associated sounds before and after learning showed no obvious change in firing rate. Among all visually responsive units, we found no unit that developed a specific response to the associated sound based on the criterion stated above.
Our findings suggest that cross-modal encoding of associations (visual-auditory) is not strongly encoded by neurons in the MTL when arbitrary stimuli are paired.

Disclosures: **M.S. Kehl:** None. **A. Racz:** None. **J. Niediek:** None. **T.P. Reber:** None. **M. Bausch:** None. **B. Samimizad:** None. **J. Boström:** None. **C.E. Elger:** None. **F. Mormann:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: Volkswagen Foundation

German Research Council (DFG)

Title: Visually and memory-selective single neurons in the human medial temporal lobe during a spatial memory task

Authors: *S. MACKAY[1], T. P. REBER[1], M. BAUSCH[1], J. BOSTRÖM[2], C. E. ELGER[1], F. MORMANN[1];
[1]Epileptology, [2]Neurosurg., Univ. of Bonn Med. Ctr., Bonn, Germany

Abstract: Certain cells in the human medial temporal lobe (MTL) have been reported to respond to specific concepts in a selective and semantically invariant manner. Such cells have been termed semantic neurons or concept cells. Since the MTL is essential for declarative memory formation, the observed semantic selectivity in this region may be related to, or even facilitate mnemonic processes. In this study, we investigated stimulus selectivity and memory effects in human MTL neurons during a spatial memory task.
The visual stimuli used in this paradigm (5 to 8 pictures) were selected from a preceding screening session so as to elicit selective responses in one or more units. Our memory task comprised several runs each of which consisted of an encoding part, a distraction task and a retrieval part. The number of stimuli presented per run was continuously adapted to the memory performance in order to obtain similar numbers of correct and incorrect trials. The encoding phase of each run consisted of the consecutive presentation of a subset of the stimuli, each displayed at a different position in a 3x3 grid. The distraction task was 15 seconds of backward counting in steps of 3. During retrieval, the images were presented consecutively and in shuffled order below the grid, and subjects were instructed to tap the location in the grid where the respective image had been shown during encoding. Object-location associations were randomized at the beginning of each run. The number of runs was restricted by a fixed total duration of the paradigm (35min).
We recorded 17 sessions of the paradigm from 5 epilepsy patients with microwires implanted in the amygdala, parahippocampal cortex, entorhinal cortex and hippocampus. For each unit we computed a 2-way ANOVA on firing rates during encoding trials with the factor stimulus identity and subsequent memory (remembered / forgotten). At an alpha level of 5% we observed a significant main effect of subsequent memory in 95 cells (7.2%, $p < .01$, binomial test). A significant main effect of stimulus identity was found in 195 cells(17.7%, $p < .01$). The number of units for which we found a significant interaction was at chance level (62 cells, 4.7%, $p = .7$). Hippocampus and entorhinal cortex were the only regions in which the number of memory-selective units was significantly above chance level (7.2%, $p < .01$ and 9.8%, $p < .01$, respectively).
Based on our findings, the absence of an interaction effect suggests distinct neuronal populations of memory and visual selectivity.

Disclosures: S. Mackay: None. **T.P. Reber:** None. **M. Bausch:** None. **J. Boström:** None. **C.E. Elger:** None. **F. Mormann:** None.

Poster

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Support: James S. McDonnell Foundation

NSERC

Title: Perceptual and conceptual object information is integrated in perirhinal cortex

Authors: *C. B. MARTIN[1], L. MAN[1], D. M. DOUGLAS[1], R. N. NEWSOME[2], M. D. BARENSE[1];
[1]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; [2]Rotman Res. Inst., Toronto, ON, Canada

Abstract: Neuropsychological and fMRI research has firmly established a link between the anterior temporal lobes, including perirhinal cortex (PrC), and the abstraction of conceptual object information. For example, representational similarity reflected in patterns of PrC BOLD activity is predicted by independent behavioral judgments of conceptual similarity between objects. However, these results remain challenging to interpret as most studies confound higher level conceptual and perceptual similarity. Specifically, objects that are conceptually related also tend to be perceptually similar (e.g., apple and cherry) and conceptually dissimilar objects are typically perceptually dissimilar (e.g., apple and shoe). The importance of this point is underscored by a substantial body of research that implicates PrC in the processing of perceptually-based discriminations between complex objects, even when stimuli have limited semantic meaning. In the current study, we used representational similarity analysis (RSA) with fMRI data to examine the extent to which the processing of conceptual and perceptual object information is related in different aspects of the ventral visual pathway, including PrC. As a first step, we generated models that captured the perceptual and conceptual similarity among 40 objects. A normalized perceptual similarity model was defined using pairwise perceptual similarity ratings. A normalized conceptual similarity model was defined using cosine similarities derived from independent feature generation data. Critically, the perceptual and conceptual models were not significantly correlated with one another, indicating that these dimensions were orthogonalized across objects. In a second stage, an independent group of participants completed a property verification task that encouraged either perceptual or conceptual processing of objects during fMRI scanning. We used RSA to quantify similarity in BOLD responses among all objects separately for both task contexts (i.e., perceptual and conceptual) and to subsequently assess how well each behaviorally-based model fit the obtained brain data. Most importantly, perirhinal cortex was the only region in the ventral visual stream to

show sensitivity to both perceptual and conceptual object information regardless of task context. This result suggests that perirhinal cortex plays a critical role in the integration of high-level perceptual and abstract conceptual object information.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH P50 MH094263

Title: Tracking dynamic connectivity shifts between memory systems during context dependent rule learning

Authors: *A. E. CHANG, A. S. WHITEMAN, C. E. STERN;
Ctr. for Memory and Brain, Boston Univ., Boston, MA

Abstract: Animal research suggests that prefrontal and striatal patterns of activity contribute to learning across differential time spans (Pasupathy & Miller 2005, *Nature*). The goal of this study was to investigate, in humans, how patterns of brain activity drive individual differences in rule-learning behavior. We developed a context-dependent rule-learning task in which object pairs were presented in one of four locations (spatial contexts) on a screen. Participants (n = 26) learned to associate different object pairs with spatial contexts through trial and error, and were scanned using fMRI during learning. Since acquisition of this task required learning a set of context dependent rules, we predicted that interactions between medial temporal, prefrontal, and basal ganglia regions would mediate successful learning strategies. Our analysis focused on understanding temporal patterns of connectivity at the individual subject level. To do this, we implemented a windowed functional connectivity analysis that tracked time-evolving relationships between brain regions using a sparse partial correlation method. This analysis allowed us to capture fairly complex relationships between regions and observe which evolving patterns of connectivity predicted learning performance within participants. Preliminary results suggest that rapid learners engaged medial temporal and orbitofrontal connectivity during early exploration that transitioned to connectivity within prefrontal cortex post-acquisition. Slower learners, by contrast, initially engaged basal ganglia structures that transitioned to medial

temporal and prefrontal cortex connectivity post-acquisition. These results suggest that shifts in connectivity patterns predict changes in individual learning behavior.

Disclosures: **A.E. Chang:** None. **A.S. Whiteman:** None. **C.E. Stern:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 361.11/KKK68

Topic: H.02. Human Cognition and Behavior

Title: The hippocampus and generalization: investigating the underlying mechanisms using 7T fMRI

Authors: ***R. KOSTER**[1], Y. CHEN[2], M. CHADWICK[1], D. BERRON[2,3], A. BANINO[1], E. DUZEL[2,3,4], D. KUMARAN[1,4];
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Abstract: Recent work suggests that the hippocampus supports performance in tasks that involve generalization across episodes, in addition to its widely acknowledged role in episodic memory. The mechanism by which it does this remains an open question: whilst generalization depends on the appreciation of the commonalities among episodes, episodic memory emphasizes the uniqueness of individual experiences. Two classes of models have been proposed: one that forms blended representations of related memories during encoding, and one that encodes episodes in a pattern separated fashion with generalization emerging through the interaction of multiple memories at the point of retrieval.
In this study we scanned participants whilst they performed the paired associate inference (PAI) task to distinguish between these models. Subjects viewed item pairs, with each pair consisting of one face and either an object or a scene. Crucially, each pair of items (e.g. pair AB) had a linked pair with one overlapping element (e.g. pair BC). Despite the fact that A and C are never presented together, subjects are capable of inferring the association between A and C via the shared B item. In our version of this task, items A and C were always faces, and item B was either an object or a scene.
Importantly, the two classes of models make divergent predictions about neural activity during test trials. Specifically, the retrieval-based model REMERGE predicts that reactivation of the intervening B item (e.g. a scene) during an AC inference trial should a) be linked to successful performance b) be evident in neural activity on the input (superficial) layer of the entorhinal

cortex (ERC) due to recirculation of the output of the hippocampal system as a new input. We leveraged high resolution 7T fMRI to empirically test these predictions, through its ability to distinguish between neural activity in different layers of the ERC. Results suggest that during AC test trials, reactivation of the intervening stimulus (e.g. scene) is present in the superficial layer of the ERC layer that provides input to the hippocampus -- despite the fact that only faces were shown on the screen. Further, the level of ERC input layer reactivation correlates with successful performance on AC trials. Our results are consistent with the retrieval-based REMERGE model that views generalization as an emergent phenomenon supported by big loop recurrence within the hippocampal system.

Disclosures: **R. Koster:** None. **Y. Chen:** None. **M. Chadwick:** None. **D. Berron:** None. **A. Banino:** None. **E. Duzel:** None. **D. Kumaran:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 361.12/KKK69

Topic: H.02. Human Cognition and Behavior

Title: Identification of nucleus reuniens in humans using probabilistic tractography

Authors: *T. A. ALLEN[1], P. C. REEDERS[1], R. P. VERTES[2], A. T. MATTFELD[1]; [1]Dept. of Psychology, Florida Intl. Univ., Miami, FL; [2]Ctr. for Complex Systems and Brain Sci., Florida Atlantic Univ., Boca Raton, FL

Abstract: The nucleus reuniens of the thalamus is a critical structure for memory. Anatomically, the nucleus reuniens bi-directionally connects the medial prefrontal cortex and hippocampus in rodents (for review Vertes et al., 2015) and primates (Anderson et al., 2015). Neurophysiological activity modulation in the nucleus reuniens can both enhance and impair details of contextual fear memory (Xu and Südhof, 2013), while inactivation of nucleus reuniens reduces trajectory-specific place cell coding (Ito et al., 2015). However, little is known about the function of midline thalamic nuclei (i.e., reuniens, rhomboid, paratenial, and paraventricular nuclei) in humans. In part, this is due to the difficulty in identifying midline thalamic regions from structural MRI data alone. Here, we attempted to identify the nucleus reuniens and associated midline thalamic nuclei using diffusion weighted imaging data and probabilistic tractography tools. The thalamus served as the seed for 5000 random walks (per voxel within each seed) toward cortical and subcortical targets. Results from the probabilistic tractography provided a value relating to the number of walks that terminated in a particular target at each seed voxel. We used a *k*-means algorithm to cluster voxels according to target connectivity feature space. This

process revealed two prominent clusters of midline thalamic regions that connect to the hippocampus, entorhinal cortex, rostral anterior cingulate cortex, and medial orbital frontal cortex. Next, the functional relevance of the midline thalamic clusters were tested using seed style resting state functional connectivity analyses. This analysis revealed correlated activity in the hippocampus and medial prefrontal cortex regions, confirming the functional relevance of the structural connectivity patterns of these midline thalamic regions, and consistent with anatomical and neurophysiological data on the nucleus reuniens in rodents. Overall, these results provide a data-driven method for the identification of the nucleus reuniens and other midline thalamic nuclei in humans using diffusion weighted imaging offering an opportunity to explore behavioral relevance.

Disclosures: T.A. Allen: None. **P.C. Reeders:** None. **R.P. Vertes:** None. **A.T. Mattfeld:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 361.13/KKK70

Topic: H.02. Human Cognition and Behavior

Title: Sequence memory predicts temporal reward discounting and both activate medial prefrontal cortex and medial temporal lobe regions

Authors: *P. C. REEDERS, T. A. ALLEN, A. T. MATTFELD;
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Abstract: Temporal reward discounting is related to temporal foresight and episodic future thought. In temporal reward discounting subjects are given the choice of a small immediate reward or a larger delayed reward. The length of delay at which the immediate reward is preferred half of the time is called the *indifference point*. However, the cognitive and neurobiological mechanisms linking episodic future thinking to temporal reward discounting are currently unknown. We suspect that medial prefrontal cortex (mPFC) and medial temporal lobe (MTL) regions play an explicit role by way of sequence memory capacities. Memory for sequences of events is critical for episodic memory and depends on mPFC and MTL regions. First, we tested if there is a relationship between temporal reward discounting and sequence memory. In temporal reward discounting, subjects (n = 31) chose between a small immediate reward ($0.10) and a larger delayed reward ($0.15) in blocks of 15 trials. Delays increased over blocks (0-128 sec). Delay discounting rates were fitted to a sigmoidal curve and the indifference point for each subject was estimated. The same subjects were tested on the sequence memory

task (Allen et al., 2014). This task includes a *sequence detection* stage consisting of predictable sequences (e.g., the letters ABCDEF), and a *sequence memory* stage consisting of novel fractal image sequences that must be learned. If an image was "in sequence" subjects were instructed to hold down the button for 1sec, if it was "out of sequence" they had to release the button before 1sec. Successful sequence memory was demonstrated if subjects responded accurately to both "in sequence" and "out of sequence" items. All subjects showed significant sequence detection and sequence memory (P's < 0.01). Regression analysis was conducted to determine if the indifference point could be predicted by sequence memory levels. A significant positive relationship was observed $F(1,31) = 5.87$, $P < 0.05$. This suggests that sequence memory is related to temporal reward discounting. A control analysis showed that sequence detection levels were not predictive of indifference points, controlling for extraneous behavioral and cognitive aspects of the task. Second, to explore the neurobiological substrates of temporal reward discounting and sequence memory we acquired BOLD fMRI data. Structural and tractography data were also collected. We found activation in the medial temporal lobe and the medial prefrontal cortex in both tasks. Overall, these results suggest temporal reward discounting relies on sequence memory and a mPFC-MTL network.

Disclosures: P.C. Reeders: None. **T.A. Allen:** None. **A.T. Mattfeld:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: Volkswagen Foundation

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FP7 602102 EPI-TARGET

Title: Activity of semantically invariant neurons in the human MTL during LFP ripples during sleep

Authors: *J. NIEDIEK[1], T. P. REBER[1], H. GAST[1], J. BOSTRÖM[2], V. A. COENEN[2], C. E. ELGER[1], F. MORMANN[1];
[1]Dept. of Epileptology, [2]Stereotaxy and MR based OR Techniques, Dept. of Neurosurg., Univ. of Bonn, Bonn, Germany

Abstract: The hippocampus is a necessary structure for the encoding of episodic memories, which can be defined as our ability to mentally relive past experiences.
Electrophysiological research in rodents and studies based on fMRI in humans have contributed to our understanding of episodic memory, but it is still unknown by which mechanism neurons in the human medial temporal lobe (MTL) enable episodic memory processes.
In rodents, sequences of activity of place-cells (spatially selective cells) during exploration of a maze resemble sequences of activity of the same cells during subsequent slow-wave sleep. These re-activations are linked to sharp-wave ripples, which are stereotypical LFP events. Disruption of sharp-wave ripples impairs spatial learning.
In humans, neurons that respond selectively to the semantic content of visual stimuli have been identified. These neurons respond to the presentation of a picture or the written name of, for example, a certain well-known person, irrespective of visual features of the presentation. These "semantic neurons" might constitute a more abstract version of rodent place-cells. Assuming some degree of analogy between rodent place-cells and human semantic neurons, we investigated similarities between neural mechanism of spatial learning in rodents and episodic memory in humans. More specifically, we hypothesized that human semantic neurons, when sequentially activated during a behavioral episode, will be re-activated in the same sequence during subsequent sleep, possibly during specific LFP events resembling sharp-wave ripples.
We used micro-electrode recordings from the MTLs of 22 epilepsy patients (55 sessions) during presurgical monitoring. We identified semantically invariant neurons in all patients. We created simple stories (customized to each individual stimulus set) that involved the identified response-eliciting stimuli. Patients memorized the stories, which involved several iterations of reading. We recorded unit activity throughout learning, recall, and subsequent sleep.
We used a data clustering approach to identify different classes of LFP events. Sleep stages were identified using standard polysomnography.
During recall, we observed internally generated activations of semantic neurons prior to vocalization. During sleep, we identified sharp-wave ripple candidate LFP events. These events occurred more frequently during NREM sleep than during other sleep stages, and the activity of semantic neurons was significantly linked to these LFP events.
Our findings thus provide evidence in favor of the hypothesis that semantic neurons contribute to episodic memory formation and consolidation.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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Title: Features in prior night's sleep relates to changes in memory representations

Authors: *E. COWAN[1], A. LIU[2,3], S. KOTHARE[2,3], O. DEVINKSY[2,3], L. DAVACHI[1,4]; [1]Ctr. For Neural Science/ New York Univ., New York, NY; [2]NYU Langone Sch. of Med., New York, NY; [3]Comprehensive Epilepsy Ctr., New York, NY; [4]Dept. of Psychology, New York Univ., New York, NY

Abstract: Theories of systems memory consolidation posit that memories are stabilized as they become more distributed throughout the cortex. Sleep has been linked with successful memory consolidation, and evidence suggests particular features in the architecture of sleep may relate to sleep-dependent memory enhancements. Recent evidence suggests a 24-hour delay promotes more distributed memory traces, as measured by greater hippocampal-cortical functional connectivity, in a manner predictive of subsequent behavioral resistance to forgetting, indicating a specific role in memory stabilization. However, it remains unknown how aspects of sleep architecture might be related to the representation of memory traces the next day, and how this relates to behavioral measures of memory. To investigate this relationship, we designed a three-day experiment utilizing overnight polysomnography recordings, fMRI, and behavior. Subjects encoded two lists of word-image pairs twice, either with an intervening period of overnight sleep (Sleep List), or a brief wakeful period (New List), such that the lists differed in the opportunity for consolidation processes. During the re-study session, subjects were presented with the previously seen word-image pairs, and a new list of pairs (Single Study List) while in the scanner. Cued source recall was probed immediately following the scan and after a 24-hour delay, providing a measure of memory stability over time. Using a multivariate pattern similarity analysis, we examined the representations of the trials from each encoding list. Overall, we found evidence for greater pattern separation within the Sleep and New Lists, relative to the Single Study List, in medial temporal lobe and cortical regions. Furthermore, pattern

differentiation in the Sleep List was greater for subsequently remembered, compared to forgotten, pairs in the hippocampus. By contrast, those same trials showed greater overlap in patterns in cortical regions. Sleep seems to be related to this change in the representations; in the hippocampus, pattern separation for remembered pairs is correlated with the duration of NREM sleep only for the Sleep List. These results suggest that consolidation may differentially effect the representations in hippocampal and cortical regions.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Program#/Poster#: 361.16/LLL3

Topic: H.02. Human Cognition and Behavior

Title: Examining the functions of the hippocampus using multimodal neuroimaging

Authors: *N. KESHAVARZIAN[1], **K. MCWILLIAMS**[2], J. PETERSEN[2], J. WILLIAMS[2], K. OSIPOWICZ[2];
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Abstract: Here, we present the findings of a number of MRI studies examining the function of the hippocampus. Functional magnetic resonance imaging (fMRI), resting state connectivity (rsfMRI) and diffusion tensor imaging (DTI) were used to map the activation, and structural and functional connectivity of the hippocampus. To activate the hippocampus, a number of tasks were evaluated, including: manipulations of emotional salience of words, sounds, pictures, and smells. Furthermore, DTI was conducted to examine the anatomical connectivity of white matter pathways associated with the fMRI activations. To evoke olfactory memories, participants were asked to envision the smell of a particular visual scene. The smell imagery paradigm revealed full bilateral hippocampus activation. We observed hippocampal resting state functional connectivity with several brain regions involved in the processing and regulation of emotionally salient events including the amygdala, regions of the default mode network. For DTI, the relationship between memory performance and tractography appeared to be primarily driven by memory for emotional stimuli. The hippocampal involvement was primarily posterior and the left hippocampus was more integrated than the right. The results help to find a general clinical method to examine the integrity and function of the hippocampus during pre-surgical planning in cases of temporal lobe seizure disorder.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Program#/Poster#: 361.17/LLL4

Topic: H.02. Human Cognition and Behavior

Title: Mediation of musical expectancies through hippocampus and amygdala interactions

Authors: *D. OMIGIE[1], S. SAMSON[2];
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Abstract: Introduction: Numerous studies have linked music-induced emotions to activity in the amygdala (AMYG) and hippocampus (HIPP). However, how these regions respond to (expectations about) unfolding musical structure has seen little research. This is unfortunate, given that musical pitch expectancy is held to be a rich source of music induced emotions. The current study explored the potential role of the HIPP and AMYG in mediating musical expectancies and thereby emotions.
Methods: Electrical activity was recorded from epileptic patients implanted with depth electrodes while they listened to melodies comprising notes characterized in terms of Information content (IC: low IC= expected, high IC=unexpected). We compared responses to low and high IC notes in terms of Granger Causality (GC) and cross-regional Phase Amplitude Coupling (PAC).
Results: GC revealed a more hub-like behavior of HIPP regions during low IC notes. Further, in line with hierarchical models that emphasize frequency specificity of bottom-up (BU) and top-down (TD) interactions: 1) TD flow (HIPP to lateral temporal (LT)) and BU flow (LT to HIPP) tended to be in the theta and gamma range, respectively and 2) PAC revealed HIPP theta phase modulation of gamma power in the LT areas but not the reverse. Finally, in stark contrast to the HIPP, and in line with the notion of AMYG as a salience detector, a more hub-like behavior was seen in the amygdala during the processing of high IC relative to low IC notes.
Conclusions: HIPP and AMYG have different but complementary roles in the processing of (expectations about) musical pitch structure. While HIPP may facilitate subjective feelings of familiarity in response to expected/ low IC notes, the amygdala may facilitate feelings of tension or surprise in response to unexpected high IC ones. The continually changing dynamics of these two regions likely comprise an important neural substrate of everyday music-induced emotions.

Disclosures: **D. Omigie:** None. **S. Samson:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Title: Recall deficits with preserved recognition memory in limbic encephalitis

Authors: *M. LAD[1], S. MULLALY[2], T. KELLY[3], T. GRIFFITHS[2,4];
[1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom; [4]Wellcome Trust Ctr. for Neuroimaging, London, United Kingdom

Abstract: Limbic Encephalitis (LE) is an inflammatory neurological disorder of the medial temporal lobe that causes a memory syndrome. LE with Voltage-Gated Potassium-Channel Complex (VGKC-LE) antibodies preferentially affects the hippocampus: neuro-imaging and post-mortem studies demonstrate hippocampal inflammation with relative sparing of the parahippocampal gyrus and other brain areas. Behaviorally, a syndrome is produced characterized by long-term memory impairment, seizures and behavioral disturbances. We report a study of nine patients with immunologically proven VGKC-LE in which we have carried out a systematic study of cognition including verbal and visual delayed recall and recognition. The work allows assessment of the specific role of the hippocampus in recall and recognition as well as in other aspects of cognition. We assessed pre-morbid intelligence [WTAR], general intelligence [short-form WAIS III], visual perception [VOSP], executive function [Letter and Category Fluency: Trails and Color-Word interference from [DKEFS; Hayling-Brixton], memory [AMIPB and Warrington Recognition Memory; Doors and People], autobiographical memory [AMI] and emotion [DASS-21]. We found consistent and significant deficits in delayed verbal and visual recall but not in recognition memory. This is consistent with models based on a critical hippocampal role in recall but not recognition. Ongoing work is further characterizing the detailed memory phenotype, including analysis of the autobiographical memory interview, and its correlation with structural changes in the medial temporal lobe.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: DFG Pl 248/4-1

Title: Memory integration in patients with hippocampal lesions

Authors: A. PAJKERT[1], C. FINKE[1], Y. SHING[2], M. HOFFMANN[1], W. SOMMER[3], H. R. HEEKEREN[4], *C. J. PLONER[1];
[1]Charité, Berlin, Germany; [2]Univ. of Stirling, Stirling, United Kingdom; [3]Humboldt-Universität zu Berlin, Berlin, Germany; [4]Freie Univ. Berlin, Berlin, Germany

Abstract: Recent research suggests that decision making between novel choice options significantly depends on hippocampus-dependent memory systems. The prevailing hypothesis is that the hippocampus provides highly flexible and associative representations that shape individual decision preferences. However, few patient studies have addressed the interrelationship of hippocampal dysfunction and decision making so far. Here, we investigated effects of hippocampal damage on associative inference, a key mechanism of memory-guided decision making. Five subjects with damage to the right medial temporal lobe and 17 controls were investigated. Subjects learned a set of overlapping associations (A-B- and B-C-associations, i.e. object-face- and face-object pairs) and were then tested for memory of either these associations (‘direct’ trials) or of inferential A-C-associations (‘indirect’ trials). The experiment was run in a blocked design consisting of four encoding/retrieval cycles. Across cycles 1 to 4, in direct trials, patients performed not significantly different from controls (77 vs. 84% correct; $p > 0.07$). By contrast, in indirect trials, patients showed a significant performance deficit (58 vs. 76 % correct; $p < 0.04$), suggesting that performance in indirect trials depended on additional computations that were not required in direct trials. These group differences however changed across cycles. In cycle 1, patients and controls only showed minor performance differences in indirect trials (65% vs. 72%). During the course of the experiment, controls progressively increased their performance up to 80% in cycle 4, while patient performance decreased to 55%. Analysis of RTs and correlation of accuracy between direct and indirect trials for each cycle suggests that all subjects based their performance mainly on retrieval-based mechanisms in cycle 1. As the experiment proceeded, controls increasingly recruited encoding-based mechanisms, while patients failed to do so. These findings show that hippocampal dysfunction indeed affects memory-guided decision making. This deficit is not merely a consequence of impaired associative memory, but also results from dysfunction of an additional integration mechanism that depends on hippocampal integrity. Our data suggest that this

mechanism mainly operates during the encoding of new associations. Thus, deficits in memory-guided decision making may critically depend on the relative contribution of encoding- and retrieval-based mechanisms to associative inference. Our findings further show that these contributions are dynamic and may change substantially even within a single behavioral context.

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Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Title: Trial timing in fMRI designs for pattern information analyses

Authors: M.-A. DE ARAUJO SANCHEZ, A. ADKE, *D. ZEITHAMOVA;
Univ. of Oregon, Eugene, OR

Abstract: In recent years, multivariate fMRI data analysis methods such as multivoxel pattern analysis (MVPA) and representational similarity analysis (RSA) have been increasingly used to decode mental states and stimulus representation. However, little is known about appropriate ways to optimize fMRI designs for these analyses, especially for memory studies in which the number of stimulus presentations is limited. Rapid event-related designs with large number of jittered trials have been the standard for univariate, condition-based analyses. Slow event-related designs may be better suited for estimating individual trial-related activation patterns, but limit the number of trials that can be fit within a given period. Here, we tested how this trade-off affects estimates of item and category representations in a network of brain regions implicated in memory during a memory encoding task. Participants encoded multiple exemplars from two categories (animals and tools) during scanning and completed an old/new memory task afterwards. Five unique sets of 12 stimuli were encoded under 5 different trial timing designs. Designs ranged from slow event-related (2 presentations per stimulus), to rapid (4 presentations per stimulus; one design with fixed trial timing and one with jitter). Each design was repeated with the same stimuli in two consecutive runs and item and category representations were compared across runs. Participants showed comparable memory performance across designs. Category decoding (tools vs. animals) was best in rapid designs with fixed trial length. Item representations (e.g., lion vs. other animals) were more reliable in slow designs than rapid designs. Item representation estimates in slow designs also tracked subsequent item memory. The jittered rapid design underperformed other designs in both category and item

representations, suggesting it may be suboptimal for condition-rich, trial-oriented analyses. Overall, the results show that design optimisation for pattern information analyses may differ from univariate analysis and also vary depending on the level of representation (item versus category) to be decoded.

Disclosures: **M. De Araujo Sanchez:** None. **A. Adke:** None. **D. Zeithamova:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls B-H

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Program#/Poster#: 361.21/LLL8

Topic: H.02. Human Cognition and Behavior

Title: Neural representations of concepts and exemplars: comparing generalization and recognition

Authors: ***C. R. BOWMAN**, D. ZEITHAMOVA;
Univ. of Oregon, Eugene, OR

Abstract: Healthy memory function involves both the ability to remember specific details of past events and the ability generalize across instances to form abstract knowledge that is applicable to novel situations. Whether memory representations of specific instances and abstract knowledge rely on the same memory systems has been of considerable debate. Exemplar models posit that abstract information can be extracted from individual instances and does not need to be maintained as a distinct representation. Prototype models posit that representations of central tendency may form separately or in the absence of exemplar representations. Alternatively, individuals may form both types of representations in parallel and deploy them based on context. The present study used category learning as a representative domain to investigate the relationship between memory specificity and generalization by comparing neural representations of category exemplars and prototypes. Participants were trained to sort exemplars into two categories, then underwent functional MRI during two tests: a recognition test that probed for memory of specific category exemplars and a generalization test that probed for category knowledge applied to novel exemplars. Exemplar and prototype models were fit to behavioral data in individual subjects and then used as regressors in neuroimaging data to identify the neural correlates of each. Initial results reveal robust prototype correlates during categorization, including portions of the hippocampus and ventromedial prefrontal cortex whose activation tracks prototype predictors. In contrast, exemplar correlates were more robust than prototype correlates during recognition. Portions of hippocampus that tracked exemplar predictors during recognition were distinct from those that tracked prototype predictors during categorization.

Taken together, the present study offers evidence of parallel neural representations of specific instances and abstractions that can be used flexibly based on task demands.

Disclosures: **C.R. Bowman:** None. **D. Zeithamova:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: McKnight Foundation Memory & Cognitive Disorders Award

Title: Does novelty detection in single neurons of the human amygdala underlie the word frequency effect in recognition memory performance?

Authors: ***J. KUHN**[1], J. T. WXTED[1], L. R. SQUIRE[1], P. N. STEINMETZ[2];
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Abstract: In the present study we investigated the coding of individual neurons in the hippocampus and amygdala of 20 epilepsy patients undergoing intracranial monitoring during a continuous recognition memory procedure. These brain regions have been posited to be involved in the detection of novel stimuli. In support of this hypothesis, we found that in the left amygdala, 25 out of 161 recorded neurons showed a significant increase in spiking activity in response to novel words as opposed to repeated words, suggesting that these cells are “novelty detectors”. Behaviorally, novelty effects are often observed in recognition memory experiments in that uncommon (low frequency) words are better recognized than common (high frequency) words, a phenomenon known as the word frequency effect. This effect was clearly observed in our behavioral data as well. Is novelty detection at the level of individual neurons related to behavioral novelty effects in either the amygdala or the hippocampus? We tested the seemingly natural prediction that neurons that are responsive to novelty will be more responsive to low frequency words than to high frequency words, but our findings suggest that, if anything, the opposite is true.

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Poster

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Title: Neural substrates of fear-conditioning induced retroactive and selective memory enhancement

Authors: *D. S. YI[1], J. E. DUNSMOOR[1], E. A. PHELPS[1,2], L. DAVACHI[1,2];
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Abstract: We are swarmed by a tremendous amount of information in our everyday lives, but we may not know what information will be valuable in the future. Accommodating such uncertainty, the synaptic tagging and capture model (Frey & Morris, 1997) suggested that we temporally store currently irrelevant information attached with weak learning tags that allows those representations to become enhanced when it acquires relevance through meaningful events in the future. Although the model is supported by a number of behavioral studies, we lack an understanding of the neural mechanism. Our study aims to examine the neural substrates that allow prior representations to be enhanced with new learning. To test this, we adapted a paradigm previously used to show that fear learning selectively and retroactively enhances memory representations (Dunsmoor et al, 2015) that allows us to track the initially weak memory context into the subsequent learning context (Gershman et al, 2013). Participants (n=19) saw a list of animal and tool pictures in the pre-conditioning phase, where their tasks were to classify the pictures. A series of natural scenes were interspersed between pictures in the pre-conditioning phase only, allowing us to look for 'scene' evidence later in the task as an indicator for reinstatement of the pre-conditioning context. In the subsequent conditioning phase, participants studied a novel list of animal and tool picture and were administered a mildly aversive shock during some of the pictures. Critically, shocks were administered only to one category of items during conditioning. A Surprise recognition memory test was administered after 24-h delay in which subjects made old/new decisions as well as confidence ratings. We see that memory is significantly better for items from the conditioning phase that were from the shocked category (CS+) (p<0.001). We also see that item recognition was significantly enhanced for items from the shocked category studied in the pre-conditioning phase (p=0.02). Imaging analyses will focus on the rest period following the conditioning phase to examine whether context reinstatement of the pre-conditioning phase is related to the memory benefit we see for preconditioning items that are related to the CS+ category during conditioning.

Disclosures: **D.S. Yi:** None. **J.E. Dunsmoor:** None. **E.A. Phelps:** None. **L. Davachi:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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Title: Memory, interrupted- Examining multi-voxel representations when perception clashes with the contents of working memory

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Abstract: Although the human medial temporal lobe has been classically viewed as supporting long-term declarative memory, there is a growing appreciation that this region also contributes to performance on perceptual discrimination and working memory tasks. The perirhinal cortex, a medial temporal lobe structure that receives strong inputs from ventral visual stream regions, has been implicated in perception, working memory, and long-term memory for faces and objects. Given that the contents of working memory and ongoing perception can be incongruent with one another, an open question is how representations in this region act in conjunction with representations throughout the ventral visual stream to successfully support both perception and working memory. In the current study, we employed a delayed match to sample task while participants underwent fMRI scanning. A study item was presented, and following a delay, participants were presented with a test item and were asked to indicate with a button press if this item differed from the study item. Critically, interfering items from a different stimulus category than the study item were presented during the maintenance phase of the task, creating conflict between the contents of working memory and ongoing perception. Using representational similarity analysis in conjunction with subject-specific regions of interest, we found that perirhinal cortex and lateral occipital / fusiform regions can be differentiated with respect to representational content during perception of the interfering item. Notably, perirhinal cortex activity best fit a model whereby working memory of the study item was reflected during

interference, whereas fusiform and lateral occipital regions best fit a model where perception of the interfering item was represented during interference. Together, these findings shed light on the division of labor within the extended ventral visual stream in the service of representations that guide memory and perception.

Disclosures: E.B. O'Neil: None. **A.C.H. Lee:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Title: Ibuprofen, but not paracetamol, enhances word recall memory in humans

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Abstract: Paracetamol is a common over-the-counter pain reliever, but the full extent of its effects on cognition remains unknown. Recent reports suggest that paracetamol has an effect on emotional behavior in humans. Paracetamol is metabolized into AM404, which acts as a cannabinoid reuptake inhibitor. This cannabinoid agonism could interfere with memory. In this study, participants (N = 56) were randomly assigned to take a placebo, 600 mg of ibuprofen, or 1000 mg of paracetamol. After 45 minutes, participants were shown a set of 20 words and asked to remember them. Participants then engaged in a mental rotations task, a perspective taking task, and several filler tasks. They were subsequently asked to freely recall the list of words. Drug condition had a significant effect on word recall ($F(2,52) = 5.672$, $p = .006$). Post-hoc tests reveal that the group receiving ibuprofen recalled significantly more words than the placebo group, whereas the group receiving paracetamol did not differ significantly from the control group. In the perspective taking task, males made more accurate judgements than females ($t(40) = 2.114$, $p = .049$). In this task, there was a non-significant trend for an interaction between drug condition and sex ($F(2, 36) = 2.95$, $p = .065$). There was a non-significant trend toward males having higher scores on the mental rotations task ($t(40) = 2.001$, $p = .052$), but there was no interaction between sex and drug condition in this task. Overall, these results suggest that paracetamol interacts with memory and spatial abilities.

Disclosures: M.S. Gallo: None. **K.A. Buckhaults:** None. **R.G. Owens:** None. **P.T. Orr:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Support: NIH T32 AG00096-31

NIH R01 MH102393

Title: Ec cortical thickness predicts cognitive decline in mci in the adni sample

Authors: *J. M. ROBERTS[1], A. J. HOLBROOK[2], N. TUSTISON[3], J. STONE[4], D. GILLEN[2], M. A. YASSA[1];
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Abstract: The entorhinal cortex (EC) has been shown to be one of the earliest sites of neurodegeneration in Alzheimer's Disease (AD). Additionally, recent work has suggested that the lateral portion of the EC (lEC) may be more susceptible to degeneration in the early stages of AD than the medial portion (mEC). In this study, we apply the ANTs cortical thickness algorithm to the ADNI dataset in order to compare longitudinal changes in the lEC and mEC and their relationship to cognitive outcomes. Using a hierarchical regression model, we find that baseline thickness and changes in thickness in both lEC and mEC are associated with cognitive decline as measured by longitudinal changes in the Mini Mental State Exam in Minor Cognitive Impairment (MCI) group of the ADNI sample, but that thickness of lEC and not mEC separates the MCI group from the AD group. We discuss these results in the context of the usefulness of EC cortical thickness as an imaging biomarker of AD.

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Poster

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Support: NIH NINDS NS 45540

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R21AG049220

NINDS R3721135

Title: High-frequency band activity in human hippocampal CA1 predicts the precision of spatial memory retrieval

Authors: *R. F. STEVENSON[1], J. ZHENG[1], S. L. LEAL[2], A. P. CHUN[1], S. VADERA[1], R. T. KNIGHT[3], J. J. LIN[1], M. A. YASSA[1];
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Abstract: Previous studies using intracranial recordings in humans have shown that correct memory judgments are associated with increased high-frequency band activity (HFB) in the medial temporal lobe (MTL). However, the relationship between HFB and memory strength, or precision, is still not well understood. In the current study, we tested pre-surgical epilepsy patients with bilateral depth electrodes implanted in the hippocampus and surrounding cortices with a task designed to elicit a broad range of spatial memory precision. During encoding, 100 objects were presented one at a time at different locations on a computer screen while participants performed an incidental encoding task. At test, the same objects were presented at the top of the screen, and participants were asked to use a mouse wheel to move the object to where it appeared during encoding. Using a high-resolution MRI anatomical template co-registered with pre- and post-implantation MRI scans, we were able to determine the location of the depth electrodes within MTL subregions. We found that there was greater gamma HFB in MTL subregions for highly precise trials at retrieval as well as greater theta-gamma phase-amplitude coupling. Moreover, we found a linear correlation between HFB, thought to reflect local neuronal activity, and spatial memory precision in the CA1 subfield of the hippocampus. These results indicate that local processing within the CA1 subfield is important for the retrieval of precise spatial memories.

Disclosures: R.F. Stevenson: None. **J. Zheng:** None. **S.L. Leal:** None. **A.P. Chun:** None. **S. Vadera:** None. **R.T. Knight:** None. **J.J. Lin:** None. **M.A. Yassa:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Title: Hippocampal-cortical networks for temporal memory precision

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Abstract: Many studies have provided evidence that the hippocampus and neocortex play important roles in memory for time. However, it remains unclear exactly how these regions work together to support temporal memory, especially for complex naturalistic events. Prior studies of temporal memory have focused mainly on whether subjects could correctly make a binary response (e.g. which object came first?). However, people can be seconds, minutes, hours, or days from the correct answer, and the magnitude of error may provide insight into networks supporting temporal precision. In the present study, participants viewed an episode of a sitcom. At test, they were shown still frames from the episode and asked to indicate when each one occurred in time. A psychophysical interactions analysis of retrieval fMRI data revealed increased functional connectivity between the hippocampus and retrosplenial and visual cortex as a function of precision during memory retrieval. This may reflect increased hippocampal-cortical communication supporting temporal context details and reinstatement of visual information.

Disclosures: **M.E. Montchal:** None. **M.A. Yassa:** None.

Poster

361. Human Long-Term Memory: Medial Temporal Lobe

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Title: Category specific phase encoding for facial expressions in the orbitofrontal cortex

Authors: *J. ZHENG[1,2], R. F. STEVENSON[3], H. ERKOL[4], M. A. YASSA[3], R. T. KNIGHT[6], J. J. LIN[5];
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Abstract: The ability to interpret facial expressions is critical for adaptive human interaction and hypersensitivity to negative emotion is commonly observed in people with major depressive disorder. This increased sensitivity is thought to be due to dysregulation of the orbitofrontal cortex, but the oscillatory dynamics underlying discrimination of facial expression are poorly understood. To explore the electrophysiological mechanisms regulating processing of facial expressions, we recorded intracranial electroencephalography from the orbitofrontal cortex in 8 human subjects. During the task, participants were asked to label or match emotions by choosing the word or facial expression on the right or left of the screen (negative, positive, neutral valence) to describe the emotional face presented in the center. We found that high gamma (HG, 70-150 Hz) amplitude in the orbitofrontal region discriminated emotional facial expressions with the highest peak analytic amplitude observed for the negative facial emotion compared to other emotions (main effect for valence $F(2, 46)=6.32$; $p=0.004$; post hoc Negative > Neutral $p=0.02$, Negative > Positive $p=0.03$). We then tested whether the orbitofrontal region showed specific phase encoding for the valence of facial expressions. We found that 81% electrodes had HG amplitude coupled to the phase of the low frequency oscillations (LFO, 2-12 Hz) for valence (significant phase amplitude coupling = z score >2). Further, the phase of LFO at which the HG activity occurred was distinct for different facial emotions ($F (2, 78)=3.12$, $p=0.049$, Watson Williams test). Post-hoc test showed that HG activity occurred at specific phases of LFO for negative and positive facial expressions (Rayleigh test $p<0.05$) but not for neutral expressions. Our findings provide evidence that the orbitofrontal cortex signals the valence of facial expressions, with category specific phase encoding of the LFO for negative and positive valence.

Disclosures: J. Zheng: None. **R.F. Stevenson:** None. **H. Erkol:** None. **M.A. Yassa:** None. **R.T. Knight:** None. **J.J. Lin:** None.

Poster

362. Cortical Control of Executive Function

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Support: NIH Grant RO1DA013165

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Title: Individual differences in abstract rule selection

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Abstract: Cognitive control requires both the ability to flexibly switch between rules and the ability to select between simultaneously activated rules. While both processes have been described as "conflict resolution" it is unclear whether they are achieved via the same neural mechanisms. The current study examined this question using an individual differences approach with a task developed previously in our laboratory. Participants performed one of two classification tasks on English words: syllable (whether a word contained 2 syllables or not) or semantic (whether a word had concrete or abstract meaning). Participants were trained that the current task depended on the relevant dimension (color or shape) of a bivalent cue presented at the beginning of each trial. A separate instruction at the beginning of each block indicated whether color or shape was currently relevant. When the cue was incongruent (shape indicated a different rule than color), participants had to remember which cue dimension was relevant to resolve the conflict, resulting in incongruence costs. Switch costs were a result of having to switch between semantic and syllable tasks from one trial to the next, regardless of cue congruence. In the present study (n=65), incongruence costs and switch costs were not correlated across participants, and they were differentially correlated with Behavioral Activation System (BAS) scores, a measure of appetitive motivation, from the BIS/BAS survey. BAS scores were negatively correlated with incongruence RT costs, but positively correlated with switch accuracy costs. These results suggest greater appetitive motivation is associated with greater ability to resolve conflict arising from cue incongruency but greater difficulty resolving conflict due to the need to switch task sets. These results suggest different types of conflict are resolved by distinct neural mechanisms and that individuals with higher BAS scores have stronger, more stable prefrontal cortical representations for the rule context, which allows for more proactive control and quick resolution of the cue incongruency. These stable cortical representations, however, may also result in prolonged activation of the previous task set and increased difficulty updating, causing lower accuracy on switch trials. According to theories on dopamine's role in cognitive

stability and flexibility, higher BAS scores may indicate greater cortical dopamine availability resulting in reduced incongruency costs. These individuals, however, may require greater transient subcortical dopamine signaling to avoid increased switch costs.

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Poster

362. Cortical Control of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: Norwegian Research Council - MI Lab, NTNU

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Title: Young adults born preterm with very low birth weight exhibit hyper-reactive cognitive control processing accompanied by disrupted white matter integrity

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Abstract: Individuals born preterm with very low birth weight (VLBW) are at high risk for perinatal brain injuries and deviant brain development, leading to increased chances of later cognitive, emotional, and behavioral problems. Although deficits within a broad range of cognitive domains have been reported, considerable evidence points towards cognitive control dysfunction as particularly prevalent in the VLBW population. Previous BOLD fMRI studies have demonstrated both hyper- and hypo activations in preterm and VLBW individuals as compared with term-born controls. Accumulated evidence from behavioral studies points towards that the preterm behavioral phenotype may be associated with predominantly reactive-, rather than proactive cognitive control processing. The present study is the first to investigate the neuronal underpinnings of both reactive and proactive cognitive control processes in young

adults with VLBW. Thirty-two VLBW (birth weight ≤ 1500g) born preterm (before 37th week of gestation) and 32 term-born controls (birth weight ≥10th percentile for gestational age) aged 22-24 years old were included in the present study. Participants performed a well-validated Not-X continuous performance test (CPT) adapted for use in a mixed block- and event-related fMRI protocol. BOLD fMRI and DTI data was acquired during the same session on a Siemens Trio scanner. Performance on the Not-X CPT was highly similar between groups. Relative to controls, VLBW adults demonstrated less pronounced proactive cognitive control activations in fronto-parietal brain regions. In contrast, VLBW adults had more pronounced reactive cognitive control activations in posterior brain regions. Within the VLBW group there was a linear relationship between lower gestational age and stronger reactive cognitive control activations. Automated Multi-Atlas Tract Extraction (AutoMATE) analyses focusing on the cingulum and anterior thalamic radiation revealed that the functional brain alterations were accompanied by disruption of white matter integrity in the VLBW group, as reflected in both reduced FA and increased MD. In summary, these results indicate that reduced white matter integrity in VLBW individuals may be associated with hyperactivity in the cognitive control system and a failure to proactively apply and maintain appropriate task-sets.

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Poster

362. Cortical Control of Executive Function

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Topic: H.02. Human Cognition and Behavior

Title: Effects of repetitive transcranial magnetic stimulation at 1 Hz on right dorsolateral prefrontal cortex on impulse control, quality of life and disability self-assessment in patients with borderline personality disorder.

Authors: *E. MORELOS SANTANA[1], J. REYES-LÓPEZ[2], K. CERRILLO-AVILA[1], R. ALCALÁ-LOZANO[1], E. REYES-ZAMORANO[1], E. MIRANDA-TERRES[1], J. RICARDO-GARCELL[3], M. GARCÍA-ANAYA[1], J. GONZÁLEZ-OLVERA[1];
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Abstract: Borderline Personality Disorder (BPD) has been linked to functional and structural abnormalities in particular regions of the prefrontal cortex (Deeping et al., 2016) and to

impairments in executive function (Ruocco, 2005, Dell'Osso et al., 2010), one of which is a marked decrease in impulse control (Krause-Utz y cols, 2014). Only a few studies (Cailhol et al., 2014; Arbabi et al., 2013) have explored the effects of repetitive transcranial magnetic stimulation (rTMS) in patients with BPD, which makes it necessary to explore the effects of this treatment on variables such as impulsivity and impulse control.
The present study was conducted on 10 patients with BPD diagnosis who received 15 sessions of rTMS at 1 Hz frequency in the right Dorsolateral Prefrontal Cortex (DLPFC) for a lapse of 15 minutes a day, equaling 900 pulses. The equipment used included a Magpro stimulator (Dantec, Denmark) and an 8 shaped coil. All patients were evaluated before and after treatment using the Barratt Impulsivity Scale (BIS), the Stop-Signal paradigm (Verbruggen et al., 2008) and the World Health Organization scales of Quality of Life (WHOQOL-BREF) and Disability Assessment Schedule 2.0 (WHODAS II). The hypotheses were a reduction in BIS and disability scale, improvements in stop-signal performance as well as increases in quality of life scores following treatment.
A statistically significant reduction in BIS score ($Z= -2.36$, $p=0.018$) and motor impulsivity domain ($Z= -1.963$, $p= 0.05$), statistically significant decreases in the total score of WHODAS ($Z= -2.31$, $p= 0.021$) and its domain of Self-care ($Z= -2.536$, $p= 0.011$), as well as in the domains from WHOQOL-BREF of physical health ($Z= -2.12$, $p= 0.011$), psychological ($Z= -2.53$, $p= 0.011$), and social relationships ($Z=-2.536$, $p= 0.011$) were found. There was also a decrease in the stop signal reaction time (SSRT), that is, the time it takes the internal processes of the subject to stop a motor response, which was marginally significant ($Z=-1.886$, $p=0.059$) and a reduction in the probability of responding to a stop signal (P), as well as an increase in the stop signal delay (SSD), however these were not statistically significant.
This study shows that rTMS at 1 Hz applied over the right DLPFC can contribute to improve certain components of impulsivity such as motor impulsivity, specifically in reducing SSRT. Even though the sample for this study was small, it contributes to the current knowledge of the effects of rTMS treatment in BPD patients by showing a reduction in impulsivity and significant increases in the quality of life and reduction in disability self-assessment scores.

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Poster

362. Cortical Control of Executive Function

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James S. McDonnell Foundation (DB)

Brown Institute for Brain Science (TMD, DB)

Title: Sequence monitoring in the frontal cortex

Authors: *T. M. DESROCHERS[1,2,3], D. BADRE[1,3];
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Abstract: Keeping track of complex sequential information is essential to everyday life. Navigating the highway to a new destination requires carefully monitoring a series of stimuli, in the correct order, to know when to take the exit. Previous work showed that sequential task monitoring and control is dependent on rostrolateral prefrontal cortex (RLPFC; Desrochers et al., 2015). FMRI of human participants during simple, repeated sequences of tasks revealed ramping activation in the RLPFC that increased through the sequence and reset at each first position. TMS showed that RLPFC activation was necessary toward the end of sequences and that it dissociated from two other frontal control regions in two separate experiments. Because both task execution and monitoring were components of this previous task, an open question remains as to whether RLPFC would be engaged by sequential monitoring alone, even if no response had to be planned or emitted. Further, it is unknown if a sequence must be monitored without external position cues (i.e. from memory) in order to engage ramping in the RLPFC.
We designed an experiment where participants monitor a repeated series of four stimuli (based on Allen et al., 2014). The response button is released if a presented stimulus is out of sequence, otherwise it is held. Participants perform two versions of this task: one with all Visible stimuli, and one where all but the last stimulus in the block are Occluded by an irrelevant distractor so participants must monitor the sequence without external position cues. Preliminary results from fMRI scanning suggest that the hippocampus is preferentially performing a ramping/monitoring function in the Occluded > Visible sequences. Previous findings in the memory literature have shown the hippocampus represents sequential information and has increased activity during retrieval, but not with the same dynamics or without external cues as in this study. Beyond hippocampus, occluded monitoring also shows ramping activation in several frontal cortical areas, but not reliably in the left RLPFC as observed in the previous study. This suggests that monitoring and executing a series of tasks may be necessary to engage the RLPFC. The trend of more caudal regions showing ramping during this simpler monitoring only task also suggests that ramping activation may follow a similar rostral to caudal gradient of more abstract to less abstract that has been found in non-sequential, hierarchical control tasks.

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Poster

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Support: R01 DA026457

Lilly Endowment, Inc.

Indiana METACyt Initiative

Title: Medial prefrontal cortex signals prediction errors across domains of pain and cognitive control

Authors: *A. JAHN[1], D. NEE[2], W. ALEXANDER[3], J. BROWN[4];
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Abstract: Recent meta-analyses suggest that the dorsal anterior cingulate cortex (dACC) as a whole is responsive to a wide variety of stimuli and psychological states, including prediction error (PE), pain, and cognitive control. In contrast, a recent study by Lieberman & Eisenberger (2015) argues that the dACC is most responsive to pain, while supplementary motor area (SMA) and pre-SMA regions are associated with higher-level cognitive processes. To address this debate, here we use an FMRI paradigm with cues signaling the probability of receiving either aversive or non-aversive levels of electrical stimulation, and cues signaling the probability of receiving a congruent or incongruent spatial Conflict stimulus. Here we show a robust dorsal-ventral distinction across the SMA/pre-SMA vs. dACC, with PE more dorsally, and pain and galvanic skin response signals more ventrally. We also find overlapping activations in the SMA and pre-SMA for PEs of pain and cognitive control. Furthermore, post-hoc analyses revealed that the observed effects for both surprising pain and surprising spatial Conflict stimuli were driven by outcomes that were worse than expected as well as by outcomes that were better than expected, in contrast to predictions from Reinforcement Learning theory and conflict monitoring theory, but instead consistent with predictions of the PRO model. These results speak to the functional organization of the mPFC and provide a framework for future modeling of this region.

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Poster

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Title: Human single-neuron correlate of error monitoring in the medial frontal cortex

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Abstract: Human executive function relies on the ability to detect and compensate for errors. The error-related negativity (ERN) is an electrophysiological correlate of this process that has been localized to the anterior cingulate cortex by EEG source localization. However, how ERN and the error-monitoring mechanism it represents are reflected in the activity of single neurons remains unknown.

We used intracranial electroencephalography (iEEG) and single unit recordings to investigate error monitoring in human subjects. 24 epilepsy surgery candidates with implanted intracranial depth electrodes performed 71 sessions of a speeded version of a color-word Stroop task. Subjects showed robust Stroop interference effect (231.5 ± 22.7ms, $F(1,76) = 103.6$, $p < 10^{-10}$, mixed-effect one-way ANOVA model with sessions as random effect) and post-error slowing (93.7 ± 19.5ms (mean ± s.e.m. across sessions), $F(1,169) = 9.63$, $p = 0.0022$, mixed-effect one-way ANOVA model with sessions as random effect).

We isolated 290 neurons in the dorsal anterior cingulate cortex (dACC) and 323 in pre-supplementary motor area (pre-SMA). 27% of all dACC neurons and 40% of all pre-SMA neurons changed their firing rate immediately after subjects made an error, but before feedback was presented. This suggested that the error signals were internally-generated, not triggered by external feedback. The neuronal error signal in pre-SMA preceded that in dACC by 172ms. Given the prominent post-error slowing effect (PES) and error signals, we hypothesized that dACC and pre-SMA are part of the neuronal circuitry that implements PES. Indeed, we found that 7% of dACC and 14% of pre-SMA neurons modulated their firing rate in the correct trials that followed an error. We also found 5% of dACC and 23% of pre-SMA neurons signaled trial congruency.

We also simultaneously recorded iEEG from macro electrode. We identified robust intracranial

error-related evoked potentials in dACC and pre-SMA. These error-related potentials had a negative and positive going component with waveform and latency similar to classical studies of the ERN (Falkenstein et al 1990, Gehring et al,1993). Theta power of iEEG increased significantly more in error trials than in correct trials. Here we again found that Ne peaks in pre-SMA preceded those in dACC by 38ms. The temporal patterns of the ERN and error-related neuronal activity, together with trial-by-trial
correlations between ERN amplitude and firing rates, suggested that we have identified a single-neuron correlate of the well-known ERN.
Our findings provided the first potential circuit-level mechanism for error monitoring and post-error adjustment in dACC and pre-SMA.

Disclosures: Z. Fu: None. **A. Mamelak:** None. **I. Ross:** None. **J. Chung:** None. **R. Adolphs:** None. **U. Rutishauser:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.07/LLL23

Topic: H.02. Human Cognition and Behavior

Title: Disentangling the relative timing of right inferior frontal cortex and anterior insula in response inhibition with intracranial recordings in humans

Authors: *E. BARTOLI[1], A. ARON[2], N. TANDON[1];
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Abstract: Responding quickly to varying environmental demands by changing our responses, such as withholding an initiated movement, is a core process of cognitive control. Stop signal tasks have been used to evaluate the process of inhibitory control, consistently reporting the recruitment of right ventral lateral prefrontal cortex, including the right inferior frontal cortex (rIFC), and the adjacent right anterior insula (rAI). Yet the relative functional roles of these two regions in stop signal processing, braking of the response, and processing the outcome are currently debated. Functional imaging techniques lack the temporal resolution to resolve these processes, as they occur in rapid succession. Here, we used electro-corticographic recordings in 12 patients performing a simple stopping paradigm. All patients had electrodes implanted within the rAI (short gyri: 12 patients) and most patients had coverage of rIFC subregions (pars triangularis: 5 patients; pars opercularis: 3 patients; both regions: 4 patients). We calculated the change in broad-band gamma power (50-150 Hz) following the onset of the stop signal. We then assessed a) the relative timing of rAI activation with respect to rIFC, and b) the timing of onset

of activity in these two regions with respect to the stop signal reaction time (SSRT), We also characterized c) the functional response in the rAI by contrasting successful and failed stop trials. First of all, we found that rAI activity generally occurred after that in the rIFC. Second, the rIFC was consistently activated before SSRT, whereas activity in rAI was more variable. Further, in the rAI there was no consistent difference for successful and failed stop trails, due to the variability in the broad-band gamma change response associated with these trials. This latter result challenges the view that anterior insula activity in stopping merely reflects error processing, which would be supported by a reliably higher response during failed stop trials. These results can help resolve the debate about the relative functional roles of frontal subregions and the insula during response inhibition.

Disclosures: **E. Bartoli:** None. **A. Aron:** None. **N. Tandon:** None.

Poster

362. Cortical Control of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: NIH R00MH090238

NIMH F30 MH105158-01A1

Title: CLOCK regulation of circadian rhythms in the human neocortex

Authors: M. FONTENOT[1], *G. KONOPKA[2];
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Abstract: For the past 20 years, the molecular mechanisms underlying circadian rhythm and its relevance to human health have been the subject of intense research. The transcription factor CLOCK is a key driver of circadian cycling. Although genome-wide transcriptomic profiling studies have focused on cyclical gene expression in the liver and the suprachiasmatic nucleus, little is known about the function of CLOCK in other areas of the central nervous system. We have previously shown that CLOCK is more highly expressed in human neocortex compared to non-human primates, raising questions concerning the role of CLOCK in human brain evolution and its relevance to human mental health. We hypothesize that neocortical expression of CLOCK in the human brain is critical for regulating novel transcriptional networks. To test this hypothesis, we are identifying the transcriptional network regulated by CLOCK through ChIP-seq in human neocortex and RNA-seq following CLOCK knockdown in human neurons.

Additionally, we will assess the role of neocortical CLOCK expression by creating a humanized mouse, which will further serve as an improved model for circadian functioning and neocortical-associated disease.

Disclosures: **M. Fontenot:** None. **G. Konopka:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.09/LLL25

Topic: H.02. Human Cognition and Behavior

Support: NSF IGERT Grant DGE-0903495

Title: A computational neural model of sequential action in the fronto-parietal network

Authors: ***N. ZARR**, J. W. BROWN;
Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: A core problem in computational and cognitive neuroscience is how a biological agent can learn to navigate an environment in order to achieve its goals, especially when goals are distant and can only be achieved by a sequence of actions. Existing evidence implicates the fronto-parietal network in this process, but few mechanistic accounts exist. We have developed a neural network model of goal directed action that provides such an account. The Goal-Oriented Learning and Sequential Action (GOLSA) model is comprised of continuous-time dynamic model neurons that adapt via associative learning based solely on locally-available information. The model quickly learns both the layout of the state space as well as a mapping from state transitions to actions. It also learns which states are associated with satisfying various drives. In some ways, the model serves as a neurally plausible instantiation of model-based reinforcement learning (MBRL), though it differs from MBRL in that the ends of behavior are conceived of as more flexible desired states rather than reward per se.
Model components can be mapped onto various cortical areas, most notably prefrontal and parietal cortex, which represent the current state and desired transitions. It thereby provides an account of how these regions work together to support goal-directed action, providing clear predictions for future fMRI studies to test and refine the model. The GOLSA model also suggests a novel functional role for neural oscillations, namely to cyclically route input to some layers from multiple distinct input sources. This allows Hebbian learning to make associations that would otherwise require non-local, biologically implausible transfer of information.

Disclosures: **N. Zarr:** None. **J.W. Brown:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.10/LLL26

Topic: H.02. Human Cognition and Behavior

Support: grant from TEVA Belgium

Title: Frontal dysfunction in resting state networks in Multiple Sclerosis.

Authors: ***J. GIELEN**[1], J. VAN SCHEPENDOM[1,2], J. LATON[1], J. DE MEY[1,3], A.-M. VANBINST[3], M. CAMBRON[1,3], M. D'HAESELEER[3,1], M. D'HOOGHE[1,4], G. NAGELS[1,3,2,4]; [1]Vrije Univ. Brussel, Brussel, Belgium; [2]Univ. de Mons, Mons, Belgium; [3]UZ Brussel, Brussel, Belgium; [4]Natl. MS Ctr. Melsbroek, Melsbroek, Belgium

Abstract: Functional MR imaging in the resting brain (RSfMRI) may offer important insights into brain function. We researched the effect of brain functional connectivity and network analysis, both topographical and topological, in MS patients and healthy controls.
Here, 27 healthy controls (HC) and 53 MS patients (MS) underwent RSfMRI scanning and cognitive testing. We used the AAL atlas to divide subject data into 95 regions of interest (ROI). In topographical analysis, connectivity between all regions was based on Pearson correlation of the mean BOLD time series within each ROI (Figure 1). Panel C shows significantly different edges ($p<0.01$, uncorrected). We split up the patients into two groups based on the median SDMT score, resulting in a cognitively preserved and impaired group (CP and CI). HC had significantly stronger edges in right frontal AAL areas, but this difference was not found between CP and CI. No relations were found between cognition and the strength of significantly different edges.
In topological analysis, networks were binarised by thresholding at correlations between 0 and 0.6, in steps of 0.025. Network measures were calculated based on these networks. Figure 2 shows the mean measures for MS and HC. In an ANOVA with factors group and threshold, a significant effect ($p<0.01$) of both was found in all parameters.
We show that network analysis of RS data is able to differentiate between HC and MS.

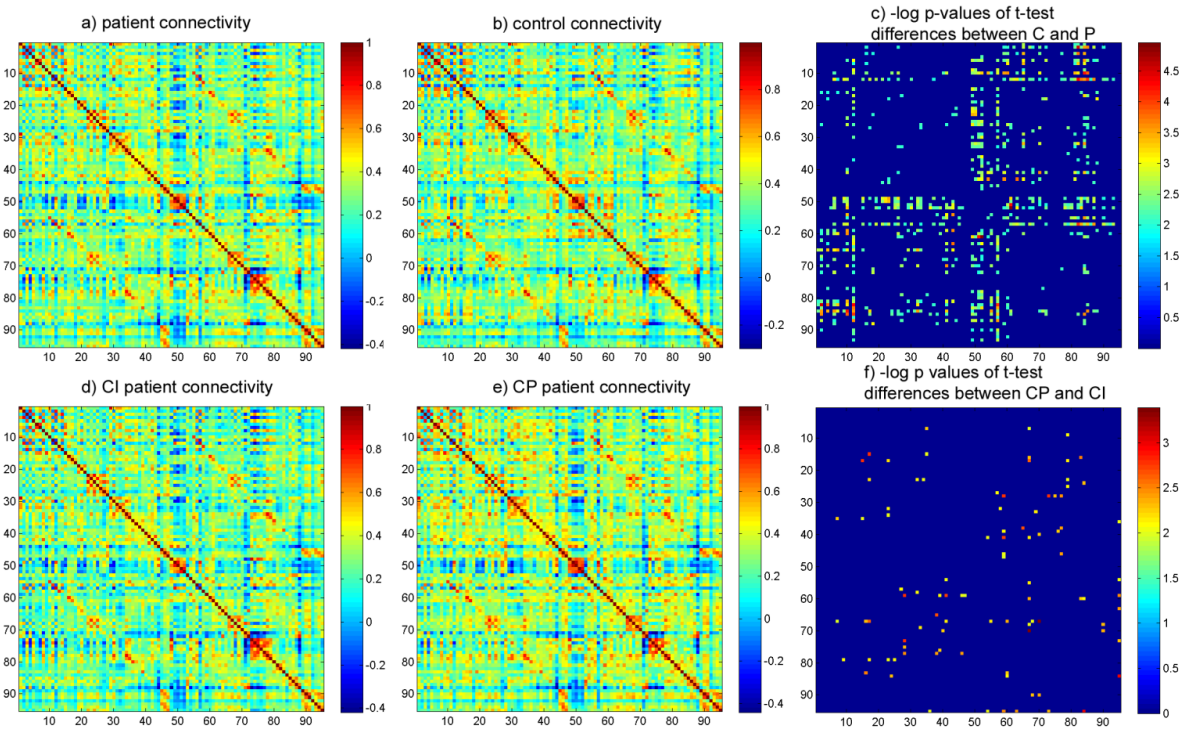

Figure 1: Connectivity matrices for a) patients, b) controls, e) cognitively imparied and e) preserved patients, as well as significant differences between c) patients and controls and f) CP and CI patients. (insignificant edges = dark blue)

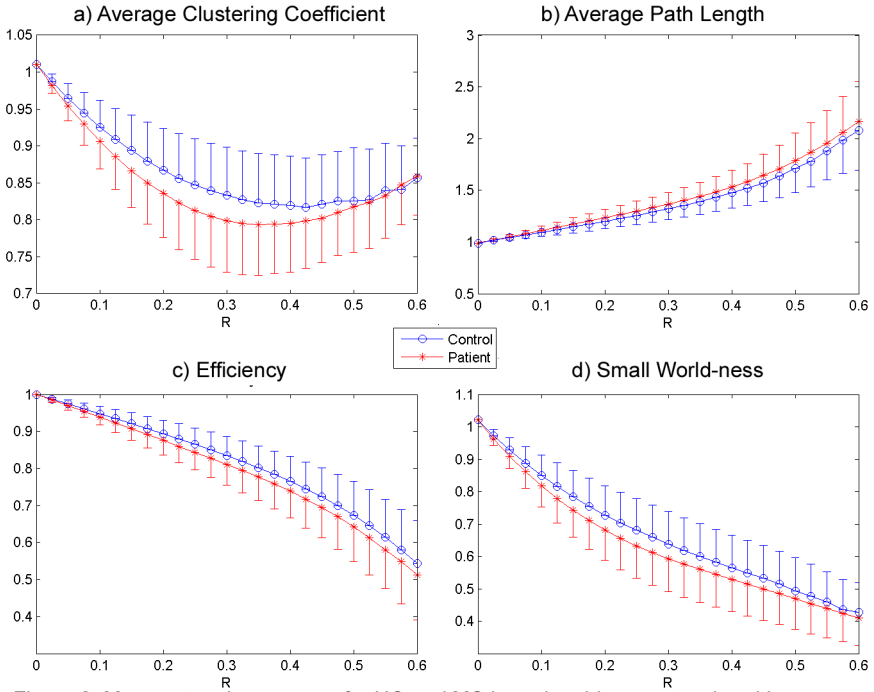

Figure 2: Mean network measures for HC and MS based on binary networks with different edge strength thresholds, error bars show standard deviation.

Disclosures: **J. Gielen:** None. **J. Van Schependom:** None. **J. Laton:** None. **J. De Mey:** None. **A. Vanbinst:** None. **M. Cambron:** None. **M. D'Haeseleer:** None. **M. D'hooghe:** None. **G. Nagels:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.11/LLL27

Topic: H.02. Human Cognition and Behavior

Title: Effects of the cholinergic agonist nicotine on cognitive flexibility and stability are modulated by baseline prefrontal functions

Authors: *C. M. THIEL, S. PUSCHMANN, S. AHRENS;
Univ. of Oldenburg, Oldenburg, Germany

Abstract: In a constantly changing environment, individuals must be able to balance between the stabilization of task relevant representations and flexible updating of these representations in response to changing demands. We have previously shown that the cholinergic agonist nicotine reduces distractor interference, i.e. promotes cognitive stability, and increases attentional shifting, i.e. promotes cognitive flexibility. The effects of nicotine varied however as a function of cholinergic and dopaminergic receptor genes. Since several studies suggest, that the dopaminergic innervation of the prefrontal cortex is critical for flexible behavior we here aimed to investigate whether interindividual differences in prefrontal cortical function modulate the effect of nicotine in a task that requires to dynamically balance between cognitive stability and flexibility. Young, healthy non-smokers trained on a sustained attention task with distractor and switch trials to gauge cognitive stability and flexibility respectively. Individual differences in prefrontal function were assessed with the intra/extradimensional set shifting task. Participants then received either a 7 mg transdermal nicotine or placebo patch in a double-blind, within-subject design one hour prior to performing the sustained attention task in the MR scanner. The behavioural data indicate that subjects with high and low prefrontal function (i.e. high and low performance in the intra/extradimensional set shifting task) benefitted differentially from nicotine. Subjects with low prefrontal function showed a significantly reduced distractor interference after nicotine, while subjects with high prefrontal function showed descriptively reduced switch costs under nicotine. Neural effects of nicotine in distractor and switch trials were analysed as a function of intra/extradimensional set shifting performance. Individual differences were evident in the inferior frontal gyrus bilaterally and the right insula in the distractor condition. Those subjects with high errors, i.e. low prefrontal function, were those showing the strongest reduction of neural activity under nicotine. In the switch condition, individual differences in intra/extradimensional task performance co-occurred with differences in neural activity in inferior frontal gyrus and angular gyrus bilaterally. Those subjects with high errors, showed the strongest reduction of neural activity under nicotine. Our results suggest that inidividual effects of nicotine on stability and flexibility depend on a differential modulation of neural activity in parieto-frontal brain regions.

Disclosures: **C.M. Thiel:** None. **S. Puschmann:** None. **S. Ahrens:** None.

Poster

362. Cortical Control of Executive Function

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Program#/Poster#: 362.12/LLL28

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant NS089729

Title: Fronto-parietal regions represent both abstract goals and goal-relevant feature information

Authors: ***N. M. LONG**, B. A. KUHL;
Univ. of Oregon, Eugene, OR

Abstract: Electrophysiological recordings from non-human primates and human fMRI evidence support the idea that prefrontal and parietal cortices jointly contribute to goal-directed processing of information. However, goal-directed processing can be decomposed into the maintenance of abstract goals and the selective representation of content that relates to current goals. While it is generally agreed that fronto-parietal regions maintain top-down goals, it is less clear whether fronto-parietal regions actively represent goal-relevant content or whether they simply bias processing within sensory regions. Here we conducted a human fMRI study using multi-voxel pattern analysis to separately test for representations of top-down goals vs. selective representation of goal-relevant features within fronto-parietal regions. We employed a task in which participants viewed a series of faces and had to make one of four decisions on each trial (‘male?’, ‘female?’, ‘happy?’, ‘grumpy?’). These goal cues appeared just prior to the face stimuli and were independent from the features of the actual face stimulus. Notably, the four goal cues reflected two orthogonal face dimensions (affect vs. gender). Thus, for trials with a goal cue of ‘male?’, gender was the goal-relevant dimension. This design allowed us to test for top-down representations of specific goals (e.g., ‘male?’) and goal-relevant dimensions (‘gender’) as well as coding of goal-relevant features (e.g., coding that a stimulus was ‘female’ when the gender dimension was relevant). Pattern classification analyses revealed that fronto-parietal activity patterns reflected top-down goals as well as the goal-relevant dimension. Moreover, fronto-parietal activity patterns also preferentially represented goal-relevant features (e.g., whether a face was male vs. female if gender was relevant). These findings help clarify how fronto-parietal regions contribute to goal-directed behavior.

Disclosures: **N.M. Long:** None. **B.A. Kuhl:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.13/LLL29

Topic: H.02. Human Cognition and Behavior

Title: The impact of poly-victimization and traumatic stress on psychological and neuropsychological functioning of undergraduate college students

Authors: A. STIVER, *W. M. MEIL;
Psychology, Indiana Univ. of Pennsylvania Dept. of Psychology, Indiana, PA

Abstract: Studies assessing the impact of trauma often focus on a single traumatic event and do not consider the impact of repeated traumas that accumulate over the lifespan. While many individuals who are victimized by exposure to traumatic stress show resiliency to these experiences, others have been identified as having greater neuropsychological impairment and increased psychopathology in the form of depression, anxiety, and PTSD. To date, there is little research exploring associations between poly-victimization and executive dysfunction. The purpose of the current study was to evaluate the cumulative experience of poly-victimization and the impact of these experiences on neuropsychological functioning, and the development of stress related disorders in a sample of 147 undergraduate college students. The Juvenile Victimization Questionnaire (JVQ) was used to measure poly-victimization and to assess previous exposure to trauma during childhood. The Frontal Systems Behavior Scale (FrSBe) and the Iowa Gambling Task (IGT) measured executive functioning. To assess anxiety and depression, the Kessler Psychological Distress Scale (Kessler-10) was administered. The Impact of Event Scale- Revised (IES-R) was used to assess PTSD symptoms. The Alcohol Use Disorders Identification Test (AUDIT) was used to measure alcohol use. Finally, the Inventory of College Students Recent Life Experiences assessed recent life hassles and stressors (ICSRLE). Hierarchical multiple linear regression revealed the addition of total number of reported traumatic events on the JVQ led to a statistically significant increase in the prediction of executive dysfunction on FrSBe total scores (R2 of .076 (1,111)= 9.64, p=.002) and FrSbe disinhibition subscores (R2 of .061, F(1,111)= 7.910, p<.006.). The total number of reported traumatic events on the JVQ did not predict executive dysfunction on the IGT. In addition, total number of reported traumatic events on the JVQ led to a statistically significant increases in the scores on the Kessler-10 (R2 of .080, F(1,111) = 10.13, p< .002.) and IES-R (R2 of .268, F(1,111) = 35.123, p< .001). Overall, these results suggest the number of traumatic events during childhood and adolescence is predictive of executive dysfunction and increased scores on measures of stress-related psychopathology. Moreover, these results suggest self-report measures such as the FrSBe, which was designed be an ecologically valid measure to detect changes

following frontal lobe damage, may be more sensitive to identifying executive dysfunction following trauma than performance-based measures, such as the IGT.

Disclosures: A. Stiver: None. **W.M. Meil:** None.

Poster

362. Cortical Control of Executive Function

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Program#/Poster#: 362.14/LLL30

Topic: H.02. Human Cognition and Behavior

Support: Polish National Science Center grant no. 2014/13/D/HS6/03015

Title: Is punishment impairing cognitive control in procrastinators? - an fMRI monetary Go/NoGo study

Authors: *M. WYPYCH[1], J. MICHAŁOWSKI[2], D. DROŻDZIEL[1,2], M. BANIA[2], M. SZCZEPANIK[1,3], A. MARCHEWKA[1];
[1]Lab. of Brain Imaging, Neurobio. Ctr., Nencki Inst. of Experimenal Biol., Warsaw, Poland; [2]Fac. of Psychology, [3]Fac. of Physics, Warsaw Univ., Warsaw, Poland

Abstract: *Introduction and methods*
Previously, in a sample of low (LPS) and high (HPS) procrastinating students, we have performed behavioral monetary Go/NoGo study with three conditions: Neutral (NEU) - where monetary gratification did not depend on the performance; Reward (REW) - where each response to Go signal and correct inhibition of NoGo trials were rewarded with small amounts of money; Punishment (PUN) - where subjects were given some money prior to the task and each error resulted in a loss of a fraction of the money.
During PUN HPS were significantly faster in the post-error Go trials (Go trials directly following error of commission to NoGo) then in Go trials after successful inhibition. At the same time LPS were slowing down after the errors in PUN. This result may suggest impaired error processing and/or increase of impulsivity in procrastinators under pressure of punishment.
In the current fMRI study we employ monetary Go/NoGo task in mixed-block design in the general population of low (LP) and high (HP) procrastinators recruited on the basis of the result in Pure Procrastination Scale (Steel, 2010).
Results
On the behavioral level, similar as in the student population, we observe after-error speeding in HP in PUN.
Preliminary fMRI results show stronger activation of anterior cingulate cortex in LP than in HP

during PUN blocks. PUN>NEU contrast revealed activation of caudate nuclei and middle frontal gyri (cf. Simões-Franklin et al., 2010) in LP but no significant clusters in HP. Between-group comparison in PUN>NEU contrast showed stronger activation in left caudate and left anterior middle frontal gyrus in LP than HP.
No differences in Neutral and Reward conditions were found.
Discussion and conclusions
Current results showed, that in the threat of punishment LP tend to activate frontal "control" regions more strongly than in neutral conditions - this is not the case in HP. This observation could be interpreted in line with recent resting-state fMRI study (Wu et al., 2016) showing decreased functional connectivity between several frontal regions in procrastinators.
Obtained data are consistent with previous results of high correlation between procrastination and "negative impulsivity" (decrease of self-control in negative-feelings situations) and literature observations that negative feelings (stress, anxiety, guilt) intensify procrastination (possibly by decrease of self-control).
The results may suggest that procrastination is related to decreased ability to intensify self-control in more demanding situations and/or impaired coping in a contexts in which punishment is likely to occur.

Disclosures: M. Wypych: None. **J. Michałowski:** None. **D. Droździel:** None. **M. Bania:** None. **M. Szczepanik:** None. **A. Marchewka:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.15/LLL31

Topic: H.02. Human Cognition and Behavior

Support: Toyota Class Action Settlement Safety Research and Education Program

Title: Complementary physiological and behavioral data streams enhance analysis of fNIRS data during a real-world driving task.

Authors: *A. GUNDRAN[1], A. M. PICCIRILLI[1], J. M. BAKER[1], J. L. BRUNO[1], L. K. HARBOTT[2], H. HOSSEINI[1], J. C. GERDES[2], A. L. REISS[1];
[1]Psychiatry and Behavioral Sci., [2]Mechanical Engin., Stanford Univ., Palo Alto, CA

Abstract: Functional near infrared spectroscopy (fNIRS) is an ultra-portable neuroimaging technique that allows imaging of brain activity in real world, dynamic scenarios. Automobile driving is one such scenario that involves several higher-order cognitive functions, such as

visuospatial working memory, divided attention, and decision-making. Because of its methodological benefits, fNIRS is an optimal platform for investigating cortical function during real world driving. However, with the ability to study the brain in realistic settings comes the loss of experimental control often present in lab-based experiments. To make up for these added confounds, we demonstrate the utility of combining data from multiple sources, including head acceleration, cardiac rate, pupil dilation, and vehicle dynamics, to enhance the interpretation of fNIRS data. A total of 15 participants completed a naturalistic driving task in which they made a series of single lane-change maneuvers in an electronic, steer-by-wire experimental vehicle driving on a course marked by cones. To investigate the neural correlates of increased mental workload during driving, the participants performed a block of lane-changes with nominal vehicle steering behavior, followed by a block of lane changes during which the steering was reversed and a final block of congruent steering trials. During the task, our suite of data streams captured all task-related changes in cortical activation (fNIRS), head movement (3-axis accelerometer), heart rate (electrocardiogram), pupillary dilation (eye-tracking), and real-time information about the vehicle dynamics (e.g., steering wheel angle, GPS tracking, etc.). Our results show a clear relationship between each data stream. Abrupt head movements caused by changes in automobile acceleration and turns resulted in artifacts in fNIRS data. However, accurate head motion data collected with an accelerometer can be used as a source of motion correction. Similarly, changes in a participant's heart rate and pupillary dilation correlate with baseline changes in fNIRS data streams. Taken together, our data provide advances in the measurement of cortical activation during driving, and are the first to establish a clear need for multiple data streams within this and other real world tasks. Indeed, as brain imaging moves to more complex and naturalistic environments, such as driving, multiple data streams will be essential to ensure the integrity of the data and for thoughtful, intelligent analysis, and to inform the design of future automotive systems that enhance driver-car interaction and improve overall safety.

Disclosures: **A. Gundran:** None. **A.M. Piccirilli:** None. **J.M. Baker:** None. **J.L. Bruno:** None. **L.K. Harbott:** None. **H. Hosseini:** None. **J.C. Gerdes:** None. **A.L. Reiss:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.16/LLL32

Topic: H.02. Human Cognition and Behavior

Support: Toyota Class Action Settlement Safety Research and Education Program

Title: Neural, physiological, and behavioral correlates of visuomotor cognitive load: a functional NIRS study

Authors: *H. HOSSEINI[1], J. L. BRUNO[1], J. M. BAKER[1], A. GUNDRAN[1], A. M. PICCIRILLI[1], L. K. HARBOTT[2], J. C. GERDES[2], A. L. REISS[1];
[1]Psychiatry and Behavioral Sci., [2]Mechanical Engin., Stanford Univ., Stanford, CA

Abstract: Visuomotor ability is crucial for complex everyday functions specifically for driving and playing sports. While several studies have examined the neural systems subserving visuomotor control in humans, less is known about the regions involved in resolving conflict in visuomotor mapping under different cognitive demands. In this study, we employed functional near infrared spectroscopy (fNIRS), in conjunction with eye-tracking, to examine the neural, physiological and behavioral correlates of visuomotor mapping under varying cognitive loads. Twenty healthy young adults completed a computer-based visuomotor task, in which they were instructed to keep a moving dot in the middle of a path using the left and right keyboard arrows. We manipulated the cognitive load by reversing the mapping between visual and motor response (right/left arrow moved the dot to the left/right) and also by increasing the speed of the moving dot. The experiment consists of 118 trials and the order of trials was pseudorandom.
A Tandem NIRSport (NIRx Medical Technologies) was used for measuring brain activity in bilateral frontoparietal network and SMI eye-tracking glasses (ETG 2) were used to measure pupil response. Mean deviation of the dot from the center of the path was considered as a measure of performance.
A 2x2 analysis of variance (ANOVA) revealed significant main effect of mapping and acceleration with poorer performance during incongruent and high-speed conditions ($p< 0.001$). Pupillometry data indicated significant increase in pupil diameter during incongruent and high-speed conditions ($P<0.05$) (Figure 1). Finally, fNIRS data revealed higher activity in the left prefrontal cortex associated with higher cognitive demands in the incongruent visuomotor mapping condition. Conversely, bilateral parietal cortices showed higher activity in response to the high-speed condition (Figure 2).
Our pupillometry results confirm previous data showing correlation between changes in pupil diameter and mental workload. Our fNIRS data further dissociate the brain regions responding to different types of mental workload (visuomotor mapping conflict vs. unexpected acceleration). Together, these data suggest that fNIRS can be used to distinguish between different aspects of mental workload. These results may have significant implications in imaging brain activity during complex, everyday visuomotor tasks such as driving.

Disclosures: H. Hosseini: None. **J.L. Bruno:** None. **J.M. Baker:** None. **A. Gundran:** None. **A.M. Piccirilli:** None. **L.K. Harbott:** None. **J.C. Gerdes:** None. **A.L. Reiss:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.17/LLL33

Topic: H.02. Human Cognition and Behavior

Support: Toyota Class Action Settlement Safety Research and Education Program

Title: fNIRS measurement of cortical activation and functional connectivity during a visuospatial working memory task

Authors: *J. BAKER, J. L. BRUNO, A. GUNDRAN, H. HOSSEINI, A. L. REISS; Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Demands on visuospatial working memory are a ubiquitous part of everyday life. As such, significant effort has been made to understand how the brain responds to these demands in real-world environments. Multiple brain imaging studies have highlighted a fronto-parietal cortical network that underlies visuospatial working memory in humans, and that appears to respond uniquely to encoding versus retrieval components. Furthermore, multiple studies have identified functional connectivity in regions of the fronto-parietal network during working memory tasks. Together, these findings have helped outline important aspects of the neural architecture that underlies visuospatial working memory.

Here, we provide results from the first functional near infrared spectroscopy (fNIRS)-based investigation of cortical activation and functional connectivity during a unique computer-based visuospatial working memory task. First, participants' psychophysical sensitivity to visuospatial change was estimated and used to equate the difficulty of "hard" and "easy" trails across participants. Next, fNIRS recording channels were positioned over the bilateral prefrontal and parietal cortices. Neural function was measured during encoding/maintenance and retrieval of visuospatial working memory information throughout our task. A total of fifteen ($n_{female} = 8$) healthy adults completed our study.

Cortical activation was greatest in the prefrontal compared to parietal cortices ($F(3,112)= 3.009$, $p= 0.033$), and was highest during retrieval ($F(1,112)= 18.229$, $p< 0.001$). Conversely, functional connectivity was highest within the parietal compared to prefrontal regions ($F(1,464)= 131.697$, $p< .001$), but did not differ significantly across task components. These results highlight an important dissociation between cortical activation and functional coherence in the frontal-parietal cortices during our visuospatial working memory task. Thus, focused interest on cortical activation alone indicates that the prefrontal cortex provides the strongest response to visuospatial working memory demands, and that these patterns vary as a function of task difficulty. Conversely, analysis of neural coherence in isolation emphasizes the role of the bilateral parietal cortex, and fails to identify any moderating effects of task demands. As both

approaches to fNIRS data analysis provide useful information about brain function, our results highlight the importance of future research to focus its efforts on elucidating the functional relationship underlying both cognitive signatures.

Disclosures: J. Baker: None. **J.L. Bruno:** None. **A. Gundran:** None. **H. Hosseini:** None. **A.L. Reiss:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

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Program#/Poster#: 362.18/LLL34

Topic: H.02. Human Cognition and Behavior

Support: This work was funded in part by the Toyota Class Action Settlement Safety Research and Education Program. The conclusions being expressed are the authors' only, and have not been sponsored, approved, or endorsed by Toyota or Plaintiffs' Class Counsel

Title: Characterizing brain and behavioral correlates of steering control during simulated driving.

Authors: *J. BRUNO[1], J. M. BAKER[1], A. GUNDRAN[1], L. K. HARBOTT[2], Z. STUART[2], A. PICCIRILLI[1], S. H. HOSSEINI[1], J. C. GERDES[2], A. REISS[1];
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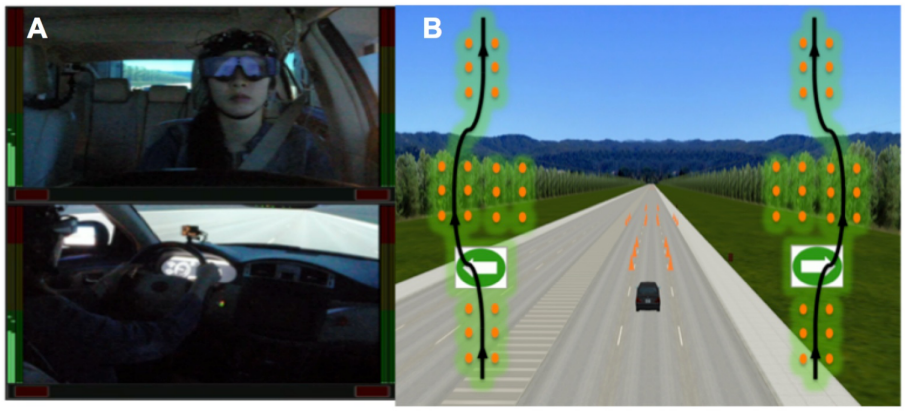
Abstract: As modern production vehicles implement increasingly automated safety systems, understanding driving behavior and its underlying neural processes has become ever more critical. Functional brain imaging during realistic driving could be useful for enhancing car-driver communication, and improving driver safety. To better understand how drivers respond to unexpected changes in vehicle control we used functional near infrared spectroscopy (fNIRS), a portable, noninvasive optical imaging technique, to examine brain activity in response to changes in vehicle steering control. Twenty-two participants completed a driving task in a realistic driving simulator involving a standard double lane change maneuver (Figure 1). During half the trials steering was reversed (incongruent steering, turning the steering wheel right steers the vehicle left, and vice versa). fNIRS data were collected from 40 channels positioned over bilateral prefrontal and parietal cortices and pupillary response was measured via eye tracking goggles. Drivers successfully completed the double lane change under both steering conditions, but demonstrated inferior performance with incongruent steering, as indicated by a less consistent path and more steering wheel reversals, compared to the congruent steering.

Correspondingly, fNIRS data indicated greater activation in the right prefrontal cortex and bilateral parietal cortex during incongruent relative to the congruent steering. Pupil diameter was also increased in the incongruent condition. These results indicate that changes in vehicle steering response are associated with increased mental workload, particularly in terms of prefrontal and parietal cortical processes. Increased activation over the course of the experiment suggests that drivers compensate for challenging driving demands by ramping up cognitive resources. Results such as these, that elucidate the neurobiological underpinnings of driver behavior, will be useful for informing the design of automated safety systems that facilitate safe and optimal driver-car communication.

Figure 1. Driving simulator task.



A. Participants performed a simulated driving task, during which fNIRS and pupillometry was collected. B. Bird's-eye view of the task, in which the participant is instructed to drive straight down the center of the lane through the first 3 pairs of cones, and then make a lane change left or right, indicated by the green direction arrow that appears in their line of sight.

Disclosures: J. Bruno: None. **J.M. Baker:** None. **A. Gundran:** None. **L.K. Harbott:** None. **Z. Stuart:** None. **A. Piccirilli:** None. **S.H. Hosseini:** None. **J.C. Gerdes:** None. **A. Reiss:** None.

Poster

362. Cortical Control of Executive Function

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.19/LLL35

Topic: H.02. Human Cognition and Behavior

Title: Brain network connectivity of a world record contender for simultaneous blindfold chess

Authors: *M. JOHNSON, J.-B. POCHON, N. REGGENTE, J. C. WEBSTER, J. RISSMAN; UCLA, Los Angeles, CA

Abstract: The ability to play chess while blindfolded has long been considered a showcase of one's skill at the game, as well as an impressive feat of human memory. Over the years, a few exceptional individuals developed the ability to play multiple blindfolded games simultaneously. In order to succeed at this endeavor, the player must keep an accurate mnemonic record of each game board and be able to flexibly shift between games without suffering from catastrophic interference. Although several studies have attempted to characterize neural correlates of chess expertise, no prior study to our knowledge has assessed the brain of someone who possesses exceptional talent at simultaneous blindfold chess. We acquired resting-state fMRI data from a highly-ranked chess grandmaster (T.G., age=28, Elo rating=2686, FIDE rating = 2611) who in 2013 demonstrated an ability to play 33 simultaneous blindfold games (29 wins; 4 draws; 0 losses), and who will soon be attempting to break the world record of 46 games. The domain-specificity of T.G.'s expertise is evidenced by the results of our neuropsychological testing, which found his working memory and visuospatial reasoning scores to be within the normal range. We were interested in whether T.G.'s blindfold chess abilities might be supported in part by distinctive brain network connectivity properties. Six minutes of echo-planar imaging data were acquired on a 3T scanner. Signal was extracted from a set of 378 nodes throughout the brain, preprocessed following standard procedures, and correlated between all pairs of nodes. We calculated the mean connectivity within six pre-defined networks of interest and also used the Brain Connectivity Toolbox to derive network-specific graph theoretical measures. T.G.'s results were compared with those from a cohort of 63 non-chessmasters who underwent the same imaging protocol. Most notably, T.G.'s brain showed especially high levels of connectivity within the fronto-parietal control network and a particularly high mean participation coefficient within the visual network. On both metrics, only one or two other subjects in the comparison group had comparable effects. While these intriguing findings will benefit from further investigation, they suggest that T.G.'s brain may have a more tightly organized cognitive control system with enhanced communication between fronto-parietal nodes. Moreover, the effect in T.G.'s visual network indicates that nodes within his visual system tend to interact more strongly with other brain modules than is typical. Taken together, these results provide novel and valuable clues about the neural characteristics of T.G.'s extraordinary ability.

Disclosures: M. Johnson: None. **J. Pochon:** None. **N. Reggente:** None. **J.C. Webster:** None. **J. Rissman:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: Mind and Life Institute Varela Research Award

Title: Executive system recruitment is specific to goal-related and intentionally directed mind-wandering: Direct evidence for the functional role of the frontoparietal control network in self-generated thought

Authors: *K. C. FOX, M. L. DIXON, M. GIRN, S. SHETH, A. HERRERA-BENNETT, K. CHRISTOFF;
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Abstract: 'Mind-wandering' (MW) has been most famously tied to activity in brain regions of the default mode network (DMN). Many studies, however, have also observed executive system recruitment during self-generated thought, but no study has directly addressed the functional role played by control regions in these largely spontaneous thought processes. We tested the hypothesis that executive regions would be more strongly recruited by self-generated thoughts that were intentionally directed by subjects, or that were related to subjects' goals and current concerns. During an fMRI scan, we let 16 subjects rest and think freely, interrupting their thinking at random intervals with occasional thought probes. Probes asked subjects about (i) whether their thoughts arose spontaneously, or whether they were intentionally directing them; (ii) whether thoughts were related to their goals and current concerns in life, or not; and (iii) whether they were emotionally pleasant, unpleasant or neutral. We used an event-related design to examine neural activity during MW (just prior to the thought probe) according to subjects' responses. We found greater executive system recruitment for intentionally directed and goal-related thoughts, as well as widespread reward system recruitment associated with emotionally pleasant vs. unpleasant and neutral thoughts. Counter to the prevailing view, even undirected thoughts were largely positively-valenced and goal-related, suggesting an adaptive function. Our results suggest that distinctive forms of self-generated thought recruit distinctive brain networks. They also speak to clinical disorders involving dysfunctional forms of self-generated thought, e.g. depressive rumination, by showing that negative, non-goal-related thoughts are neurally distinct from positive, goal-related thinking.

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Poster

362. Cortical Control of Executive Function

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.21/LLL37

Topic: H.02. Human Cognition and Behavior

Title: Fractionating the frontoparietal network into distinct anterior and posterior systems based on stable and dynamic network architecture

Authors: *M. L. DIXON, K. CHRISTOFF;
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Abstract: The frontoparietal control network (FPCN) contributes to the ability to flexibly shift attention between internal thoughts and external perceptual inputs. However, the network architecture underlying this capacity has yet to be fully explicated. Here, we address this issue by applying graph theoretical analyses to resting-state and task-based functional connectivity data. A stable architecture was observed across rest and six different tasks, with the FPCN fractionating into distinct anterior and posterior subsystems. The anterior FPCN was preferentially connected with the default network (DN), suggesting a role in controlling internal attention, whereas the posterior FPCN was preferentially connected with the dorsal attention network (DAN), suggesting a role in controlling external perceptual attention. Additionally, using a time-resolved approach, we tracked the dynamic evolution of network interactions and found that periods of increased anterior FPCN-DN coupling were associated with stronger specialized processing capacity (i.e., clustering) within the DN, whereas periods of increased posterior FPCN-DAN coupling were associated with stronger specialized processing capacity within the DAN. These findings provide novel evidence that the FPCN can be fractionated into distinct anterior and posterior systems, and demonstrate that dynamic interactions involving the FPCN are associated with changes across time in the strength of information processing within the DN and DAN. This study offers a novel understanding of the systems-level neural architecture underlying the control of attention.

Disclosures: M.L. Dixon: None. **K. Christoff:** None.

Poster

362. Cortical Control of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: National Center for Mental Health, Ministry of Health & Welfare

Title: Neurophysiological correlates of executive function in children and adolescents with attention-deficit hyperactivity disorder: a preliminary qEEG study

Authors: *K. JHUNG[1], J. PARK[2], J. CHOI[3], J. SONG[4];
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Abstract: Objectives: Attention-deficit hyperactivity disorder (ADHD) is a widely prevalent condition in school-aged children, and precise and reliable diagnosis is an important clinical issue. Executive performance tasks have been useful tools to aid in the diagnosis of ADHD. Quantitative EEG (qEEG) has been increasingly used to evaluate patients with ADHD. This study aimed to assess the correlation of qEEG during the resting state with executive performance tasks in patients with ADHD. Methods: Twenty-two children and adolescents with ADHD were recruited. Electroencephalography was recorded during the resting-state, and qEEG data were obtained in both eyes-open and eyes-closed state. Comprehensive Attention Test (CAT), Stroop Color-Word Inference Test (Stroop CWIT), Trail Making Test (TMT), and Wisconsin Card Sorting Test (WCST) were used to test exeutive performance of all subjects. Korean version of the ADHD Rating Scale (K-ARS) and Korean Child Behavior Checklist 6-18 (K-CBCL) were assessed. Results: In general, alpha, beta and gamma power positively correlated with the Attention Quotient (AQ), while delta and theta power negatively correlated with the AQ scores from the CAT, in the frontal, central and parietal regions. In the Stroop CWIT, delta and theta powers were decreased, while alpha, beta and gamma powers were increased in frontal, central and parietal regions, in relations to higher executive performance. Power of the gamma band in the central and parietal regions decreased with higher TMT performance. Moreover, delta, theta and gamma power of the frontal and central region negatively correlated with higher performance on the WCST, while the alpha band was positively correlated with WCST performance. Conclusions: These preliminary findings suggest that qEEG of the resting state may be a useful tool for evaluating ADHD, in relations to their executive function.

Disclosures: K. Jhung: None. **J. Park:** None. **J. Choi:** None. **J. Song:** None.

Poster

362. Cortical Control of Executive Function

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.23/LLL39

Topic: H.02. Human Cognition and Behavior

Support: CREST, JST

Title: A method for prediction of performance errors from single-trial EEG data

Authors: *H. ORA, Y. MIYAKE;
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Abstract: Performance errors may have serious results. For example, while driving a car, if the intention is to stop and the accelerator pedal is mistaken for the brake pedal, a serious traffic accident may result. If neural mechanisms that cause performance errors are identified, we may be able to avoid performance errors by detecting the error precursor. We have already revealed a neural sign of performance errors in our previous study (Ora et al., 2015). In the previous study, we applied a spatiotemporal analysis to high-density electroencephalogram (EEG) signals recorded during a visual discrimination task, a d2 test of attention, and we demonstrated that, during trials with error outcomes, a positive deviation of scalp amplitude with a latency of approx. 30 ms was observed in frontal regions, a positive deviation with a latency of approx. 125 ms was observed in parietal regions, and then a positive deviation with a latency of approx. 160 ms was observed in the occipital region. In this study, we propose a single-trial prediction method of performance errors by detecting the neural sign of performance errors.
We used non-linear support vector machine (SVM) to predict performance errors from EEG signals just before reactions. To evaluate the method, we applied the method to the data reported in our previous study. In the previous study, participants (n=10) performed a d2 test of attention during EEG recording (128 channels).
The non-linear SVM classifier was able to detect trials with error outcomes (mean AUC=0.68) from single-trial EEG data.
The results suggest that the non-linear SVM classifier was able to predict trials with error outcomes during d2 test of attention. With progress of the line of this study, we may be able to avoid performance errors by recognizing the error precursor.

Disclosures: H. Ora: None. **Y. Miyake:** None.

Poster

362. Cortical Control of Executive Function

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Program#/Poster#: 362.24/LLL40

Topic: H.02. Human Cognition and Behavior

Support: K08MH092697

Title: Sustained vs. instantaneous connectivity differentiates processing speed and fluid intelligence

Authors: *J. B. KING, A. K. MALLIK, L. M. SHAH, J. S. ANDERSON;
Univ. of Utah, Salt Lake City, UT

Abstract: Introduction: Traditional functional connectivity MRI measures synchrony of intrinsic activity between brain regions. This method has been used to identify resting state functional networks that underlie cognitive function. However, little is known about how the duration of connectivity between brain regions, or sustained connectivity, affects cognition. We used the width of cross-correlation curves between fMRI time series of brain activity as a metric of the relative duration of synchronous activity.
Methods: Resting state time series data from 839 typically developing individuals were selected for analysis from the Human Connectome Project S900 Release. Following preprocessing, a cross-correlation curve was generated for each individual between pairs of time series for 7 bilateral subcortical regions (from subject-specific FreeSurfer parcellation), 7 bilateral cerebellar networks, and 7 resting state networks. Peak and width values were extracted from each curve. Behavioral data, consisting of fluid intelligence and processing speed, were also selected for analysis. Correlations were drawn between regions of interest and behavioral data. Connections were considered significant if they satisfied false discovery rate $q<0.05$.
Results: Significant negative correlations were found between processing speed and width of cross correlation curve in numerous region pairs across the cortex and cerebellum suggesting that sustained levels of connectivity may be related to slower processing speed. Additionally, significant positive correlations were found between a progressive matrices task and the peak of cross correlation curve specifically between the bilateral caudate and cerebellar and cerebral networks indicating a relationship between peak instantaneous connectivity and fluid intelligence.
Conclusions: We found that decreases in the cross correlation curve width are associated with increases in processing speed across the cerebellum and cortex and that fluid intelligence was associated with increased cross correlation peak in corticostriatal and striato-cerebellar connections. We believe that these distinctive aspects of functional connectivity may encode different cognitive domains. Sustained connectivity may represent a novel approach to the study of cognition and psychopathology in patient groups.

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Poster

362. Cortical Control of Executive Function

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01GQ1001C

DFG grant SFB 940/1 2015

Exc 257

KFO247

Title: Who does what? Neural representations of identity and ownership of one's own and a partner's subtasks.

Authors: *D. PISCHEDDA[1,2,3,4], S. SEYED-ALLAEI[2,3], K. GÖRGEN[1,4,5], J.-D. HAYNES[1,4,5,7,6], C. F. REVERBERI[2,3];
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Abstract: In daily life, humans often coordinate their actions to perform collaborative tasks (Sebanz, Bekkering, & Knoblich, 2006). Previous studies have shown that people co-represent different parts of a general task when they carry it out together, even if representing the partner's subtask is not necessary to perform their own part of the task (e.g., Atmaca, Sebanz, & Knoblich, 2011). However, it is still an open question how these task representations are encoded at the neural level. In this study, we examined task encoding when people have to work together to achieve a common goal. Specifically, we investigated where and how the human brain represents a task that is performed by the subject or by their partner.
Twenty-six participants played a collaborative game in pairs. In the game, they had to coordinate to reach a common goal. For each pair, each subject performed the game once while undergoing functional magnetic resonance imaging (fMRI) and once using a computer in a room adjacent to the fMRI scanner. Each participant performed one part of a shared task. To win the game, the players had to consider both their own and the other's subtask. The shared task consisted in moving two pawns on a graphic path to match their positions. Each player moved one of the two pawns as specified by the subtask assigned to them. The players had to compute the combination of moves that allowed both of them to reach the goal. Importantly, the same subtask was assigned to one subject on some trials and to their partner on other trials. This paradigm allowed us (i) to identify neural representations of one's own and the other's subtask and (ii) to evaluate whether task representations differ depending on whom the task is assigned to (by comparing trials in which the same subtask was assigned to the subject vs. to their partner).
We applied multivariate decoding methods (e.g., Haynes & Rees, 2006) to fMRI data. Preliminary results show that the identity of the subtask assigned to either the subject or to their partner is represented in distinct frontal and parietal regions: ventrolateral and rostrolateral prefrontal cortex (PFC) encoded only the identity of one's own task, while medial PFC and postcentral gyrus specifically represented the identity of the other's subtask. Information about

who performed a specific subtask was contained in orbitofrontal cortex, superior parietal lobule, and inferior parietal lobule. These findings suggest that task ownership determines how information is represented across the brain.

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Poster

362. Cortical Control of Executive Function

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Program#/Poster#: 362.26/LLL42

Topic: H.02. Human Cognition and Behavior

Title: Neural correlates of role switching: a functional MRI study

Authors: *H. KADOTA, Y. KOKAGE;
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Abstract: The switching of role depending on the situation is required in both daily life and sports in order to encourage appropriate behavior. This ability is important because switching between offense and defense sometimes decides victory or defeat in a competitive game or sport. The purpose of this study was to investigate the neural correlates of role switching using an fMRI (Siemens Verio, 3T). Twenty healthy participants (seventeen men and three women) participated in this experiment. We used a game of tag with two persons (one was a participant, and the other was the author). Participants were required to control a circular object on the screen while the author controlled a square object. The object color indicated the role of the player—that is, white represented the escape role, and red represented the chase role. When one object touched the other, the color changed, and the role was reversed (switch condition). The experimental design in the session was as follows. A fixation point was presented for the first and last thirty seconds as a rest, and a game of tag was played for four minutes. This session was carried out four times. Moreover, a control experiment was conducted with the same design except that the participants' roles did not change—that is, the role was only escape or only chase (no switch condition) with two sessions each. The order of condition was counter-balanced. We used SPM12 for fMRI data preprocessing and statistical analysis. The performance result showed that the number of touches was 19.5 (±3.4) times in the switch condition and 19.7 (±3.4) times in the no-switch condition. There was no significant difference between the two conditions. The fMRI result showed that the right inferior temporal gyrus, right middle temporal gyrus, bilateral precuneus, and dorsal premotor cortices activated during the switching condition compared with during the no-switching condition. These results suggest that these areas are

involved in the switching behavior and can have an important role in flexible response according to circumstances.

Disclosures: H. Kadota: None. **Y. Kokage:** None.

Poster

362. Cortical Control of Executive Function

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Department of Veterans Affairs, VASDHS, Office of R&D

Career Development Award (D.M.S. & L.D-W)

Department of Defense Award (L.D-W)

Title: Neural correlates of response inhibition in mild-moderate traumatic brain injury: An fMRI study

Authors: *K. A. HOLIDAY[1,2], L. T. EYLER[2,3,4], R. T. KIM[2], S. SORG[2], A. M. CLARK[1,2], L. DELANO-WOOD[2,4], D. M. SCHIEHSER[2,4];
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Abstract: Introduction: Response inhibition has been theorized to be impaired in individuals with Traumatic Brain Injury (TBI), yet many studies fail to detect group differences in performance on response inhibition tasks between individuals with TBI and healthy controls. It is presumed that cognitive compensation secondary to neuronal injury may contribute to this observed behavior. However, this has yet to be experimentally tested in mild-moderate TBI (mmTBI). Thus, the objective of our study was to examine the neural correlates of response inhibition in mmTBI.
Methods: Sixty-nine Veterans with mmTBI and 41 Veteran Controls (VC) participated in a go/no-go task while undergoing event-related functional magnetic resonance imaging (fMRI). After field inhomogeneity and motion correction, spatial smoothing, and registration to standard atlas space, changes from baseline in the blood oxygen-level dependent signal during the no-go trials were calculated at each voxel and maps were compared between groups with independent

sample t-tests. Task performance was measured by d-prime, or discriminability accuracy, calculated by subtracting the z-score for the false alarm rate from the z-score of the hit rate. **Results**: The mmTBI participants did not significantly differ from VC on go/no-go task performance (d prime). During the inhibition trials ("no-go"), VC exhibited greater deactivation in the right anterior cingulate cortex (rACC) compared to mmTBI participants. Within the mmTBI group, worse performance was associated with greater rACC activation. **Discussion**: Compared to controls, mmTBI Veterans exhibited difficulty deactivating the rACC, and this impaired deactivation was associated with worse response inhibition. These findings suggest that mmTBI disrupts brain function necessary for adequate response inhibition and underscore the importance of the rACC in response inhibition.

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Poster

362. Cortical Control of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: JSPS Kakenhi 26540074

Title: Neural substrate of shifting visual-spatial attention during task-switching

Authors: *S. IWAKI[1], K. RANA[2], L. M. VAINA[2,3];
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Abstract: Task switching (TS) usually includes both shifts in visuospatial attention and contextual rules (Monsell, 2003), however, the neural mechanisms underlying these reallocation of mental resources are not fully understood. The current study focused on visualizing the brain areas responsible for handling increased TS costs due to spatial attention shifting during a cued TS paradigm. Twenty-two subjects (23.8±2.1y.o; 4 females) performed two three-alternative forced-choice tasks. Each task started with a cue consisting of a 500 ms RDK pattern of either expanding or contracting dots moving at 3 deg/s, followed by a 500 ms delay. Immediately after a pair of numbers was presented in one visual hemifield and a pair of simple shapes (either with round or angular outlines) in the other visual hemifield. **1.** If the cue portrayed an expanding RDK, the observers's task was to attend to the numbers and indicate whether they were both odd,

even, or mixed. **2.** Otherwise, the task was to attend to the shapes and indicate whether both were round, angular, or mixed. The tasks and target locations were randomly changed between 240 trials to switch contextual rules and spatial location of attention separately. We considered 4 switching conditions a) task-changed/spatial attention-changed (TCAC), b) task-changed/attention-retained (TCAR), c) task-retained/attention-changed (TRAC), and d) task-retained/attention-retained (TRAR). The fMRI data were acquired on a 3-T scanner (Philips Ingenia 3T). A single-shot EPI sequence was used to acquire 27, 3.6 mm axial slices, TR of 2,000 ms (TE 35 ms, 90 deg flip angle). Reaction times for TCAC (2.094±0.165 s; $p<0.0001$), TCAR (2.085±0.152s; $p=0.0004$), and TRAC (2.060±0.158 s; $p=0.03$) were significantly longer than for TRAR (2.033±0.158 s). We observed significant increase of brain activities in bilateral posterior cingulate cortices (PCC) both in the TCAC vs TRAR and the TCAR vs TRAR contrasts. Activities in left inferior frontal gyrus (IFG), intraparietal sulcus (IPS), and supplementary motor area (SMA) were observed only in the TCAC condition. No supra-threshold voxels were observed in TRAC vs TRAR contrast. The results indicate that increased switching cost associated with the spatial shift of attention engaged the left IFG, IPS and SMA regions. These results are consistent with recent findings suggesting that IPS is involved in directing spatial attention (Husain, 2007) and IFG controls stimulus category-switching during TS (Philipp, 2013). Bilateral activation of PCC in the TCAC and TCAR conditions may be interpreted as implementing flexible and adaptive behavior characterizing cognitive control.

Disclosures: **S. Iwaki:** None. **K. Rana:** None. **L.M. Vaina:** None.

Poster

362. Cortical Control of Executive Function

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 362.29/LLL45

Topic: H.02. Human Cognition and Behavior

Title: Neural networks associated with response selection Revealed by a flanker task with averted eye-gazes serving as response cues

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Abstract: Gaze direction can indicate not only where people is looking at but also what her intention might be. Therefore, the ability to correctly perceive/understand other's gaze is important for inferring others' mental states. Neuroimaging studies also indicate that reorienting attention in response to gaze shifts involves neural networks different from those driven by symbolic cues such as arrowheads, including the superior temporal sulcus, middle temporal

gyrus and inferior parietal lobule. In this study, we would like to test if direction information delivered by human gaze will cause differential effects and trigger different neural networks for processing response selection, as compared to the traditional version of a flanker task using symbolic cues.
We constructed a social version of a flanker task by using human gaze, i.e., the left-averted and right-averted gazes for the left and right response cues. Several behavior effects were revealed. In general, RT of the congruent trials was shorter than that of the incongruent trials. However, RT of the social version was shorter than that of the traditional version. RTs of the neutral trials of the two version behaved differently, suggesting that human gaze triggered a different system for processing. Brain activity patterns of the two flanker task versions were different. The traditional one caused higher activation in the bilateral premotor cortex, right inferior frontal cortex and superior parietal sulcus. The social version showed enhanced activities in the middle temporal gyrus, inferior parietal lobule, ventral medial prefrontal cortex, and the parahippocampal regions. The neural networks associated with this social flanker task mostly overlapped with the TOM neural networks. The results revealed robustness of the social neural networks, even for response/action selection.

Disclosures: H. Lee: None. **F. Lin:** None. **W. Kuo:** None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

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Program#/Poster#: 363.01/LLL46

Topic: H.02. Human Cognition and Behavior

Support: NIH NS080565

Title: Intracranial electrophysiology demonstrates hippocampal activation in verbal fluency tasks

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Abstract: BACKGROUND: Many studies of language production have demonstrated the importance of a large network of neocortical structures including the inferior frontal and superior temporal regions (e.g. Edwards et al., 2010; Korzeniewska et al., 2011; Cervenka et al., 2013; Pei et al., 2011). However, clinical and fMRI studies demonstrate that hippocampus is also activated in some language production tasks (Pihlajamaki et al. 2000; Gleissner and Elger, 2001; Glikmann-Johnston et al., 2015). Given the established role of the hippocampus in semantic

memory, the finding of decreased hippocampal connectivity in the semantic variant of primary progressive aphasia (Agosta et al., 2014) and the discovery that individual neurons in the mesial temporal lobe can respond selectively to specific percepts with multimodal invariance (Quian Quiroga et al. 2009), we hypothesized that the hippocampus is activated in semantic, but not phonemic, verbal fluency tasks.
METHODS: We used depth electrode recordings to study the activation patterns in the dominant hippocampus in a patient undergoing presurgical evaluation for intractable epilepsy. Four-contact platinum-iridium depth electrodes were placed in the bilateral mesial temporal regions under neuronavigation. Recordings were obtained at rest and during semantic and phonemic verbal fluency tasks using a Natus XLTEK acquisition system, with a sampling frequency of 500Hz. EEG data under each of condition were epoched in 3 second nonoverlapping segments with 1 second windows in between. Power spectra were determined in the 75Hz-115Hz frequency range using Chronux toolkit in MATLAB (http://chronux.org).
RESULTS: We observed a broad increase in power in the 85Hz-105Hz range in the left hippocampal region in both verbal fluency tasks by comparison to the resting condition. No difference was noted between the semantic and the phonemic verbal fluency conditions.
DISCUSSION: The hippocampal high gamma activity seen in the active word production conditions support prior claims that this structure may be involved in word production. Our findings are consistent with prior studies that report hippocampal activation during linguistic generation tasks (e.g. Hamamé et al., 2014). No difference was noted between semantic and phonemic verbal fluency, suggesting that the role of the hippocampus in language production may not be specific to tasks involving semantic processing.

Disclosures: S.T. Williams: None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 363.02/LLL47

Topic: H.02. Human Cognition and Behavior

Title: Inner speech activates auditory cortex: an fmri study

Authors: *K. OKADA, G. HICKOK;
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Abstract: Recent models of speech production suggest that motor commands generate forward predictions of the auditory consequences of those commands, that these forward predications can be used to monitor and correct speech output, and that this system is hierarchically organized

(Hickok, 2011; Pickering & Garrod, 2013). Recent psycholinguistic research has shown that internally generated speech (i.e., imagined speech) produces different types of errors than overt speech (Oppenheim & Dell, 2008; 2010). These studies suggest that articulated speech might involve predictive coding at additional levels than imagined speech. The current fMRI study presents neuroimaging data in support of this hypothesis. Twenty-four participants from UC Irvine were recruited for the study. Participants were scanned while they were visually presented with a sequence of words that they reproduced in sync with a visual metronome. On each trial, they were cued to either silently articulate the sequence or to imagine the sequence without overt articulation. As expected, silent articulation and imagined speech both engaged a left hemisphere network previously implicated in speech production. A contrast of silent articulation with imagined speech revealed greater activation for articulated speech in inferior frontal cortex, premotor cortex and the insula in the left hemisphere, consistent with greater articulatory load. Although both conditions were silent, this contrast also produced significantly greater activation in auditory cortex in dorsal superior temporal gyrus in both hemispheres. We suggest that these activations reflect forward predictions arising from additional levels of the perceptual/motor hierarchy that are involved in monitoring the intended speech output.

Disclosures: K. Okada: None. **G. Hickok:** None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

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Program#/Poster#: 363.03/LLL48

Topic: H.02. Human Cognition and Behavior

Title: The role of feature type and semantic domain in effective connectivity underlying semantic retrieval

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Abstract: Increasing evidence shows that the brain semantic network involves large-scale connections among frontal, parietal and temporal regions. The cerebral organization of conceptual knowledge remains a controversial issue. We used Psycho-Physiological-Interaction (PPI) analyses to assess the role of feature type (motion and visual form/surface) and domain (living and non-living) on the strength of effective connectivity among the key regions recruited by a verification task. Thirty-eight healthy subjects were asked to report whether concept-feature sentences (e.g. “The saw cuts trees”) are true or false during functional magnetic resonance

imaging. Each feature type was contrasted on exactly the same exemplars, and each category was contrasted on exactly the same features. Unlike previous studies, we could thus evaluate the extent to which brain activity and connectivity underlying semantic retrieval are driven by feature type and concept domain, with exactly the same materials and task. We first assessed the effect of feature type vs. semantic domain on brain activity underlying semantic retrieval. Direct comparisons highlighted widespread effects of feature type, with stronger activation of bilateral fronto-parietal networks for visual than. motion features, while the left lateral posterior temporal cortex and inferior frontal gyrus (pars triangularis) were activated in the opposite comparison. The effects of semantic domain were less extensive, and involved the left medial temporal pole for living compared to non-living items, and the left lateral temporal cortex alongside ventral premotor cortex in the reverse comparison. To further understand their role, we assessed how connectivity to/from these regions is modulated by semantic features vs. domain processing. PPI analyses showed that effective connectivity among these regions is driven exclusively by feature type, with no modulatory effect by semantic domain. Even when testing the connectivity to/from the regions associated with living vs. non-living comparisons, we observed significant modulations only by feature types.These findings provide further support to a modality-specific account of the brain semantic network, whose causal organization is driven by specific kinds of features rather than taxonomic domains.

Disclosures: S.F. Cappa: None. **E. Catricala':** None. **E. Zanin:** None. **A. Falini:** None. **N. Canessa:** None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: European Research Council (ERC-2009-AdG 249425-CriticalBrainChanges)

German Research Foundation (DFG, SFB 538)

Title: Neural correlates of semantic and syntactic processing in sign language

Authors: *A.-L. STROH, F. ROESLER, G. DORMAL, N. SKOTARA, B. HAENEL-FAULHABER, B. ROEDER;
Univ. of Hamburg, Hamburg, Germany

Abstract: Even though signed and spoken languages share similar characteristics at an abstract linguistic level, including phonological, semantic and syntactic organization (Sandler & Lillo-Martin, 2006), they differ in the way they express grammatical relations: Whereas spoken languages use word-order and/or inflectional morphology to distinguish grammatical referents, signed languages express grammatical relations by means of spatial loci and hand orientations. Thus, it could be hypothesized that the neural correlates of the core aspects of language processing (i.e. semantic) are similar across spoken and signed languages (Caselli & Cohen-Goldberg, 2014), while other higher level language processes (i.e. syntactic) are influenced by surface form differences between the two modalities.
In the present study we investigated the neural correlates of semantic and syntactic processing during sign language comprehension in order to assess whether specific aspects of linguistic processing depend upon sensory processing mechanisms of a language. We conducted a functional magnetic imaging (fMRI) experiment with a group of 16 native signers (8 deaf/ 8 hearing). Participants performed a sentence judgement task on sign language sentences which were correct or contained either syntactic or semantic violations.
Analyses performed across groups revealed evidence for modality-dependent and modality-independent effects for the two types of linguistic violations. Semantic violations compared to correct sentences elicited increased activation in low-level visual areas, suggesting modality-specific processing mechanisms. Semantic violations compared to syntactic violations elicited activation in the left inferior frontal gyrus. This concurs with findings from spoken and written language studies, suggesting modality-independent processing mechanisms. Syntactic violations compared to correct sentences involved activation in the right inferior parietal cortex, possibly indicating spatial processing mechanisms that are specific to signed syntax. This activation was violation specific, as syntactic violations compared to semantic violations elicited increased activation in this area as well. Taken together, the present findings suggest that the neural correlates of language processing are partly shaped by biological constraints – regardless of language modality -, but that they may additionally be influenced by the unique processing demands of the language modality.

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Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: DFG GE 2777/2-1

Title: Earlier than expected: functional and structural connectivity of early auditory cortex influences broca's involvement during speech production

Authors: *P. FRIEDRICH, S. OCKLENBURG, C. FRAENZ, O. GUNTURKUN, E. GENC; Dept. of Biopsychology, Ruhr-University, Bochum, Germany

Abstract: Human speech is a dynamic process involving both speech executive and sensory processes, associated with a wide spreading cortical network. Though inferior frontal gyrus (IFG) is a key contributor to speech production, it is not yet clarified to which extent temporo-parietal regions mediate involvement of the IFG during speech perception and production. Using fMRI in combination with DTI we examined if the activation pattern of IFG during speech perception and production is influenced by its functional and structural connectivity with temporo-parietal regions.

BOLD activation was measured in 105 right-handed participants during a passive word perception and silent word generation task. An anatomical-landmark-based segmentation was used to determine regions of interests (ROIs) located in IFG (pars opercularis and pars triangularis (broca)) and temporo-parietal regions (transversetemporal gyrus (trans), planum temporale (planu) and supramarginal gyrus (supra)). For all connectivity analyses broca was used as target and temporo-parietal areas as seed regions. We first extracted BOLD activity in broca and than tested whether this activity pattern is modulated by functional PPI connectivity from temporo-parietal regions. In addition, DTI probabilistic-tractography between target and seeds was used to test if pathway properties of these tracts were able to predict the extent of broca activity. Functional connectivity analysis revealed that individual pattern of activity in broca during word generation was related to functional PPI connectivity from all ROIs of temporo-parietal regions (trans-to-broca, $r = .35$, $p < .01$; planu-to-broca, $r = .26$, $p < .01$; supra-broca, $r = .26$, $p < .01$). In addition, we found that the individual pattern of activity in broca was associated to variability of tract size of connections between planu-to-broca ($r = .27$, $p < .01$) and supra-to-broca ($r = .34$, $p < .01$). Since all PPI functional and DTI structural connectivities were associated to each other ($r = .26$ - $.86$, $p < .01$), we performed a multiple regression analysis with all PPI and structural connectivities as independent variables and broca activity as the dependent variable. Surprisingly, only trans-to-broca PPI functional connectivity provided unique contributions to the prediction of broca activity ($ß = .24$, $p < .05$; other connectivities, $p > .12$). No associations were found for homologous ROIs in the right hemisphere, as well as for both hemispheres during the word perception task.

Our results document that the pattern of activity of IFG as key region involved in speech production are closely linked to the input of the early auditory cortex.

Disclosures: **P. Friedrich:** None. **S. Ocklenburg:** None. **C. Fraenz:** None. **O. Gunturkun:** None. **E. Genc:** None.

Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: Walton Family Foundation, Inc.

NIDCD T32DC000038

Title: Children's language exposure predicts their neural activation during language processing

Authors: *R. R. ROMEO[1,2,3], J. A. LEONARD[2,3], S. T. ROBINSON[2,3], M. L. ROWE[4], A. P. MACKEY[2,3], J. D. E. GABRIELI[2,3];
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Abstract: Research provides increasing evidence that a child's experience early in life shapes their neuroanatomy and corresponding cognitive functions. One critical experience is language exposure; the quantity and quality of the language children hear during these early years impacts their overt language abilities throughout childhood and into adulthood. However, there is little evidence that directly relates children's language experience to specific aspects of the underlying brain function. Here, we examined how variation in spoken language experience at home is related to variation in brain functions associated with spoken language comprehension. A socioeconomically-diverse group of families with typically-developing, native English-speaking children in pre-Kindergarten and Kindergarten (ages 4 to 6 years) were recruited. Both children and their primary caregiver completed behavioral assessments of their verbal and non-verbal cognitive abilities. Children then completed a functional magnetic resonance imaging (fMRI) session, including a task involving listening to simple stories and the same stories played backwards. Finally, families completed two full days of real-world audio recordings from the child's perspective (via a child-worn audio recorder), from which two measures were calculated: the number of words spoken by an adult, and the number of conversational turns between the child and an adult. While the number of adult words is a purely quantitative measure, 'conversational turns' is both quantitative and qualitative, because it implies a balanced, time-locked discourse. By contrasting activation for typical speech to incomprehensible backwards speech, we identified brain regions related to higher-level language comprehension, i.e., the semantic and syntactic processing necessary to understand the stories. This contrast yielded a significant positive correlation between the peak number of hourly conversational turns at home and activation in Broca's area, formed by left pars triangularis and pars opercularis regions. This relationship held when controlling for measures of socioeconomic status (SES). Behaviorally, conversational turns also correlated with children's scores on a standardized language

assessment. Similar but statistically insignificant relations were found with simple number of words spoken by an adult at home. This suggests that language exposure is not only related to children's behavioral verbal abilities, but also to the brain function underlying language processing; furthermore, it suggests that conversational *quality* has more impact than sheer numbers of words.

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Poster

363. Cortical Mechanisms of Language

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Program#/Poster#: 363.07/LLL52

Topic: H.02. Human Cognition and Behavior

Title: Comprehension-dependent cortical activation during speech comprehension tasks with multiple languages: functional near-infrared spectroscopy (fNIRS) study

Authors: M. LEI[1], T. MIYOSHI[1], Y. NIWA[1], I. DAN[2], *H. SATO[1];
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Abstract: With the aim of investigating cerebral hemodynamics during auditory language comprehension, we measured brain activation of 46 normal right-handed Japanese adults with functional near-infrared spectroscopy (fNIRS) while performing speech comprehension tasks. In the experiment, a picture and four statements were given as stimuli to a subject, and the subject was asked to choose a statement that correctly describes the picture. Statements were given in three languages: English (second language), Japanese (native language), and Chinese (unknown language). Significant differences of oxygenated-hemoglobin (oxy-Hb) activations are found among three different languages particularly in the left hemisphere. Moreover, particularly in the English tasks, there are significant differences in oxy-Hb activations between the case when subjects correctly answered questions and the case when they didn't. These results suggest that hemodynamic response differ in whether a subject understands given speeches or not.

Disclosures: M. Lei: A. Employment/Salary (full or part-time): Hitachi, Ltd. **T. Miyoshi:** A. Employment/Salary (full or part-time): Hitachi, Ltd. **Y. Niwa:** A. Employment/Salary (full or part-time): Hitachi, Ltd.. **I. Dan:** None. **H. Sato:** A. Employment/Salary (full or part-time): Hitachi, Ltd..

Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 15K16414

Title: Fractional anisotropy decreases at extended segments in the left arcuate fasciculus in people who stutter

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Abstract: The left arcuate fasciculus connects motoric and perceptual language systems and the left ventral premotor and motor cortex. In people who stutter (PWS), the fractional anisotropy (FA) of the white matter is reduced in the left arcuate fasciculus near the ventral motor cortex related to speech (Sommer *et al.*, 2002; Chang *et al.*, 2008; Watkins *et al.*, 2008; Cykowski *et al.*, 2010, Connally *et al.*, 2014, Cai *et al.*, 2014). Among these studies, however, the areas of significant FA reduction were subtly different. In this study, the whole-brain fractional anisotropies (FA) were calculated from the diffusion weighted magnetic resonance imaging (DWI) of 64 directions and of no DWI (b0) acquired with a 3.0 T scanner (Skyra, Siemens) using a 64-channel head coil. FMRIB's Diffusion Toolbox (FDT) in the FMRIB Software Library (FSL, FMRIB, Oxford, UK) was used for analysis. Tract-based spatial statistics of FA was applied to the whole comparison between the two groups. During preprocessing, brain-edge artifacts were removed. All FA images were aligned and registered to the standard space. After creating the mean 3D image of all the FA images, a skeletonizing mask was created with the threshold of 0.3. Two-group voxel-wise unpaired *t*-test was applied to the masked (skeletonized) FA data of the PWS and control subject groups. More elongated segments (up to 30 mm) of significantly decreased FA ($p < 0.05$, corrected) was found near the operculum and the angular gyrus (AG) in the left superior longitudinal fasciculus (SLF) than previously reported. The present results indicate that the somewhat inconsistent localizations of decreased FA in previous studies are parts of the more elongated segments of decreased FA in the left arcuate fasciculus. The decreased FA near the presumed mental lexicon area (AG) is likely to correspond to the decreased functional activation around this area in PWS (Mori *et al.*, J Phon Soc Jpn, 20:61, 2016) and may explain the stuttering symptoms with familiar words.

Disclosures: **K. Yasu:** None. **R. A:** None. **K. Mori:** None. **N. Sakai:** None.

Poster

363. Cortical Mechanisms of Language

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Changjiang Scholars Programme of China

Title: The shared mechanism underlying music and reading

Authors: *M. YU[1], M. XU[2], X. WANG[2], J. LIU[1];
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Abstract: Human musical ability is proposed to play a key phylogenetical role in the evolution of language, and the similarity of hierarchical sound structure has further led to a speculation of shared mechanisms. However, previous studies on the relation of these abilities usually treat language and music as a whole, and the results are controversy. Because both language and music have a complex structure, consisting of multiple relatively independent components, we reason that the relation may be stably revealed at the component level, where the correlation will not be diluted by unrelated components. To test this intuition, we examined the relation between reading components (e.g., semantic processing) and music components (e.g., melodic analysis) at both behavioral and neural levels. Behaviorally, we compared the ability of processing semantic and phonemic information of language between individuals with music training and those without. We found that individuals with music training were better at semantic processing than those without, whereas no difference was found in phonemic processing, suggesting that the semantic, not phonetic, component of language may be related to music. To further specify the relation, we conducted correlation analyses between two abovementioned language components and two music components (melodic and rhythmic analysis). As expected, phonetic processing did not correlate with any of music components tested. Importantly, semantic processing correlated with melodic, not rhythmic, analysis of music, suggesting a shared mechanism underlying semantic processing of language and the melodic analysis of music. To further explore the neural basis of the shared mechanism, we used resting-state fMRI (rs-fMRI) with a

measure on the magnitude of spontaneous neural fluctuation called fALFF. We found that both behavioral performance in semantic processing of language and that in melodic analysis of music positively correlated with fALFF of bilateral precentral gyrus (PCG) and superior temporal plane (STP) at the regional level, and the functional connectivity (FC) of left PCG with left supramarginal gyrus (SMG) and the FC of left PCG with left superior temporal gyrus (STG) at the network level. In short, our study provides direct evidence revealing an intrinsic link between music and reading, which may rely on a shared mechanism of automatic sound processing and auditory-motor integration.

Disclosures: **M. Yu:** None. **M. Xu:** None. **X. Wang:** None. **J. Liu:** None.

Poster

363. Cortical Mechanisms of Language

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Program#/Poster#: 363.10/LLL55

Topic: H.02. Human Cognition and Behavior

Title: fMRI inter-trial variability as behavioral predictor in reading

Authors: ***J. SCHEFF**[1,2], S. BAILEY[2], M. RICHMOND[2], L. CUTTING[2];
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Abstract: Current studies examining the neurobiological correlates of reading interventions have largely used means-based analysis methods for functional MRI (fMRI); however, recent studies have suggested that inter-trial variability of functional neuroimaging data may provide additional insights into neurocognitive function. For example, studies have found unique correlations between inter-trial variability and autism (Milne, 2011), behavioral variability (McIntosh, Kovacevic, & Itier, 2008), and variability in the evoked neurological response (Fox, Snyder, Zacks, & Raichle, 2006). This study set out to test whether inter-trial variability in the fMRI BOLD response could predict longitudinal changes in reading-related behavioral tasks--i.e., whether variability at time 1 in the fMRI signal could predict changes in measures of phonological processing or other reading-related measures between time 1 and time 2. Using longitudinal data from 41 children starting in first grade, we built a general linear model that included inter-trial variance of single-trial betas as the independent variable, longitudinal change in phonological processing as the dependent variable, and controls such as age and socioeconomic status. Findings indeed suggest that inter-trial variability may have added value in predicting behavioral outcomes.

Disclosures: **J. Scheff:** None. **S. Bailey:** None. **M. Richmond:** None. **L. Cutting:** None.

Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: NSF/CRNS IIS-1208203

NEI 1R01EY019684-01A1

Title: The visual-linguistic interface: anatomically aligned semantic representations of vision and language

Authors: A. G. HUTH, S. F. POPHAM, N. Y. BILENKO, *J. L. GALLANT;
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Abstract: Information about visual categories is represented in the anterior occipital cortex. Similarly, information about semantic categories in narrative speech is represented in the nearby posterior temporal and parietal lobes. These two types of representation meet at the boundary of visual cortex. We investigated the anatomical relationship between these two types of representation by examining voxel-wise models trained to predict BOLD fMRI responses based on semantic properties of visual and linguistic stimuli. Six subjects watched two hours of natural movie clips, each second of which was labeled with object and action categories that were present. In separate scanning sessions, the same subjects listened to over two hours of naturally spoken, narrative stories, which were transcribed and aligned to find when each word was spoken. Each category label in the movies and each word in the stories was projected into a shared 985-dimensional semantic word embedding space. Then regularized linear regression was used to estimate separate voxel-wise encoding models for the movie data and for the story data. Directly comparing visual and linguistic models on a voxel-by-voxel basis shows that some voxels near the boundary of visual cortex have similar semantic selectivity in both modalities. However, closer examination of representations of specific categories reveals a more complex organization. For nearly any visual category that is represented on the posterior side of the boundary, we find an adjacent representation of the same linguistic category on the anterior side of the boundary. In many cases, voxels in a narrow region immediately on the boundary of visual cortex represent the same semantic category in both modalities. This suggests that the boundary of visual cortex constitutes both an anatomical and functional interface between visual and linguistic representations.

Disclosures: A.G. Huth: None. **S.F. Popham:** None. **N.Y. Bilenko:** None. **J.L. Gallant:** None.

Poster

363. Cortical Mechanisms of Language

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Ministry of Education, Culture, Sports, Science and Technology of Japan; Number: 26860675

the SENSHIN Medical Research Foundation

Title: Contribution of the motor system to McGurk effect —event-related fMRI and TMS studies—

Authors: *T. MURAKAMI[1], J. FUJIWARA[1], Y. SAKAMOTO[1], M. OKAMOTO[1], T. MIZUOCHI[2], T. IWABUCHI[2], M. MAKUUCHI[2], M. ABE[3], H. KUBO[1], N. MATSUDA[1], S. KOBAYASHI[1], Y. UGAWA[1];
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Abstract: Introduction Incongruent phonetic sound and facial movement can elicit audiovisual illusion, so called ‘‘McGurk effect’’. For example, a combination of auditory /Pa/ and visual /Ka/ results in the perception of a third syllable of /Ta/. Recent neuroimaging studies proposed that left posterior superior temporal sulcus (pSTS), a hub region of audiovisual integration, is involved in McGurk effect. However, the mechanisms still remain under debate. **Methods** 27 right-handed healthy volunteers received audiovisual incongruent (auditory /Ba//Pa/ and visual /Ga//Ka/) and congruent (both /Ba//Pa/) perceptual tasks, and then they were asked the syllables listened to. We investigated activated brain regions in event-related functional magnetic resonance imaging (fMRI) study. In addition, we applied single-pulse transcranial magnetic stimulation (TMS) over the lip or foot area of the primary motor cortex (M1) in an event-related design and we measured McGurk susceptibility. **Results** Behaviorally, audiovisual incongruent trials resulted in higher illusion susceptibility and longer reaction time than congruent trials. FMRI study revealed brain activations in audiovisual areas, dorsal premotor cortex (dPMC) and inferior frontal gyrus (IFG), bilaterally in both audiovisual stimuli. Activations in dPMC and IFG were more prominent in incongruent task, whereas stronger activations of the pSTS in congruent task. Left IFG activation correlated negatively with McGurk susceptibility. Psychophysiological interaction analysis demonstrated increased effective connectivity between the left IFG and M1 lip area in incongruent task. Event-related TMS over M1 lip area showed significant reduction of

McGurk susceptibility when compared with TMS over M1 foot area or the control condition (no TMS). **Conclusions** Findings indicate that the motor system contributes to the recognition of audiovisual inputs and that it is more active in the incongruent condition, while pSTS is more active in the congruent condition. The left IFG-M1 network plays some roles in detecting and resolving multisensory incompatibility to reduce illusion.

Disclosures: **T. Murakami:** None. **J. Fujiwara:** None. **Y. Sakamoto:** None. **M. Okamoto:** None. **T. Mizuochi:** None. **T. Iwabuchi:** None. **M. Makuuchi:** None. **M. Abe:** None. **H. Kubo:** None. **N. Matsuda:** None. **S. Kobayashi:** None. **Y. Ugawa:** None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant 81171016

NSFC Grant 81371206

NSFC Grant 31571158

Title: The left dorsal premotor cortex in foreign visual-word processing for beginner readers

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Abstract: For reading acquisition in a native language, visual-words are learnt by mapping to the already developed spoken language captured in the visual-words. But for beginner readers of a foreign language, the acquisition of the foreign visual-words are largely different from that of the native visual-words, because its spoken system is yet to develop. The foreign words are often learnt by repeating articulation of the words, and by connecting to the native language to access the semantics. Although much research has explored the neural substrates underlying reading in a native language, it remains unknown how foreign visual-words of which the spoken system is undeveloped are processed in the human brain. Here we addressed this issue by recruiting children from primary schools who learnt to read in their native language and in a foreign language. Forty-nine children in fourth or fifth grade were scanned when several categories of visual stimuli (including Chinese character, English word, face, house, etc.) were presented in a

block paradigm. The participants were asked to look at the screen and press a button when a target star popped up. Their reading skills in Chinese and English were measured after the scanning with standard tests. As shown by the activation contrasts, although both languages activated a similar reading network, the processing of English words, as compared to Chinese characters, did not activate the left superior temporal sulsus, a region involved in phonological decoding. It may suggest that the foreign phonological system is not matured enough to access in foreign visual-word processing. Since foreign language acquisition involves a large number of practices of articulation, we hypothesized that the regions involved in articulation would be important for the foreign visual-word processing. We thus conducted correlation analyses between activation and reading skills, which revealed that the activation in the left dorsal premotor cortex (dPM) for English words (not for Chinese characters) was positively correlated with English reading performance and with rapid automatic naming score. The role of the left dPM in foreign visual-word processing was further supported by a correlation between activation in the left dPM and in the VWFA for English words (not for Chinese characters). These results indicate the tight link of articulation to foreign visual-word processing and learning for beginner readers.

Disclosures: L. Li: None. **X. Feng:** None. **X. Meng:** None. **G. Ding:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Arizona State University

Title: Functional plasticity of sentence-processing brain networks: an fMRI study of late American Sign Language acquisition

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Abstract: It is well documented that the neural circuitry supporting second language (L2) acquisition highly overlaps with that of an individual's native language (L1), particularly when L2 proficiency is high and age of L2 acquisition is low. It is perhaps not surprising that brain networks that are tuned to the complex hierarchical auditory signals of L1 are recruited by the complex hierarchical auditory signals of L2. However, it is unclear if established language

networks specialized for auditory languages are flexible enough to be responsive to hierarchical visual-spatial information, such as syntactic structures in signed languages. The present study addresses this question by studying late L2 normal hearing learners of American Sign Language (ASL). Twenty native English-speaking undergraduates enrolled in American Sign Language courses viewed videos of the following four conditions during fMRI acquisition: signed sentences, signed word lists, English sentences and English word lists. Participants also completed an ASL proficiency test from which proficiency in ASL syntax and vocabulary was calculated. Stimuli contained vocabulary and syntactic constructions covered in the participants' ASL coursework and were signed by a fluent ASL instructor. Standard fMRI preprocessing, voxel-wise regression and correlational analyses were conducted using SPM8. Preliminary results include: L2 ASL and L1 English recruited highly overlapping brain networks, including Broca's area and bilateral superior temporal regions. ASL syntactic processing (as measured by a contrast of ASL sentences versus ASL word lists) recruited bilateral anterior and posterior temporal regions as well as medial fronto-parietal attention networks. Portions of Broca's area and anterior temporal cortex, known sentence-processing regions, were significantly activated to both ASL and English sentences. Notably, Broca's area was more responsive to ASL sentences than English sentences. Greater ASL proficiency was significantly correlated with activation in the left posterior superior temporal and inferior parietal cortex (i.e. area Spt) and Broca's area, replicating previous findings regarding the neural correlates of spoken language L2 proficiency. Lower ASL proficiency was associated with activation of attention and working memory networks (e.g. dorsolateral prefrontal cortex) as well as visual-spatial parietal-occipital networks. These initial findings suggest that well-established fronto-temporal auditory language networks involved in sentence processing exhibit functional plasticity and are not modality-dependent.

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Poster

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Support: W.M. Keck Foundation Grant

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Title: Infants' sensitivity to visual rhythmic-temporal patterning of language: An integrated fNIRS neuroimaging, thermal infrared imaging, and eye tracking investigation

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Abstract: Sensitivity to rhythmic temporal patterning at the size of the syllable in language (~1.5Hz) is hypothesized to be a core mechanism for infant linguistic segmentation and categorization (Baker et al 2006; Petitto et al 1991; 2001; 2004). The STG is argued to be a key neural site driving this peaked sensitivity (Petitto 2007; Petitto et al 2000; 2012) and may also drive emotional arousal and engagement, directing infants' attention to language and constituting a marker of "readiness to learn." Controversy prevails about whether infants' sensitivity to rhythmicity is initially predisposed to syllabic patterning or arises from language experience. Hearing babies--no sign language experience--viewed meaningless but well-formed phonetic-syllabic units in signed language (SL) and identical syllabic information in point-light scenes (PL; stripped of surface phonological features but with global syllabic rhythms).
Methods. 6-month-old babies (N = 6), block design, 4 conditions: (1) Real human signed syllable, presented at 1.5Hz, and PL at (2) 0.5Hz, (3) 1.5Hz, (4) 3.0Hz. Time-locked recorded neural activity (fNIRS), psychophysiological (IR thermal imaging), and cognitive attention (eye tracking).
Results. fNIRS differed by condition: Real human 1.5Hz SL and syllabic patterned 1.5Hz PL elicited similar bilateral STG/MTG activity and laterality indices (Seghier, 2008), while nonlinguistic patterned PL at 0.5Hz and 3.0Hz each elicited different LH STG/MTG activity and laterality indices indicating low-level temporal signal processing (Zatorre & Belin, 2001). IR thermal data aligned with fNIRS: Real human 1.5Hz SL showed peaked parasympathetic activity indicating social engagement (Manini et al, 2013. Syllabic patterned 1.5Hz PL elicited a similarly peaked but inverted response involving sympathetic activation, indicating error detection/conflict resolution. Nonlinguistic patterned PL 0.5Hz and 3.0Hz both elicited IR responses indicating varying levels of disengagement. Eye tracking: no differences across PL, longer looking in SL.
Preliminary findings suggest that temporal lobe sites, including STG, may be attuned to rhythmic temporal patterning relevant to syllabic structure at ~1.5 Hz with yoked psychophysiological responses involving social engagement, disengagement, and error detection, revealing a first-time important language acquisition mechanism. As the hearing infants had no experience with signed languages but showed specific engagement with abstract human and point-light syllabic patterning, it provides compelling support for the hypothesis that infants may be predisposed to syllabic patterning unique to language.

Disclosures: **A. Stone:** None. **B. Manini:** None. **G. Kartheiser:** None. **C. Langdon:** None. **A. Merla:** None. **L. Petitto:** None.

Poster

363. Cortical Mechanisms of Language

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Language in Interaction Gravitation Grant 024-001-006

Spinoza Prize to P.H.

Title: Oxytocin modulates semantic integration in speech comprehension

Authors: *Z. YE[1], A. STOLK[2], I. TONI[2], P. HAGOORT[2];
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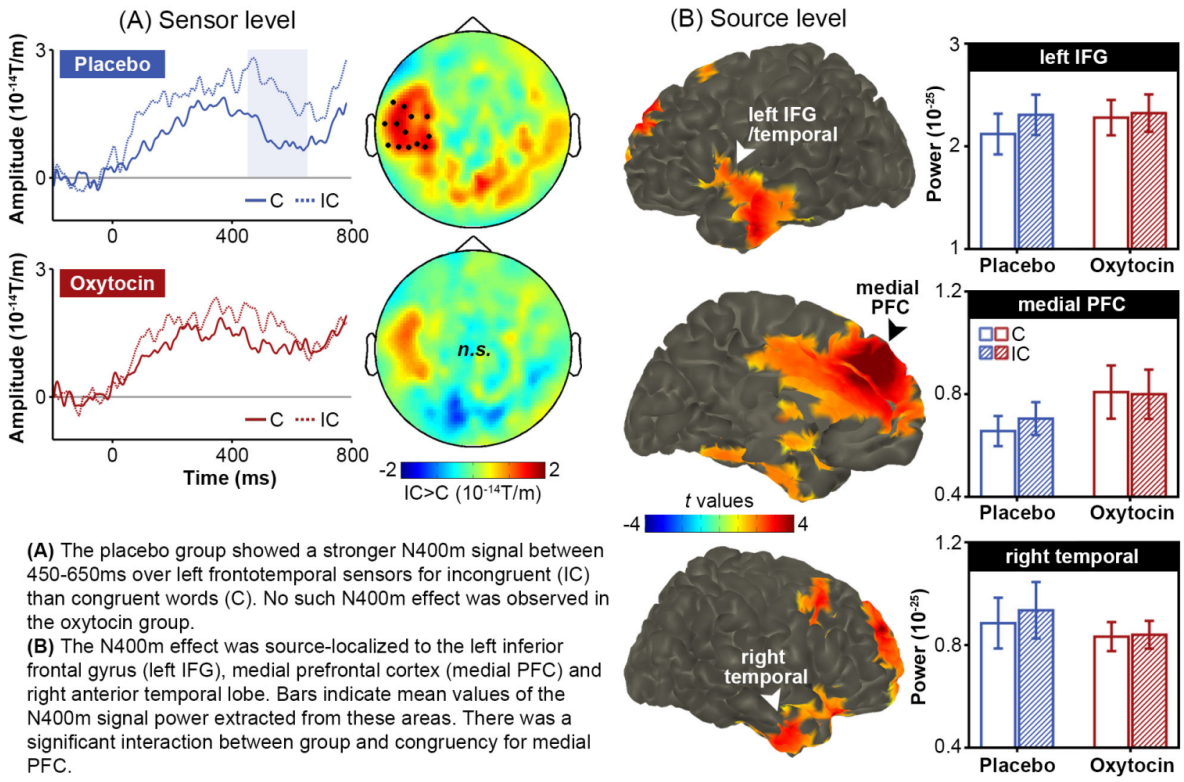
Abstract: **Background:** Listeners interpret utterances by integrating information from linguistic (word-level semantics) and extra-linguistic sources (world knowledge). When the semantics of an expression conflicts with his/her world knowledge, the listener may have to search through the conceptual space for alternative possible-world scenarios that can make the expression more acceptable. Such cognitive exploration could be costly in terms of computational resources and might depend on motivational factors. Oxytocin is a neuropeptide known to influence social motivation by reducing social anxiety and enhancing affiliative tendencies. This study explores whether and how oxytocin can facilitate the integration of world knowledge and word meanings.
Methods: The study used a between-subject double-blind randomized placebo-controlled design. Forty-five healthy males participated in a speech comprehension task with sentences that were either congruent or incongruent with facts of the world after receiving intranasally delivered oxytocin or placebo. Semantic integration was quantified with magnetoencephalography (MEG) through the N400m marker.
Results: Compared with congruent sentences, world-knowledge incongruent sentences elicited a stronger N400m signal (N400m effect) over left frontal and temporal sensors in the placebo group (FigA). The N400m effect was source-localized to the inferior frontal and anterior temporal regions and medial prefrontal cortex (FigB). Oxytocin administration abolished the N400m effect at both sensor and cortical source levels throughout the experiment, in a state-like manner. Additional analyses suggested that the absence of the N400m effect in the oxytocin group is unlikely due to the lack of early sensory or semantic processing or attention.
Conclusion: Our findings suggest that oxytocin may have a motivational in language comprehension. Oxytocin might drive listeners to resolve challenges of semantic integration, possibly via facilitating the cognitive exploration of alternative possible-world scenarios.



(A) The placebo group showed a stronger N400m signal between 450-650ms over left frontotemporal sensors for incongruent (IC) than congruent words (C). No such N400m effect was observed in the oxytocin group.
(B) The N400m effect was source-localized to the left inferior frontal gyrus (left IFG), medial prefrontal cortex (medial PFC) and right anterior temporal lobe. Bars indicate mean values of the N400m signal power extracted from these areas. There was a significant interaction between group and congruency for medial PFC.

Disclosures: **Z. Ye:** None. **A. Stolk:** None. **I. Toni:** None. **P. Hagoort:** None.

Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: CONACyT Frontiers Grant 225

LANIREM, INB

Title: Development of an assessment tool for the study of pragmatic language comprehension for use in neuroimaging studies

Authors: A. V. CARRILLO-PEÑA[1], G. L. LICEA-HAQUET[2], D. E. VALLES-CAPETILLO[2], *M. GIORDANO[2];

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Abstract: Human language is one very good example of how culture shapes the brain. Once a child is born into a human cultural group, she is exposed to sounds and utterances that become associated with specific meanings. Areas in the brain become specialized for comprehension and expression of language, processes that also involve auditory and motor components. Words are then organized, spoken language becomes immersed in the context in which words are uttered. Besides the processing of the semantic meaning of the utterances, language comprehension involves the processing of information about context and intentions. The discipline that studies the principles that guide the use of language for communication is known as Pragmatics, and the term Neuropragmatics was coined to define the study of the neurocognitive architecture of Pragmatics. Studies with patients with pragmatic deficits have led to hypothesis regarding the cognitive functions required for pragmatic comprehension, but few studies in healthy subjects have evaluated which cognitive functions are necessary or sufficient for pragmatic comprehension, and which neural areas and networks are involved. In the present study we selected 521 Spanish proverbs that were evaluated by 599 subjects using Likert scales in four dimensions: how understandable, familiar, literal versus metaphorical they were, and their emotional valence. In a separate group of participants (n=48), we evaluated a battery of psychological and neuropsychological tests to measure general and specific domain cognitive functions that could underlie pragmatic comprehension. The results of the proverb selections resulted in 54 of highly comprehensive proverbs, from that group we selected those rated high and low on familiarity. Subjects had difficulty classifying the proverbs as literal or metaphorical, and assigning an emotional valence. Comprehensibility ratings were validated against the subject's interpretation of each proverb. With regard to the battery of tests we found that all executive functions correlated significantly with the general capacity index (WAIS-IV), and that working memory correlated with mental flexibility, and verbal fluency. With regard to specific functions, the test for the emotional component of theory of mind correlated significantly only with the test for the cognitive component; the IRI empathic concern scale correlated significantly only with the fantasy and personal distress scales (Spearman's rho, $p<0.05$). With these results we will now proceed to scan our participants using magnetic resonance imaging and measure the blood-oxygen-level-dependent signal during the pragmatic comprehension task.

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Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: R01DC010145

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BCS-1262297

Title: Language lateralization assessed by magnetoencephalography imaging using three different language tasks

Authors: *E. DE WITTE[1], L. HINKLEY[2], D. MIZUIRI[2], C. GARRETT[2], S. HONMA[2], H. KIRSCH[3], J. HOUDE[4], M. BERGER[5], S. NAGARAJAN[6];
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Abstract: INTRODUCTION: Magnetoencephalography (MEG) is a valuable alternative to the invasive intracarotid amobarbital procedure (also known as the Wada test) for defining preoperative language lateralization in neurosurgical candidates. Laterality indexes (LI) measured by MEG imaging have shown high concordance with Wada data. In addition, MEG provides temporal information about the transient nature of hemispheric dominance of language. Verb generation is typically used during preoperative language mapping, but might be too difficult for some patients with preoperative language deficits. Therefore, other language tasks such as repetition and picture naming have been administered. As for all mapping techniques task selection may impact the interpretation of results and possibly the calculated LI. A preoperative MEG paradigm has been developed to determine language lateralization with excellent predictive value using the verb generation task (Findlay et al., 2012). However, this paradigm has not yet been tested nor adapted for other language tasks.
METHODS: In this study, 7 glioma patients (6 left-handed, 1 right-handed) underwent both the Wada test and MEG in the preoperative phase. MEG was recorded using a 275-channel whole head biomagnetometer (CTF Systems) during three different language tasks: verb generation, picture naming (Hinkley et al., 2012) and non-word repetition. MEG data was reconstructed in source space using an adaptive spatial filtering technique in Nutmeg (www.nitrc.org/projects/nutmeg). Optimized time windows and regions of interest for each task were determined by examining oscillations in the beta band (12-30Hz), which were concordant

with the Wada data. LI was estimated by comparing changes in beta power across frontal and temporal ROIs for both stimulus and response locked analyses.
RESULTS: As has been previously reported, increased beta suppression was observed across the frontal and temporal lobes for verb generation representative of hemispheric dominance for language. Using identical time windows and regions for analysis in the picture naming and non-word tasks, we identified concordance with the Wada test in 5/6 subjects (picture naming) and 6/7 subjects (non-word repetition). Time windows and ROIs were adjusted to ensure full concordance. The new paradigms for repetition and picture naming will be illustrated.
DISCUSSION: This pilot study allows us to define the optimal parameters including selected time windows and regions of interest for MEG laterality calculations in language tasks outside of verb generation. In addition, new insights into the spatiotemporal dynamics of language lateralization are provided.

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Poster

363. Cortical Mechanisms of Language

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Program#/Poster#: 363.19/LLL64

Topic: H.02. Human Cognition and Behavior

Title: Noninvasive measurement of language-related frontal gamma band activity with magnetoencephalography

Authors: *H. HASHIMOTO[1], Y. HASEGAWA[2], T. ARAKI[2], T. YANAGISAWA[1], S. YORIHUJI[2], M. HIRATA[1];
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Abstract: Gamma band (>50Hz) activity is a key oscillatory phenomenon of brain activation, and is useful for precise functional brain mapping and brain-machine interfaces. However, the non-invasive way to precisely detect language-related brain areas has not been established yet. Previously, we revealed that a silent reading task induces event-related desynchronizations (ERD) in the low gamma band (25-50 Hz) in the Broca area with magnetoencephalography (MEG) and that the low gamma ERD well predict language dominance. Also, high gamma band activity recorded by electrocorticograms has been shown to well reflect functional localization. In the present study, we investigated the language-related gamma band activity using a 160-channel whole-head MEG system equipped with SQUID gradiometers (MEG vision NEO, RICOH, Japan).Fifteen healthy, right-handed native Japanese volunteers participated in this

study. A verb generation task and a silent reading task were used. In the verb generation task, participants were instructed to silently read each presented word once immediately after word-presentation, and then to recall a verb associate with the word. In the silent reading task, participants were instructed to read each presented word once without phonation. A total of one hundred words were presented serially. The same words series were used in both the verb generation task the silent reading task. To assess the spatio-temporal distributions of the cerebral oscillatory changes, we used a group analysis of the result from a beamforming method and a time frequency analysis. In the verb generation task, high gamma band activity was observed in the posterior part of the left middle frontal gyrus. A time frequency analysis showed high gamma band activity at 650ms and 950ms after word stimuli. In the silent reading task, a similar result was shown but the response was weaker.In this study, language-related high gamma band activity was able to be detected non-invasively. However, we have to improve detection sensitivity of the language-related high gamma band activity, so that it can be applied to clinical examination for individuals.

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Poster

363. Cortical Mechanisms of Language

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 363.20/LLL65

Topic: H.02. Human Cognition and Behavior

Title: How can the time length of being bilingual affect the white matter of the brain? connectometry approach

Authors: ***M. DOLATSHAHI**[1], A. ANJOMSHOA[2], A. KAMALIAN[2], F. RAHMANI[2], N. HOSSEINI[2], M. AARABI[3];
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Abstract: **INTRODUCTION:** It has been shown that lifetime bilingualism maintains white matter (WM) integrity in older adults. On the other hand, increased FA values following intensive second language vocabulary training have been observed unless the training is discontinued. Considering these facts, it can be inferred that the time length of bilingualism (here we call it T) is associated with WM integrity. Analyses in bilinguals using T as a regressor have produced no significant results but in this study, we have applied connectometry, not suffering

some limitations of previous methods. Instead of finding the difference in tracks, connectometry tracks the difference associated with the study variable in local connectome. **METHOD:** Diffusion MRI connectometry was conducted for 17 young (mean age of 31), highly immersed late bilinguals using a multiple regression model considering immersion in bilinguals. A percentage threshold of 50 % was used to select local connectomes correlated with T. A deterministic fiber tracking algorithm was conducted to connect the selected local connectomes. A length threshold of 65 mm was used to select tracks. The seeding density was 20 seed(s) per mm3. To estimate the false discovery rate, a total of 500 randomized permutations were applied to the group label to obtain the null distribution of the track length. **RESULT:** The analysis results showed some fiber tracks (IFOF, AF, genu and body of CC bilaterally) with increased qa related to T with an FDR of 0.0753, which shows a trend toward significance. It means that the high variation in brain connection patterns, which cause higher FDR, will disappear if the number of subjects is increased. **CONCLUSION:** Association between amount of immersion in bilingualism and WM integrity in fiber tracts is implicated in our study for the first time. This result can be observed due to "time-dependent" process of neural plasticity, especially occuring in those fiber tracts associated with semantic (IFOF) and phonological (AF) language pathways and also executive function (CC). However, further studies using larger samples are required.

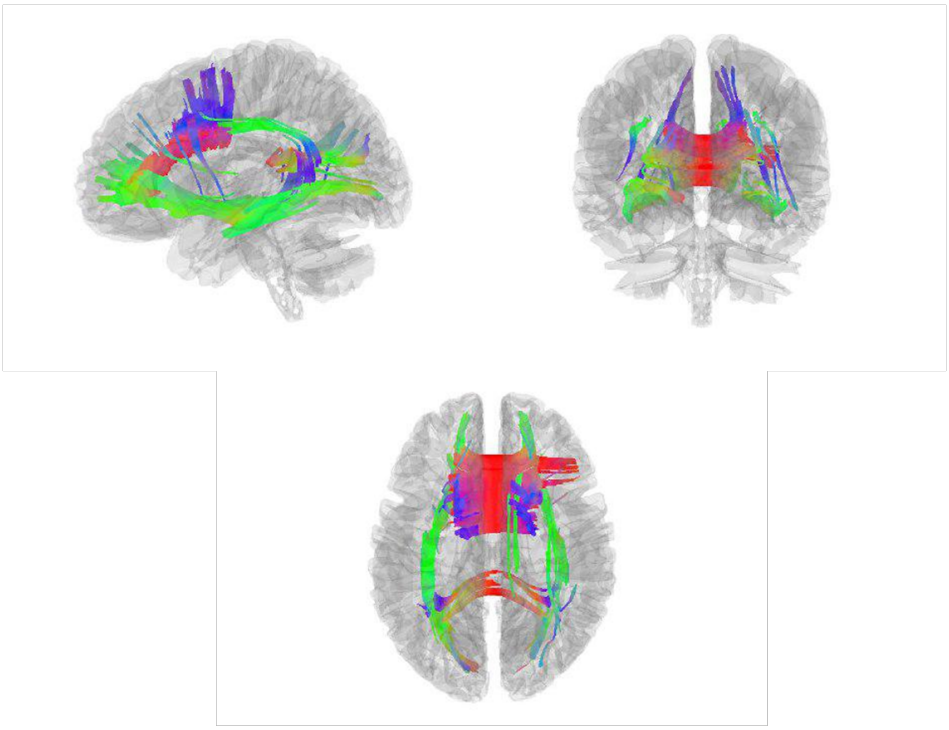

Figure. White matter fiber tracts associated with time length of immersion in late bilinguals in three views. Some fibers of IFOF, genu and body of CC and AF are seen.

Disclosures: **M. Dolatshahi:** None. **A. Anjomshoa:** None. **A. Kamalian:** None. **F. Rahmani:** None. **N. Hosseini:** None. **M. Aarabi:** None.

Poster

363. Cortical Mechanisms of Language

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Program#/Poster#: 363.21/LLL66

Topic: H.02. Human Cognition and Behavior

Title: Resting-state EEG as joint indicator of language performance and age-related cognitive decline

Authors: *C. BEESE, L. MEYER, B. VASSILEIOU, A. FRIEDERICI;
Max Planck Inst. Cognitive and Brain Sci., Leipzig, Germany

Abstract: Successful encoding of verbal information is a prerequisite for successful language comprehension, in both young and older adults. While language comprehension is often thought to remain rather stable across age, memory is known to decline. In the current study on young and older adults, this apparent paradox was addressed by combining a memory-intensive language-comprehension task with resting-state EEG as an electrophysiological indicator of both cognitive functioning and age-related cognitive decline. Whereas one line of research has shown decreased resting-state theta power to predict good general cognitive abilities, other studies showed that oscillatory power decreases with age across frequency bands, which coincides with age-related cognitive decline. We directly compared resting-state theta power linked to language performance in 19 young (mean age: 24) and 19 older adults (mean age: 65). As age-related effects, we predicted that, first, theta power decreases with age and that, second, language comprehension under high memory demands also decreases with age. Third, we predicted resting-state theta power to negatively correlate with language performance. The data revealed both decreased theta power and decreased language performance for older as compared to young adults. As a general effect, we found that across age groups, decreased theta power predicted higher language performance. Further analyses showed that the brain regions for which decreased resting-state theta power predicted successful language performance resembled that of the fronto-temporal language network. In sum, we show that decreased resting-state theta power is an electrophysiological indicator of both cognitive decline and good language performance. In other words: While decreased resting-state theta power indicates good language performance, language performance suffers when theta drops further during aging.

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Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: Max Planck Society

Croucher Foundation

Title: The right inferior frontal gyrus supports processing of nested structures in music

Authors: *V. K. CHEUNG[1,2], L. MEYER[1], A. D. FRIEDERICI[1], S. KOELSCH[3];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [3]Univ. of Bergen, Bergen, Norway

Abstract: Hierarchical cognitive processing requires the resolution of dependencies between non-adjacent elements. A classic example in natural language is an embedded sentence, where its processing is supported by the left inferior frontal gyrus (IFG). Recently, it was shown that humans can perceive nested harmonic dependencies in music. However, as the underlying neuroanatomy is still unknown, we here assessed brain responses to the processing of nested tone sequences using fMRI.
Data were collected from 17 musicians (7 male). First, participants were trained to extract the underlying nested grammar from tone sequences so that violations to the musical syntax could be reliably detected. Nested sequences had a maximum of 2 levels of embedding. Participants underwent fMRI scanning once the grammar was mastered. During scanning, participants were asked to determine the grammaticality of new auditory stimuli.
Data were analyzed in a factorial design with factors 'Grammaticality' (ungrammatical/grammatical) and 'Length' (1-/2- levels of embedding). Main effects of 'Grammaticality' were observed in the bilateral IFG —with a right-hemispheric lateralization, bilateral insula, and the supplementary motor area. Main effects of 'Length' were observed in the bilateral inferior parietal lobule (IPL) (including right angular gyrus), bilateral dorsal lateral prefrontal cortex, anterior cingulate cortex (ACC), and the basal ganglia. There was no significant interaction. A gPPI analysis further revealed task-modulated connectivity between the right IPL and the ACC.
Our results establish the functional neuroanatomy of processing nested dependencies in music, with a dominant role of the right IFG. This finding not only complements results from previous neuroimaging studies on processing dependency violations in music, but also provides striking evidence that the functional neuroanatomy of musical syntax is a mirror image of the functional neuroanatomy of natural language syntax.

Disclosures: **V.K. Cheung:** None. **L. Meyer:** None. **A.D. Friederici:** None. **S. Koelsch:** None.

Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 363.23/LLL68

Topic: H.02. Human Cognition and Behavior

Support: NWO VICI Grant 277-70-010

Title: Learning new words in a second language from spontaneous speech: an N400 study

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Abstract: In spontaneous speech, words are often pronounced in a reduced form, in which certain speech sounds are weakened or even absent. This may cause serious comprehension problems for second language (L2) learners. Here, we used EEG to examine how L2 learners process reduced variants of novel words that they have learned either predominately in the full form or in the reduced form. We expected that the N400 response would decrease during training for either the reduced or the full form, but that the N400 response would decrease less when the form during testing did not match the form during training.
Forty native Dutch speakers were trained on 3 consecutive days to learn 32 novel nouns by listening to short stories spoken by a native English speaker. Each story contained 8 tokens of a novel word and was presented along with a picture of an existing object. Half of the participants listened to stories in which 6 of the 8 target word tokens were presented in a reduced form (i.e., without schwa; RED group), whereas the other half of the participants listened to the same stories in which 6 of the 8 target word tokens were presented in their full form (i.e., with schwa; FULL group). EEG data was collected prior to training (on day 1) and after training (on day 3) using a cross-modal priming task, in which the pictures from the training were paired with four different auditory primes: (1) the English name of the existing object, (2) a semantically related English word, (3) the novel word for the object, and (4) a pseudoword. Participants were asked to indicate whether the object on the screen was man-made or nature-made. The novel words were presented in their reduced form only in this task.
Before training, the novel words induced a more negative N400 response than the real English words for both the RED and the FULL group. After training, the N400 responses induced by the learned novel words and the real English words were comparable for the RED group, but not for the FULL group. In the FULL group, the learned novel words still induced a more negative

response than the real English words. In addition, EEG recorded during the training revealed a gradual decrease of the N400 response over the 8 presentations in a story for both groups. These findings demonstrate that neural responses to new words, presented in their reduced form, were comparable to neural responses to real English words after three training sessions in a natural language learning task but only when participants were exposed to mostly reduced forms during training. This suggests that the learned pronunciation form of a word does not generalize to other pronunciation forms.

Disclosures: **M. Noordenbos:** None. **M. Ernestus:** None.

Poster

363. Cortical Mechanisms of Language

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Topic: H.02. Human Cognition and Behavior

Support: University of Florida Goldman Research Award

University of Florida Ring Research Award

Title: False belief and complementation: an electroencephalography study

Authors: ***Y. GUAN**[1], M. J. FARRAR[2], A. KEIL[2];
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Abstract: The goal of the present study was to investigate electrophysiological correlates of false belief and complementation syntax using electroencephalography (EEG), focusing on indices of oscillatory brain activity and large-scale connectivity. Behavioral studies of false belief understanding have indicated that complementation syntax is needed for representing a false belief. We utilized a standard false belief task, the Sally-Ann unexpected location scenario, implementing a false belief condition and a true belief control. Likewise, a complementation task was adopted from de Villers and Pyers (2002), to contain a false complementation condition and a true complementation control condition. There were 12 cartoon stories in each of the false belief, true belief control, false complementation, and true complementation control condition, presented in random order. Each story contained five vignettes (picture-sound pairs) with the story narrative in the first three vignettes. A question was then asked in the 4th vignette for false belief and true belief control conditions (e.g., "where does Sally look for her book when she comes back?") and the two types of complementation tasks (e.g., "what did Minnie tell Goofy she had?"). Participants were asked to answer the question

verbally in the 5th vignette and press a button to go to the next story. A total of 46 young adults (mean age =19.40 months; 18 male; 42 left-handed; 29 White, 9 Hispanic, 6 Asian, 1 African American, 1 Middle-Eastern) were recruited and watched the 48 cartoon stories in animated vignettes after two practice stories.
Wavelet analyses on single trials were conducted based on data collected on the critical 4th vignette across participants. Results demonstrated strong modulation of parietal alpha power (8-12 Hz) by the experimental manipulations: Importantly, heightened sustained alpha power was observed in the false belief condition, compared to false complementation, which in turn showed higher sustained alpha
power compared to the true belief control and true complementation control tasks. In addition, the time-varying power in the beta range (13-20 Hz) decreased strongly during 500-1000 ms in the false belief and true belief control conditions and sustained this pattern until the end of the vignette. Together with ongoing analysis of functional connectivity, these results suggest that neural population activity in the alpha and beta bands show complementary sensitivity to syntax type, with alpha sensitive to mental effort and cognitive complexity of the task, and beta dynamics reflective of the syntax type.

Disclosures: Y. Guan: None. **M.J. Farrar:** None. **A. Keil:** None.

Poster

363. Cortical Mechanisms of Language

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ADRC P50 AG23501

Title: Induced high-gamma oscillations during speaking distinguish variants of primary progressive aphasia

Authors: *L. B. HINKLEY[1], M. CAHILL-THOMPSON[1], Z. MILLER[2], K. RANASINGHE[2], D. MIZUIRI[1], C. GARRETT[1], S. HONMA[1], B. MILLER[2], K. VOSSEL[2], J. HOUDE[1], M.

GORNO-TEMPINI[2], S. S. NAGARAJAN[1];
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Abstract: Primary progressive aphasia (PPA) is symptomatically characterized into three main subtypes: a non-fluent variant (nfvPPA) associated with apraxia of speech and agrammatism, a logopenic variant (lvPPA) with a loss of phonological abilities, and a semantic variant (svPPA) with loss of word and object conceptual knowledge. Each of these variants is also associated with atrophy in the left hemisphere, with inferior frontal lobe degeneration in nfvPPA, parietal-temporal lobe degradation in lvPPA and anterior temporal lobe atrophy in svPPA. Despite these characterizations, the neurophysiological mechanisms that serve these deficits are not clear. Here, we use MEG during linguistic tasks to investigate phases of speech encoding and preparation in these PPA variants and matched controls. MEG data was collected using a 275-channel whole head biomagnetometer (CTF) during a nonsense word repetition task with overt speech production. Data were collected in nfvPPA, lvPPA and svPPA participants, as well as controls. Data were reconstructed in source space using adaptive spatial filtering techniques in the beta (12-30Hz), gamma (30-55Hz), and high-gamma (65-90Hz, 90-115Hz, 125-150Hz) frequency bands following the auditory presentation of a nonsense word (speech encoding) and prior to the subject repeating the word (speech production). In the high-gamma band, MEG source space reconstructions localized an increase in high-gamma power over the left posterior superior temporal gyrus (STG) 75ms following stimulus presentation in the nfvPPA, svPPA and control groups but not the lvPPA group. In addition, an increase in high-gamma suppression bilaterally over the inferior frontal gyrus (IFG) 475ms following stimulus presentation and 475ms prior to the response was present in the lvPPA, svPPA and control groups but absent in the nfvPPA group. Increased high-gamma suppression was also observed over the right frontal lobe in the svPPA group. The temporal resolution of MEG allows us to separately examine activation in these brain regions during different phases of the task in detail. Our findings show an absence of STG engagement during stimulus encoding in lvPPA patients consistent with a neurological basis for phonological errors. A lack of IFG engagement in nfvPPA patients is a potential source of their motor speech and grammatical errors. Increased right-hemisphere high-gamma suppression in svPPA could possibly reflect interference with word comprehension. The findings demonstrate that MEG imaging can provide distinct neurophysiological signatures across these aphasia variants.

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Poster

363. Cortical Mechanisms of Language

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Program#/Poster#: 363.26/MMM1

Topic: H.02. Human Cognition and Behavior

Support: Aalto ABC Grant

IUAP P7/11

Title: Using the N400 as a neural distance metric for unsupervised clustering of words

Authors: *M. VAN VLIET[1], M. M. VAN HULLE[2], R. SALMELIN[1];
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Abstract: The lexicon in our semantic memory is thought to have an underlying structure which competing theories have sought to delineate . For example, in a structure based on word associations, words that quickly come to mind given a cue word (DOG→BARK) are semantically closer than words that do not (DOG→FERN). To evaluate a theory against neural-level measures, the semantic distances predicted by the proposed structure may be compared with, e.g., the strength of an electrophysiological response reaching the maximum at ~400 ms after word onset, found to reflect semantic processing and labeled the N400 in electroencephalography (EEG) recordings.

Recently, advances in multivariate processing have boosted the signal-to-noise ratio (SNR) of EEG, diminishing the need for averaging and hence increasing the number of data points produced in a single study. This enables an exciting paradigm shift in how theories may be evaluated with the help of electrophysiological data. A researcher may now approach the data analysis in an "unsupervised" manner, i.e., rather than labeling the data with experimental conditions, we can now generate enough data points to learn the underlying clustering from the data distribution.

Here, we propose an unsupervised approach that may elucidate the structure of the mental lexicon. With the N400 amplitude as the distance metric, we performed an unsupervised hierarchical clustering of the following 14 words: GIRAFFE CHAIR CLOSET ZEBRA LION COUCH RHINO HIPPO TIGER DESK TABLE ELEPHANT DOOR BED. All possible combinations of 2 written words were presented in a sequential fashion to 16 participants who indicated by button press whether they thought the words were related for any reason (delayed response; preparation for finger movement did not influence the N400 window).

The EEG recording during the presentation of the second word was analyzed using a variant of beamformer filtering to estimate the N400 amplitude with a sufficient SNR. The filter combined a template of the shape of the N400 with the covariance matrix of the recording to estimate the

amplitude of the component in each trial. For an unbiased N400 template, we used a previous recording on another group of 10 participants who were presented with 800 word-pairs of varying forward association strength, in a manner identical to the current experiment.
The unsupervised analysis revealed a top-level division into two clusters where one incorporated all animate objects and the other all inanimate objects (within-cluster versus between-cluster N400 amplitudes: $p = 0.005$), as expected. This approach thus holds great promise for investigating the semantic structure at the neural level.

Disclosures: **M. Van Vliet:** None. **M.M. Van Hulle:** None. **R. Salmelin:** None.

Poster

363. Cortical Mechanisms of Language

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Support: Elizabeth H. Solomon Center for Neurodevelopmental Research

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Title: Enhancement of high-gamma power in 9-month-old infants with early active acoustic experience suggests accelerated phonemic mapping

Authors: *S. C. ORTIZ-MANTILLA, T. REALPE-BONILLA, J. DICICCO-BLOOM, A. A. BENASICH;
Rutgers The State Univ. of New Jersey, Newark, NJ

Abstract: In order to become efficient processors of language, infants must construct detailed phonemic maps within auditory cortex. This "phonemic mapping", is accomplished by 12 months-of-age, via the interplay between maturation and experience. It has been suggested that high-gamma power elicited in Superior Temporal Gyrus represents a unique neural signature for phonemic mapping. Further, our previous studies have shown that interactive acoustic experience, using temporally modulated non-speech stimuli, significantly impacts both accuracy and speed of discrimination of key pre-linguistic acoustic cues in 7-month infants, and that this enhanced processing generalizes to novel non-speech as well as speech (i.e. phonemes). It is unknown, however, if such experience-dependent effects of early auditory engagement on speech processing persist at 9 months or how these effects might impact phonemic mapping. Following the same cohort studied at 7 months, 9 month-old infants who had received active

(AEx) or passive (PEx) non-speech acoustic experience between 4 and 6 months-of-age were presented with a consonant-vowel contrast in a passive auditory oddball paradigm and compared to 9-month naïve controls (NC) with no such experience. Dense array EEG/ERP (128 sensors) was collected and mapped onto an age-appropriate brain template. Source modeling placed dipoles in both auditory cortices. Temporal-spectral analyses were conducted in source space within the 2-90 Hz frequency range using 1 Hz-wide frequency bins and time resolution of 50 ms. Changes in frequency band amplitude as a function of time relative to stimulus presentation and consistency of phase alignment across trials were evaluated using TSE (temporal spectral evolution) and ITPL (inter-trial phase locking). Significant differences were found between groups in the gamma range. When processing the standard stimulus, which relates to evolving phonemic maps, the AEx group generated greater high-gamma power than NC. Further, the AEx group had larger high-gamma phase synchronization in the left auditory source, whereas PEx and NC groups had more ITPL in the right source. When processing the deviant stimulus, which captures discrimination of segmental information, NC showed higher levels of late low-gamma power than PEx, while the AEX group generated more power than NC in the early low-gamma range. These results demonstrate that plastic effects of early non-speech acoustic experience persist until 9 months-of age and appear to confer a significant processing advantage, which facilitates earlier establishment of phonemic representations and supports more efficient left auditory cortex processing.

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Poster

363. Cortical Mechanisms of Language

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 363.28/MMM3

Topic: H.02. Human Cognition and Behavior

Title: Kohonen networks for unsupervised identification of unique multimodal spectral-temporal profiles

Authors: *K. FORSETH, N. TANDON;
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Abstract: This study develops a novel method for identifying patterns of multimodal spectral-temporal activity in an unsupervised manner. Kohonen networks are trained over 2033 electrodes, each with coarse spectrograms from multiple language tasks. The stable topography of the map encourages electrodes with similar profiles to cluster.

Disclosures: **K. Forseth:** None. **N. Tandon:** A. Employment/Salary (full or part-time): Memorial Hermann Hospital.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 364.01/MMM4

Topic: H.03. Schizophrenia

Title: A novel Muscarinic M4 receptor modulator PGM039678 inhibits Dopamine D1 receptor signalling pathways *In vivo*

Authors: *T. R. PATEL, S. BECHAR, S. STAFFORD, R. FOSBEARY, M. SHEARDOWN, L. WALSH, J. REEVES, P. RUPRAH, M. BARNES;
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Abstract: The muscarinic M4 receptor has been previously targeted for the treatment of Schizophrenia through the modulation of dopaminergic transmission. Previous studies have highlighted this modulation through the co-localisation and converging signalling pathway of the D_1-M_4 receptors present in the striatum with the use of Xanomeline, an M1/M4 agonist (Jeon et al., J NeuroSci (2010)). Through the development of an in-house M4 positive modulator the aim of this study was to characterise the compound through in-vitro screens and the thermal fixation model to confirm that the M4 selectively modulates the D_1 induced dopamine signal. The D_1 agonist, SKF82958 (3 mg/kg, s.c, dH_2O, 10 minutes ptt), was used to activate the D_1-G_s coupled signalling cascade in male Sprague Dawley rats. Thermal fixation was employed to preserve the increases in the striatal pCREB signalling marker, resulting in a robust and reproducible response. Pre-treatment with the novel M_4 positive allosteric modulator, PGM039678; *in vitro* EC_{50} 150 nM human, 228 nM rat) dose-dependently inhibited SKF82958 induced pCREB. Data are expressed as % pCREB inhibition, analysed by one-way ANOVA with Dunnett's (post-test compared to SKF82958). PGM039678 (p.o., 0.5% methylcellulose, 60 minutes ptt) n=6-9, -9%, 27%, 80% ($p<0.0001$) for 3, 10 and 30 mg/kg respectively. These data indicate that selective *in-vivo* activation of the $G_{i/o}$-coupled M_4 signalling pathway attenuates D_1-G_s induced signal. Following 14 days of twice daily oral dosing with PGM039678, a trend for reduction in pCREB was observed at 3 and 30 mg/kg (23.1% and 45.3% respectively). However, an acute 30mg/kg dose significantly inhibited pCREB (91%, $p<0.0001$ vs SKF82958), which was also significantly greater than the chronic 30 mg/kg group ($p<0.01$ unpaired t-test), suggesting desensitisation of the response following chronic M_4 positive allosteric modulation. Furthermore the PK-PD relationship of PGM039678 supports this conclusion as there is a clear difference in the

inhibition of the pCREB response at equivalent plasma drug concentrations when comparing the two 30 mg/kg groups. The present findings demonstrate that the M_4 compound is able to modulate the cAMP-pCREB signal from in-vitro to in-vivo models. Furthermore the selectivity of the compound provides additional confidence that the M_4 receptor is responsible for ameliorating the D_1 induced dopaminergic response and can demonstrate a link between receptor engagement and modification of behavioural outputs (data not shown).

Disclosures: **T.R. Patel:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **S. Bechar:** A. Employment/Salary (full or part-time): Takeda Cambridge ltd. **S. Stafford:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **R. Fosbeary:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **M. Sheardown:** None. **L. Walsh:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **J. Reeves:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **P. Ruprah:** A. Employment/Salary (full or part-time): Takeda Cambrdige Ltd. **M. Barnes:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd..

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

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Title: Effects of repeated aripiprazole treatment on $D2^{High}$ receptors and the Akt-GSK3β signaling pathway in preadolescent and adult rat

Authors: *M. L. BECKER, V. REAL, A. D. HARDIN, C. A. CRAWFORD, S. A. MCDOUGALL;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Aripiprazole (ARI) is a D2 receptor partial agonist that is commonly used to treat schizophrenia and bipolar disorder in adult populations as well as in children and adolescents. Although there is a growing literature assessing the effects of ARI during adulthood, few preclinical studies have examined the effects of repeated ARI treatment in young animals. In an early study, Seeman (2008) reported that daily exposure to ARI caused a near doubling of dorsal striatal $D2^{High}$ receptors in adult rats. The elevated number of $D2^{High}$ receptors is potentially

important, because alterations in these high affinity receptors have been linked to DA supersensitivity and psychosis. Whether repeated ARI treatment produces similar changes in the $D2^{High}$ receptors of young rats is unknown. Therefore, one purpose of the present study was to determine whether repeated ARI treatment increases the percentage of $D2^{High}$ receptors in the dorsal striatum of preadolescent rats. To determine the potential 'down-stream' actions of these receptor changes, the effects of ARI on the Akt-GSK3β signaling pathway were assessed in preadolescent and adult rats. Male and female rats were pretreated with vehicle, ARI (10 mg/kg, ip), or the D2 receptor antagonist haloperidol (HAL, 1 mg/kg, ip) once daily on PD 10-20 (young rats) or PD 70-80 (adult rats). After an additional 4 or 8 days, rats were decapitated and their dorsal striata were bilaterally dissected and stored at -80 °C until time of assay. The percentage of $D2^{High}$ receptors was measured in the dorsal striatum using [^{3}H]-domperidone; whereas, levels of Akt, phospho-Akt, GSK3β, and phospho-GSK3β were measured using immunoblotting. Repeated treatment with ARI and HAL significantly increased the percentage of dorsal striatal $D2^{High}$ receptors in preadolescent rats. Despite neither drug affecting Akt levels, ARI and HAL modestly increased Akt phosphorylation in the dorsal striatum of both age groups. In contrast, only HAL, but not ARI, significantly enhanced GSK3β activity in preadolescent and adult rats. The HAL-induced increase in GSK3β activity was most pronounced in preadolescent rats tested after 4 drug abstinence days. In sum, the present results show that ARI and HAL have persistent effects on $D2^{High}$ receptors as well as on the phosphorylation of Akt and GSK3β (HAL only). Whether drug-induced changes in $D2^{High}$ receptors are independent from, or causatively linked to changes in the Akt-GSK3β signaling pathway will require further study. Although a few age-dependent differences were apparent (i.e., p-GSK3β), the bulk of the evidence suggests that ARI and HAL affect the Akt-GSK3β signaling pathway in a generally similar manner across ontogeny.

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Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

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USPHS grant K12 HD43489-14

Title: Targeted deletion of both kynurenine aminotransferase II and kynurenine 3-monooxygenase in mice: Implications for studying kynurenine pathway metabolism

Authors: *A. POCIVAVSEK[1], M. A. R. THOMAS[1], F. GIORGINI[2], R. SCHWARCZ[1]; [1]Maryland Psychiatric Res. Center, Dept of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Dept. of Genet., Univ. of Leicester, Leicester, United Kingdom

Abstract: In the mammalian brain, kynurenine aminotransferase II (KAT II) and kynurenine 3-monooxygenase (KMO), key enzymes of the kynurenine pathway (KP) of tryptophan degradation, form the metabolites kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK), respectively. Cognitive processes are modulated by fluctuations in the brain content of KYNA, an antagonist at both NMDA and α7nACh receptors. Elevated KYNA levels are seen in cerebrospinal fluid and brain of individuals with schizophrenia (SZ) and may be causally related to cognitive impairments in the disease. Reductions in KMO activity, as seen in the brain of SZ patients, shift KP metabolism towards KYNA accumulation, and a polymorphism in *Kmo* has been linked to cognitive endophenotypes in humans. To study these phenomena further, we generated a mouse wherein both enzymes were eliminated by knockout of both the *Kmo* and *Kat2* genes and began to compare the new mutant animals (*Kat2*$^{-/-}$/*Kmo*$^{-/-}$) biochemically and behaviorally to wild-type, *Kat2*$^{-/-}$, and *Kmo*$^{-/-}$ mice. In adulthood, the levels of plasma kynurenine were significantly elevated in *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ mice compared to the other three groups. Interestingly, both female *Kmo*$^{-/-}$ and *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ animals had higher plasma kynurenine levels than corresponding male mice. Plasma KYNA levels were significantly lower in *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ mice compared to *Kmo*$^{-/-}$ mice, but significantly higher than in wild-type or *Kat2*$^{-/-}$ mice. In the brain, 3-HK levels were substantially lower in *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ and *Kmo*$^{-/-}$ mice than in wild-type and *Kat2*$^{-/-}$ mice, while KYNA levels were significantly lower in *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ compared to *Kmo*$^{-/-}$ but higher than in wild-type or *Kat2*$^{-/-}$ mice. Both *Kmo*$^{-/-}$ and *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ females contained significantly higher forebrain KYNA levels than corresponding males. In separate adult animals, we tested contextual memory using the passive avoidance paradigm. Male and female *Kmo*$^{-/-}$ mice displayed a significant reduction in avoidance latency during the retention trial compared to wild-type or *Kat2*$^{-/-}$ mice, while *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ mice were not impaired in the task. Taken together, our results provide evidence that both KMO and KAT II play key regulatory roles in the KP. *Kat2*$^{-/-}$/*Kmo*$^{-/-}$ mice can be used to study tissue-, sex-, and behavioral phenotype-specific functions of KP metabolites in health and disease.

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Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Program#/Poster#: 364.04/MMM7

Topic: H.03. Schizophrenia

Title: F17464, a preferential D3 antagonist 5-HT1A agonist with antipsychotic and procognitive properties : mechanistic study on plasma prolactin induction in rats.

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Abstract: Antipsychotic agents with higher D_3 vs. D_2 receptor affinity could improve the management of negative and cognitive symptoms while exhibiting a lower propensity to produce extrapyramidal side effects. F17464 is a preferential D_3 antagonist with high affinity for human recombinant dopamine D_3 receptors (Ki = 0.17 nM), lower affinity for hD_2L (Ki = 12.1 nM) and hD_2S (Ki= 6.5 nM) and high affinity for h5-HT_{1A} receptors (Ki = 0.16 nM). F17464 is a full antagonist for hD_3 (pK_B =9.13), and hD_2S (pK_B = 7.87) and a 5-HT_{1A} partial agonist (pEC50=7.99). It has similar affinity for rat striatal D_2 (Ki=4.8 nM) and lower affinity for rat hippocampal 5-HT_{1A} receptors (Ki=1.14 nM). In mice, F17464 increased dopamine metabolite levels in the limbic forebrain and prefrontal cortex 1h after single i.p. administration. Moreover F17464 blocked amphetamine- and MK-801-induced hyperactivity at doses that produced little or no effects on spontaneous activity in mice. F17464 also reversed MK-801-induced deficits in social interactions in mice, and showed procognitive effects in a passive avoidance test in rats. All these effects of F17464 in rodents were in the 0.16-2.5 mg/kg i.p. dose range. We studied the effect of F17464 on plasma prolactin (PRL) in rats in comparison with reference antipsychotics and D_3 and 5-HT_{1A} ligands, to investigate the possible implication of D_3 and 5-HT_{1A} receptors. F17464, 30 min after i.p. administration, dose-dependently increased PRL in rats with a maximal effect at 2.5 mg/kg. This effect was compared with the effect of haloperidol, risperidone, clozapine, aripiprazole, quetiapine, amisulpiride, BP 897 (D_3 partial agonist) and ABT 925 (a selective D_3 antagonist). F17464 2.5 mg/kg i.p. reached a maximal effect after 30 min, at 2h levels were 50% lower and at 24h they were not different from controls. The kinetics of PRL followed closely the kinetics of F17464 levels in the plasma. The selective 5-HT_{1A} antagonist WAY100635 (0.16-0.63 mg/kg s.c.) did not antagonize F17464 2.5 mg/kg i.p. induced PRL, suggesting a lack of involvement of 5-HT_{1A} receptors. WAY100635 had no effect on PRL by itself. F17464/haloperidol interaction studies were also carried out to investigate the potential involvement of D_3 related control on tuberoinfundibular dopaminergic neurons and the final effects on PRL.

Disclosures: **C. Cosi:** None. **P. Heusler:** None. **J. Martel:** None. **L. Leriche:** None. **M. Marien:** None. **S. Gatti-McArthur:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: H.03. Schizophrenia

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Title: Activation of the VPAC2 receptor inhibits neurite outgrowth and branching of cortical neurons by a PKA-dependent mechanism

Authors: H. HASHIMOTO[1], *Y. AGO[1], A. HAYATA-TAKANO[1], T. KAWANAI[1], R. YAMAUCHI[1], J. A. WASCHEK[2];
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Abstract: Clinical studies have shown that microduplications at 7q36.3, containing *VIPR2*, confer significant risk for schizophrenia. *VIPR2* gene encodes the VPAC2 receptor for VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase-activating polypeptide). Lymphocytes from patients with these mutations exhibited higher *VIPR2* gene expression and VIP responsiveness, but mechanisms by which overactive VPAC2 signaling may lead to these psychiatric disorders are unknown. We have previously found that repeated administration of a selective VPAC2 receptor agonist Ro 25-1553 in the mouse during early postnatal development caused synaptic alterations in the prefrontal cortex and cognitive impairment. This study aimed to clarify the effects of VPAC2 receptor activation on neurite outgrowth in cultured primary

mouse cortical neurons. All VIP and PACAP receptors (PAC1, VPAC1 and VPAC2 receptors) were expressed in primary mouse cortical neurons. VIP and Ro 25-1553 caused reductions in total numbers and length of neuronal dendrites and length of axon in cortical neurons, while PACAP38 facilitates dendritic, but not axonal, elongation of cortical neurons. These effects of VIP and Ro 25-1553 were blocked by a VPAC2 receptor antagonist PG 99-465 and abolished in VPAC2 receptor-deficient mice. In addition, Ro 25-1553-induced decreases in axonal and dendritic outgrowth were blocked by a PKA inhibitor H89, but not by a PKC inhibitor GF109203X or a MEK inhibitor U0126. PACAP38-induced facilitation of dendritic outgrowth was blocked by U0126. Furthermore, an atypical antipsychotic drug clozapine prevented Ro 25-1553-induced decreases in axonal and dendritic outgrowth. These results suggest that activation of the VPAC2 receptor inhibits neurite outgrowth and branching of cortical neurons by a PKA-dependent mechanism. These findings also imply that the *VIPR2*-linkage to mental health disorders may be due in part to deficits in neuronal maturation induced by VPAC2 receptor overactivation.

Disclosures: H. Hashimoto: None. **Y. Ago:** None. **A. Hayata-Takano:** None. **T. Kawanai:** None. **R. Yamauchi:** None. **J.A. Waschek:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: H.03. Schizophrenia

Title: Characterization of PGM039678, a positive allosteric modulator of the muscarinic M4 receptor, in animal models of schizophrenia

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Abstract: The muscarinic M_4 receptor is an attractive therapeutic target for schizophrenia as demonstrated by the preclinical and clinical data achieved with Xanomeline, a mixed M_1/M_4 orthosteric site agonist (Shekhar A (2008) Am. J. Psych. 165:1033). However, agents targeting the muscarinic receptors have demonstrated significant cholinergic related adverse events such as salivation, bradycardia and nausea. More recent drug discovery efforts have focused on the development of positive allosteric modulators (PAMs) to overcome the challenges of obtaining

muscarinic receptor subtype selectivity. We have identified selective muscarinic M_4 PAMs with >100 fold selectivity over other muscarinic receptor subtypes.
The goal of this study was to evaluate the efficacy of PGM039678 in two animal models relevant to the symptomology of schizophrenia: the conditioned avoidance response (CAR) task and the PCP-induced social withdrawal model. In addition, the efficacy of PGM039678 was investigated in the CAR test when combined with either risperidone or olanzapine. CAR experiments were performed in Wistar rats trained with 30 trials per day until they reached a performance criterion of 80% of correct responses on at least 2 consecutive days. Social withdrawal was evaluated using the social interaction (SI) test in Long Evans rats after a 7-day treatment with phencyclidine (PCP, 5 mg/kg, bid) followed by a 7-day wash-out period.
In the CAR test, PGM039678 dose-dependently inhibited avoidance responses with ED50 ~ 30 mg/kg, ip or 40 mg/kg, po. Co-administration of an inactive dose of PGM039678 (10 mg/kg, po) with risperidone or olanzapine lowered the dose-response curve of each antipsychotic by factors of 20 and 13, respectively. In the subchronic PCP model, PGM039678 dose-dependently reversed the SI deficit with ED50 ~ 0.5 mg/kg, ip or 0.3 mg/kg, po. Reversal of the SI deficit by PGM039678 was maintained after chronic treatment (1 mg/kg, po, bid) for 2 weeks.
These studies have demonstrated the synergistic effects of a selective M_4 PAM and antipsychotic compounds. While the clinical significance remains to be established, the possibility of reducing the dose of atypical antipsychotics in a co-dosing paradigm suggests the utility of M_4 PAM molecules in minimizing potential adverse events.

Disclosures: **E. Cayre:** A. Employment/Salary (full or part-time): Biotrial Pharmacology. **D. Parachou:** A. Employment/Salary (full or part-time): Biotrial Pharmacology. **B. Méot:** A. Employment/Salary (full or part-time): Biotrial Pharmacology. **B. Rion:** A. Employment/Salary (full or part-time): Biotrial Pharmacology. **C. Drieu La Rochelle:** A. Employment/Salary (full or part-time): Biotrial Pharmacology. **M. Sheardown:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **P. Ruprah:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **L. Walsh:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **J. Reeves:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **R. Fosbeary:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **M. Barnes:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd. **T. Patel:** A. Employment/Salary (full or part-time): Takeda Cambridge Ltd..

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 364.07/MMM10

Topic: H.03. Schizophrenia

Title: Neonatal phencyclidine (PCP) in the rat induces psychotomimetic effects in adulthood which can be inhibited by chronic treatment with antipsychotics.

Authors: *V. CASTAGNÉ, A.-M. HERNIER;
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Abstract: Epidemiological and preclinical data suggest that schizophrenia results from abnormal neurodevelopment. Inhibitors of the N-methyl-D-aspartate (NMDA) receptor induce psychosis-like behavior in various animal species, including humans. An animal model of schizophrenia-related deficits induced by early interference with NMDA receptor functions may therefore have translational value for drug development. We evaluated the long-term consequences of neonatal treatment with phencyclidine (PCP) in the rat and their reversal by antipsychotics. We also evaluated the effects of neonatal treatment with ketamine. In the first experiment, rat pups received injections of PCP (5, 10 or 20 mg/kg s.c.) on postnatal days 7, 9 and 11 and were evaluated during adulthood for spontaneous or pharmacologically stimulated behavior. The second experiment evaluated the effects of chronic treatment during adulthood with haloperidol (0.25 mg/kg i.p.) or clozapine (1 mg/kg i.p.) on the consequences of neonatal PCP injections. A third experiment evaluated the effects of neonatal treatments with ketamine (25 mg/kg s.c., administered on postnatal days 7, 9 and 11). The data of the first experiment in rats neonatally treated with PCP reveal an age-dependent increase in locomotion as well as a strong increase in reactivity to an acute pharmacological challenge with PCP. Rats treated with PCP at 5 mg/kg displayed exaggerated stereotypies during postnatal weeks 6 and 12. This same effect was observed during week 12 in rats receiving the intermediate dose of PCP and during weeks 6, 9 and 12 in rats treated with the highest dose. During the second experiment, we observed a complete reversal of PCP-induced long-term effects by a sub-chronic treatment with haloperidol during adulthood. A similar treatment with clozapine only partially reversed the psychotomimetic effects of neonatal PCP. The results of the third experiment suggest that neonatal treatment with ketamine does not induce psychotomimetic-like effects, even if minor behavioral differences were observed in adult rats, compared with control animals receiving physiological saline on postnatal days 7, 9 and 11. The neonatal PCP model of psychosis-related behavior in the rat can therefore be used in the evaluation of new chemical entities for antipsychotic-like activity. The neurodevelopmental character of this model based on the long-term effects of PCP makes it a valuable secondary preclinical test during the drug development process, in particular in evaluating antipsychotic-like activity after sub-chronic administration.

Disclosures: V. Castagné: None. **A. Hernier:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Program#/Poster#: 364.08/MMM11

Topic: H.03. Schizophrenia

Title: Selective M_1 potentiation improves PCP-induced deficits in working memory and pattern separation in rats

Authors: *E. P. LEBOIS[1], D. VOLFSON[3], D. BUHL[3], S. GRIMWOOD[2], J. EDGERTON[3]; [1]Neurosci. Res. Unit, [2]Pfizer, Inc., Cambridge, MA; [3]Pfizer, Inc, Cambridge, MA

Abstract: The M_1 muscarinic receptor is a Gq-coupled G-protein coupled receptor (GPCR) known to be heavily expressed in the hippocampus and neocortex of the mammalian brain. M_1 is known to be expressed post-synaptically on principal cells and mediate excitatory effects of ACh, but it has only recently become evident that the M_1 receptor is also expressed post-synaptically on parvalbumin (PV)-containing interneurons. Therefore, M_1 activation may be highly relevant to disease states of interneuron dysfunction. Schizophrenia is characterized by cognitive impairments that are thought to arise in part from compromised function of PV-containing interneurons. In humans, NMDA receptor antagonists such as phencyclidine (PCP) have been shown to both induce acute psychotic symptoms and decrease PV levels. Given M_1 activation is known to potentiate NMDA receptor signaling, we hypothesized that M_1 activation would be beneficial for improving both molecular and cognitive deficits induced by PCP. We observed that 3.2 mg/kg PCP dosed once-daily s.c. for 5 days was sufficient to induce persistent deficits in working memory and pattern separation 48 hours later when rats were tested in a trial-unique nonmatching-to-location (TUNL) touchscreen task with variable 1 and 6 second delays. Rats were found to be selectively impaired at the longer 6 second delay. Preliminary data indicate that the above regimen of 3.2 mg/kg PCP also appears to decrease PV levels in the mPFC, suggesting that the observed behavioral deficits may be due in part to a deficit induced in inhibitory circuitry. Intermittent dosing with PCP for 5 weeks was sufficient to maintain the observed working memory deficit at the 6 second delay, which was significantly reversed by the selective M_1 potentiator, PF-06745366. Additionally, rat performance was tracked for up to 1 month post-treatment during a washout period with no PF-5366 dosing. The performance of rats previously receiving only a PCP regimen deteriorated further to chance levels, with severe impairments in working memory and pattern separation becoming evident. Rats that received the same regimen of PCP, which were also administered PF-5366 on alternating days, never deteriorated in performance, which was virtually identical to rats only ever dosed with saline. These data indicate M_1 activation holds promise for bolstering cognition in disorders exhibiting interneuron dysfunction.

Disclosures: **E.P. Lebois:** A. Employment/Salary (full or part-time): Pfizer, Inc. **D. Volfson:** A. Employment/Salary (full or part-time): Pfizer, Inc. **D. Buhl:** A. Employment/Salary (full or part-time): Pfizer, Inc. **S. Grimwood:** A. Employment/Salary (full or part-time): Pfizer, Inc. **J. Edgerton:** A. Employment/Salary (full or part-time): Pfizer, Inc.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Topic: H.03. Schizophrenia

Support: COGNITO grant, Danish Research Council

Title: Positive allosteric modulators of the alpha7 nicotinic acetylcholine receptor reinstate cognitive control and potentiate glutamate levels in prefrontal cortex

Authors: *D. PHENIS[1], J. D. SCHUMACHER[1], V. VALENTINI[2,1], J. P. BRUNO[1];
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Abstract: Deficits in cognitive control (attention; working memory) are considered core symptoms in several neuropsychiatric disorders, including schizophrenia. The search for cognition enhancing therapeutics is intensive and significant given the poor cognitive efficacy of conventional antipsychotics and the fact that the severity of these cognitive impairments predicts patient outcome. Deficits in cognitive control can be modeled in rats by an acute elevation of the alpha7 nicotinic acetylcholine receptor (α7nAChR) negative modulator/NMDA antagonist kynurenic acid (KYNA) or after sustained elevation of KYNA during a sensitive period of development. In one model, male adult rats were trained to criterion on a non-match-to-sample operant working memory task with a 5, 10, and 15 sec delay. After acquiring the task each rat was tested under 3 treatment conditions: 1) vehicle; 2) kynurenine (KYN; 100 mg/kg,i.p.) the bioprecursor of KYNA; and 3) KYN + PAM-2 (1.0 mg/kg, i.p.). In the second model, juvenile male rats received daily injections of KYN (100 mg/kg, i.p; adolkyn) for 8 days (PD35-PD42) prior to the onset of adolescence and were tested as adults in an attentional set-shifting task (ASST). Prefrontal glutamate release is necessary for performance of both of these tasks, so, the ability of PAM-2 to potentiate evoked glutamate release was determined in a third group of rats using an enzyme-based glutamate biosensor. An acute injection of KYN led to delay-dependent deficits, particularly at the long delay (vehicle = 85% hits; KYN = 70% hits). Pretreatment with PAM-2 (1.0 mg/kg) alleviated the deficit (KYN + PAM-2 = 82%). Adults, treated with chronic KYN injections prior to entering into adolescence exhibited deficits in the extra-dimensional shift stage of the ASST relative to controls (adolcon) injected with saline (trials to criterion:

adolcon = 15.5; adolkyn = 30.2). Administration of PAM-2 (2.0 mg/kg, i.p.) eliminated this impairment (adolkyn + PAM-2 = 18.3 trials). Initial neurochemical analyses with the glutamate-sensitive biosensor revealed that administration of PAM-2 has the capacity to potentiate extracellular glutamate levels. Intra-accumbens shell infusions of NMDA (0.05, 0.30 ug/0.5 uL) increased basal glutamate by 4.93 and 11.42 uM, respectively. PAM-2 potentiated the effects of NMDA on glutamate levels by 75% and 64%, respectively. Collectively, these data are consistent with the roles of prefrontal cholinergic and glutamatergic transmission in cognitive control and further support continued research into the cognition-enhancing potential of α7nAChR PAMs in conditions in which deficits in cognitive control emerge.

Disclosures: D. Phenis: None. **J.D. Schumacher:** None. **V. Valentini:** None. **J.P. Bruno:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: FAPESP Grant 2014/16634-1

Title: Atypical antipsychotic clozapine reversed deficit on prepulse inhibition of the acoustic startle reflex produced by microinjection of DOI into the inferior colliculus in rats

Authors: *R. B. SILVA, R. OLIVEIRA;
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Abstract: Prepulse inhibition (PPI) is the reduction in the startle response caused by a low intensity non-startling stimulus (prepulse) which is presented shortly before the startle stimulus (pulse), and is an operational measure of sensorimotor gating. The inferior colliculus (IC) is a critical part of the auditory pathway mediating acoustic PPI. Deficits in PPI have been observed in schizophrenia and can be induced in rats by microinjections of GABA or NMDA blockers in the IC. The aim of this study was to investigate the role of serotonergic transmission in the IC on the expression of acoustic PPI. For that we investigated whether 5-HT2A receptor stimulation or blockade would affect this response. Thirty one male Wistar rats (300 g) were unilaterally implanted with stainless steel guide cannula in the IC. Five days after the surgery, the animals received unilateral intracollicular microinjections of the 5-HT2A receptor agonist DOI (10.0 µg/0.3 µl; N = 6), of the 5-HT2A receptor antagonist ritanserin (4 µg/0,3 µL; N= 7) or of vehicle (0.3 µl; N = 6). Microinjection of ritanserin into the IC did not alter PPI while microinjection of

DOI into this structure disrupted PPI. We also examined the ability of the atypical antipsychotic clozapine (5 mg/kg; i.p.) to reverse the disruption of PPI produced by microinjection of DOI into the IC. For that, two groups of animals received pretreatment with intraperitoneal injection of clozapine (N=6) or vehicle (N=6) ten minutes prior to the DOI microinjection of MK-801 into the IC. Pretreatment of clozapine blocked DOI-induced disruption of PPI. The present findings suggest that activation or blockade of 5-HT2A receptors of the IC differentially affects PPI response. DOI disrupted PPI when microinjected directly to the IC. In contrast, microinjection of ritanserin into this structure did not seem to interfere with PPI. Pretreatment with clozapine blocked DOI-induced disruption of PPI. Altogether, these results suggest that serotonin-mediated mechanisms of the IC are involved in the expression of PPI in rodents and that this response is sensitive to atypical antipsychotic clozapine.

Disclosures: R.B. Silva: None. **R. Oliveira:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: NIH Grant P51OD11132

NIDA Grant DA031246

Title: Pharmacological state-dependent functional connectivity MRI in conscious nonhuman primates: validating a translational model for evaluating antipsychotics.

Authors: *E. MALTBIE[1,2], K. GOPINATH[1], D. KEMPF[2], L. HOWELL[1,2];
[1]Emory Univ., Atlanta, GA; [2]Yerkes Natl. Primate Res. Ctr., Atlanta, GA

Abstract: The NMDA-receptor antagonist ketamine is of great interest for its ability to induce psychotic-like symptoms which make it useful for modeling psychosis. This study builds on previous work using fMRI to determine ketamine-induced brain activation in conscious nonhuman primates as a translational model for evaluating antipsychotics. fMRI data were obtained from four adult female rhesus monkeys while they received a bolus i.v. infusion of 0.345 mg/kg of ketamine followed by continuous i.v. infusion of 0.256 mg/kg/hr. All subjects were responsive to tactile and auditory stimulation, indicating that dosing was well below the anesthetic range. Subjects were scanned in a Siemens Trio 3 Tesla magnet while laying prone in a custom built MRI cradle with individually molded head restraints fitted to a custom-designed

transmit-receive volume NHP head coil. fMRI data were collected utilizing a whole-brain gradient echo single-shot echo planar imaging sequence (TR/TE/FA = 3000ms/32ms/90; 1.5mm isotropic resolution). Each subject underwent 3 separate scans: a 10-minute baseline scan and two 55-minute ketamine infusion scans: one with and one without pretreatment of the antipsychotic risperidone (0.06 mg/kg) one hour prior to scanning. Functional connectivity under different pharmacological conditions were assessed with global brain connectivity (GBC) which is the average of standardized cross-correlation coefficients of a given voxel's time-series with all other gray matter voxels' time-series in the brain, i.e., the degree of connectedness of a given brain region with all other regions. Ketamine-induced increased GBC all over the brain compared to baseline, with the strongest increases seen in anterior and subgenual cingulate cortices (ACC and SgCC), dorsolateral prefrontal cortex (dlPFC), and the entire striatum, consistent with human studies that report global hyperconnectivity after subanesthetic ketamine administration and in schizophrenia. Pretreatment with risperidone blocked ketamine-induced GBC increases in striatum, amygdala, dorsal midbrain, and thalamus. Interestingly risperidone combined with ketamine exhibited decreased GBC compared to baseline in all these regions, while (like ketamine) exhibiting increased GBC in the frontal lobe. Thus the results indicate that the antipsychotic effects of risperidone are mediated through dopaminergic pathways, consistent with its pharmacology. Together, these results show a remarkable concordance between nonhuman primates and human subjects.

Disclosures: **E. Maltbie:** None. **K. Gopinath:** None. **D. Kempf:** None. **L. Howell:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: Sumitomo Dainippon Pharmaceuticals

Title: The atypical antipsychotic drug lurasidone to prevent and reverse the impairment in novel object recognition in phencyclidine-treated mice

Authors: *M. HUANG, L. RAJAGOPAL, S. KWON, H. Y. MELTZER;
Psychiatry and Behavior Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Abnormalities in cortical, hippocampal and striatal dopaminergic, cholinergic and glutamatergic receptor stimulation, e.g. decreased D_1, alpha 7 nicotinic receptor, and N-methyl-D-aspartate (NMDA) receptor stimulation, have been postulated to contribute to some types of

cognitive impairment associated with schizophrenia (CIAS). CIAS has been modeled in rodents by acute or sub-chronic (sc) administrations of the NMDAR non-competitive antagonist, phencyclidine (PCP), which produces transient or prolonged impairment, respectively, in novel object recognition (NOR), a measure of spatial memory, as well as loss of spines on excitatory neuron synapses. These effects are reversed by acute treatment with atypical antipsychotic drugs (AAPDs), e.g. lurasidone and olanzapine, but not typical APDs, e.g. haloperidol, consistent with the greater benefit of AAPDs for treating CIAS. We investigated the ability of pretreatment with lurasidone to block the acute effects of a single dose of PCP on efflux of key neurotransmitters in mouse cortex and dorsal striatum, in wild type and scPCP-treated male C57BL/6J mice, utilizing in vivo microdialysis. We also investigated the ability of acute lurasidone to prevent the ability of scPCP to cause an enduring NOR deficit and of sclurasidone to produce prolonged reversal of the effect of scPCP. Lurasidone (1.0 mg/kg) alone significantly increased cortical, but not dorsal striatal acetylcholine (ACh), and cortical and dorsal striatal dopamine (DA), and glutamate (Glu) efflux, in drug naïve, as well as in scPCP-treated mice. Acute PCP (10 mg/kg, i.p.) produced significant increases in cortical and dorsal striatal DA, norepinephrine (NE), 5-HT and Glu efflux. The increased 5-HT and Glu, but not DA or NE, were blocked by pretreatment with lurasidone in both regions. Pretreatment with lurasidone (3 and 10 mg/kg) prior to each dose of scPCP (10 mg/kg, bid, for 7 days) prevented the development of an NOR deficit for 1 and 2 weeks, respectively. Sub-chronic lurasidone, 3 mg/kg, bid for 7 day after scPCP washout, reversed scPCP-induced NOR deficit for up to 4 weeks, after which the NOR deficit returned. These results indicate that lurasidone, by enhancing the release of moderate levels of DA, ACh, and Glu, and by blocking the ability of PCP to markedly increase Glu efflux, was able to prevent PCP-induce abnormalities in synaptic structure and function, which is the likely basis for the deficit in NOR. The time dependent loss of the restorative effect of lurasidone on the scPCP-induced NOR deficit represents a model of relapse and development of cognitive deficit related to withdrawal of medication in remitted patients.

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Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: NIH Grant K22MH099164

Title: Serotonergic hyperfunction in an NMDAR hypofunction mouse model of schizophrenia

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Abstract: Auditory hallucination is a cardinal neuropsychiatric symptom in schizophrenia. However, basic auditory function, including auditory steady-state response (ASSR), is also impaired. The mechanisms underlying hallucinations and sensory processing dysfunction in schizophrenia are poorly understood. To explore the potential mechanisms, we used an NMDAR hypofunction model in mice, where Grin1 gene encoding the indispensable NMDAR subunit is deleted in ~50% of cortical and hippocampal GABA neurons in early postnatal development (Belforte JE et al., 2010). First, we examined DOI (5-HT2A/2C receptor agonist)- and 5-hydroxytryptophan (5-HTP, precursor of 5-HT)-induced head-twitch response (HTR), a behavioral assay for 5-HT2A receptor activation in the brain. Both DOI- and 5-HTP-induced HTRs were more frequently detected in the mutants compared to the control mice (DOI; n= 3 for each, $p< 0.05$, 5-HTP; n=7 for mutant and n=5 for control, $p< 0.05$). Next, we investigated whether activity-regulated cytoskeletal associated (Arc) gene is activated in a tone- and 5-HT-dependent fashion in the auditory cortex. Following habituation in the dark/sound-proof chamber for 12 hours, ten auditory click trains were applied every 20 sec to a free-moving animal. Click stimuli (85dB, white noise) were 40-Hz click trains in which each click was 2 ms in duration delivered every 25 ms for a total of 500 ms. The click train-evoked Arc expression was induced in deep layer pyramidal neurons of auditory cortex, but not in the visual cortex, of the control Arc-dVenus mouse reporter line, and it was peaked at about 3-4 hours after tone application and disappeared by 8 hours after. In contrast, in the mutant-Arc mice, which were obtained by crossing the Arc-dVenus line to the mutant line, the tone-evoked Arc expression lasted over 10-15 hours in the deep layer neurons of the auditory cortex. Notably, the prolongation of Arc expression in the mutant-Arc mice was blocked by prior administration of M100907 (selective 5-HT2A receptor antagonist) to the tone stimuli. The same prolongation of Arc expression was observed in the control Arc mice by pre-treatment of psychoactive drugs including DOI and TCB-2 (selective 5-HT2A receptor agonist) prior to click train application. These results suggest 5-HT2A receptor activation in the auditory cortex deep layer in the mutant mice, contributing to the prolongation of tone-induced Arc expression. Taken together, Grin1 deletion in the cortical GABA neurons appears to confer hyper serotonergic activity in the auditory cortex pyramidal neurons, which may potentially be associated with the auditory dysfunction in schizophrenia.

Disclosures: **K. Nakao:** None. **S. Yamaguchi:** None. **K. Nakazawa:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Program#/Poster#: 364.14/MMM17

Topic: H.03. Schizophrenia

Support: DBT, Government of India

Intramural funding from National Centre for Biological Sciences

Title: Antipsychotics and 5-HT_{2A} receptor: Insights from the 5-HT_{2A} knockout mouse

Authors: *R. JOSHI, M. M. PANICKER;
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Abstract: Antipsychotic treatment has been on the forefront of battle against mental illness such as Schizophrenia. Unfortunately, antipsychotics are often associated with severe side effects such as sedation, extrapyramidal symptoms and metabolic disorders. Yet, the underlying mechanism of therapeutic effects or side effects of antipsychotics is poorly understood. My work has given the first few insights on how the pharmacological factors like 5-HT_{2A} receptor and non-pharmacological factors like environmental context affect the sedative side effect of antipsychotics. Additionally, a c-fos reporter mouse strain has allowed us to identify novel cellular targets of antipsychotic drugs in certain brain areas.

My work focuses on the sedation induced by a prototypical antipsychotic- Clozapine. 5-HT_{2A} is one of the prime targets of Clozapine. To specifically address the role of 5-HT_{2A} in clozapine induced sedation (CIS) our group has generated a 5-HT_{2A} knockout mouse at our institute. We find that *Htr2a*$^{-/-}$ mice are more resistant to CIS than the *Htr2a*$^{+/+}$ mice. Interestingly, the genotype alone is not enough to explain the sedative effect of clozapine. Environmental context during the behaviour is yet another regulator of the CIS. Importantly, the context dependency of CIS is not uniform across the antipsychotics, but probably depends on the unique GPCR binding profile of each drug.

Recently we have begun to explore the cellular basis of the actions of antipsychotics. We have used a c-fos reporter line which allows us to visualize neurons and in turn the brain areas which respond to antipsychotics. We have identified several brain regions which exhibit unique activity to certain antipsychotics, for example the striatum showed increased activity in response to typical (catalepsy inducing) antipsychotics- haloperidol and loxapine but not to the atypical drugs- clozapine and olanzapine. We have also identified a subset of glial cells along the ventricles as a novel target of the atypical antipsychotic clozapine. In addition the cre-lox reporter line in 5-HT_{2A} knockout background should help expand on the role of 5-HT_{2A} in the neurochemical action of antipsychotics.

Disclosures: **R. Joshi:** None. **M.M. Panicker:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Title: Comparison of the discriminative stimulus properties of the antipsychotics amperozide and amisulpride in C57BL/6 mice and antagonist activity at dopamine D_2 and serotonin 5-HT_2 receptors in Xenopus oocytes

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Abstract: The benzamide derivative amisulpride (Solian®) is an atypical antipsychotic drug used in Europe that is clinically effective in the treatment of both positive symptoms (e.g. delusions, hallucinations) and negative symptoms (e.g. flat affect, anhedonia) of schizophrenia with a low incidence of extrapyramidal motor side effects. Also, at low doses, amisulpride has been shown to be effective for the treatment of dysthymia. It displays a high binding affinity to dopamine D_2 and D_3 and serotonin 5-HT_7 and 5-HT_{2B} receptors. While the diphenylbutylpiperazine amperozide was investigated in clinical studies and appeared to have an atypical profile in schizophrenic patients, it was never adopted clinically; however, it does remain in use in veterinary medicine. In contrast to amisulpride, amperozide has a very low binding affinity to dopamine D_1 and D_2 receptors, but binds with high affinity to 5-HT_{2A} receptors. Amperozide has a low binding affinity for 5-HT_{1A} receptors, $alpha_1$ and $alpha_2$ adrenoceptors, and cholinergic M_1 and M_2 receptors. In study 1, C57BL/6 mice were trained to discriminate 10 mg/kg amisulpride from vehicle in a two lever drug discrimination task with full generalization (> 80% drug lever responding) at 10 and 20 mg/kg (ED50 = 0.56 mg/kg). However, amperozide did not generate amisulpride-appropriate responding at any of the tested doses (0.25 - 4.0 mg/kg). In study 2, amisulpride and amperozide were tested in *Xenopus* oocytes to measure their functional antagonism at D_2 and 5-HT_{2A} receptors. Amperozide was more potent at 5-HT_{2A} receptors blocking serotonin effects, but with limited efficacy, while it was less potent at the D_2 receptor blocking dopamine effects. Amisulpride, on the other hand, had a full range of efficacy at both receptors and higher potency at blocking the effects of dopamine at D_2 receptors. These results demonstrate that amisulpride and amperozide do not share discriminative

stimulus properties (i.e. subjective effects) and differ in their antagonism at dopamine D_2 and serotonin 5-HT_{2A} receptors.

Disclosures: **T.J. Donahue:** None. **J. Younkin:** None. **J.C. King:** None. **D.E. Logothetis:** None. **J.H. Porter:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: NIH Grant MH103421-01A1

Title: Does the antipsychotic-like effect of acute oxytocin on prepulse inhibition persist with chronic administration?

Authors: ***P. D. SHILLING**, G. MELENDEZ, B. ROBERTS, J. TRAN, A. AVALOS, A. DAQIAN, A. NARWAN, B. KIAEI, D. FEIFEL;
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Abstract: Background: There is a great need for improved treatments for schizophrenia. One promising molecule addressing this need is oxytocin (OT). Human studies administering OT to patients with schizophrenia intranasally over 2-8 weeks have produced mixed results and this may be due to the development of some tolerance with chronic administration. In preclinical studies, a single peripheral administration of OT administered to rats with naturally low prepulse inhibition (PPI) or PPI reduced by psychomimetic drugs produces antipsychotic-like effects in the PPI paradigm. Because chronic administration is more relevant to the treatment regimen used for patients with schizophrenia, it is important to also investigate the chronic effects of OT on PPI. To investigate the antipsychotic-like effects of chronic OT, we administered daily OT to female Brown Norway rats, an animal model that exhibits natural deficits in PPI. Methods: Female Brown Norway rats were administered either subcutaneous saline, 0.04, 0.2 or 1.0 mg/kg OT once/day for 22 days. On days 1 and 22, baseline PPI was measured 30 min after OT injection. Baseline PPI was also tested 1 day after the last OT treatment and on the subsequent day, 30 minutes after the administration of a PPI lowering dose (2.0 mg/kg) of PCP. Results: Acute and chronic administration of 1.0 mg/kg OT significantly facilitated PPI ($P < 0.05$). Two days after stopping chronic OT treatment, a single injection of OT reversed ($P < 0.01$) PCP-induced PPI deficits. Discussion: Similar to the effects of OT in male Brown Norway rats, acute OT facilitates PPI in female Brown Norway rats. To the best of our knowledge, this is the first

report that the antipsychotic-like effects of acute OT treatment persist after chronic administration in female rats. Furthermore, OT antipsychotic-like effects persist for at least 2 days after discontinuation of OT administration. These results are not consistent with the only other reports on the effects of chronic OT on PPI in rodents. In these studies, chronic OT did not facilitate PPI in mice. However, the effects of acute OT on PPI were either not reported (Huang et al. 2013) or acute OT did not facilitate PPI deficits (Teng et al. 2015). In contrast to these two studies, our results are consistent with the antipsychotic-like potential of OT after chronic administration.

Disclosures: **P.D. Shilling:** None. **G. Melendez:** None. **B. Roberts:** None. **J. Tran:** None. **A. Avalos:** None. **A. Daqian:** None. **A. Narwan:** None. **B. Kiaei:** None. **D. Feifel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DF is a named inventor on a patent application for the therapeutic use of oxytocin, filed on his behalf by UCSD..

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Topic: H.03. Schizophrenia

Support: NSERC

CIHR

Title: Chemogenetic activation of CCK-GABA neurons: implications for schizophrenia

Authors: ***P. D. WHISSELL**, I. KHAN, J. KIM;
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Abstract: Schizophrenia is a serious mental health disorder characterized by impairments in executive function, recognition memory and social interaction. Disruption of γ-aminobutyric acid (GABA) neurotransmission is frequently observed in schizophrenia and may contribute to behavioral dysfunction. Recently, animal models of schizophrenia have suggested that GABAergic neurons which express the neuropeptide cholecystokinin (CCK-GABA neurons) play a particularly important role in the disorder. As reduced CCK-GABA neuron activity may contribute to behavioral deficits in schizophrenia, it is possible that activating CCK-GABA neurons may have a therapeutic effect and restore normal behavior. Here, we used a chemogenetic approach to selectively activate CCK-GABA neurons during behaviors relevant to

schizophrenia. Transgenic mice were generated which expressed the synthetic excitatory receptor, hM3Dq, selectively in CCK-GABA neurons (hM3Dq::CCK-GABA mice). These mice underwent behavioral testing following an injection of either the synthetic hM3Dq receptor-specific agonist clozapine-N-oxide (CNO) or saline. The results showed that hM3Dq::CCK-GABA mice treated with CNO had slightly increased anxiety in the elevated plus maze but enhanced performance in the novel object recognition, puzzle box and fear conditioning tests. Injection of CNO in mice not expressing hM3Dq receptors had no effect on behavior. These surprising results indicate that activating CCK-GABA neurons does indeed have beneficial effects on behavior, particularly cognitive function, though at the expense of increased anxiety. The hM3Dq::CCK-GABA mouse line presents as a useful model to study the behavioral functions of CCK-GABA neurons, as well as the mechanisms of behavioral impairments in schizophrenia.

Disclosures: P.D. Whissell: None. **I. Khan:** None. **J. Kim:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: CIHR MOP-130393

Title: Amelioration of working memory deficits induced by prefrontal GABA hypofunction by D1 receptor agonists and D-govadine

Authors: *J. MECCIA[1], M. AUGER[1], A. G. PHILLIPS[2], S. B. FLORESCO[1];
[1]Psychology, [2]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Perturbed prefrontal GABA signalling is thought to contribute to cognitive impairment associated with schizophrenia. Recent work from our group and others has found that pharmacological reductions in GABA-A receptor activity in rodent prefrontal cortex (PFC) recapitulate many cognitive abnormalities observed in schizophrenia, including deficits in working memory assessed with delayed-response tasks. It is possible that compounds that reverse impairments in cognition induced by diminished PFC GABA signalling may have utility in treating cognitive dysfunction associated with the disorder. Moderate stimulation of PFC dopamine D1 receptors enhances interneuron excitability and GABAergic inhibition of PFC pyramidal cells, which may potentially reverse the effects of diminished PFC GABA signalling. D-govadine is a recently characterized tetrahydroprotoberberine that promotes mesocortical

dopamine release and enhances working memory in intact rats performing a delayed-response version of the radial maze task. However, whether this drug can ameliorate cognitive deficits in perturbed systems is unclear. Here, we investigated whether D-govadine could mitigate impairments in working memory induced by PFC GABA-A receptor antagonism and compared these effects to those induced by the D_1 agonist SKF81297. Male Long Evans rats were well-trained on an operant delayed non-match to position task, prior to implantation with bilateral cannulae in the medial PFC. The task consisted of a sample phase in which one lever is extended, and a choice phase in which the rat must select the opposite lever, separated by a variable delay (1-24s). On separate test days, rats were pre-treated with either saline or d-govadine (0.5-1.0 mg/kg) or SKF 81297 (0.03-0.3 mg/kg) prior to receiving infusions of either saline or the GABA-A receptor antagonist, bicuculline (50 ng). Reducing PFC GABA activity caused delay-independent deficits in working memory. Importantly, these deficits were ameliorated by pre-treatment with d-govadine (1.0 mg/kg), in a manner similar to certain doses of SKF81297. d-govadine did not affect performance when administered prior to intra-PFC saline infusions. In a separate experiment, intra-PFC bicuculline impaired performance of a reference/working memory version of the radial arm maze task, and this effect was attenuated by SKF81297. Whether d-govadine induces a similar rescue is currently being assessed. These results further highlight the therapeutic potential of d-govadine in the treatment of schizophrenia-related cognitive impairment, and suggest that these effects may be mediated in part by facilitation of D_1 receptor activity.

Disclosures: J. Meccia: None. **M. Auger:** None. **A.G. Phillips:** None. **S.B. Floresco:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Support: NIH grant RO1 AA022448

School of Pharmacy, University of Southern California

Title: Ivermectin, a positive modulator of P2X4 receptors, interacts with D1 receptors in modulation of prepulse inhibition of acoustic startle reflex

Authors: *S. KHOJA[1], L. ASATRYAN[2], M. W. JAKOWEC[3], D. L. DAVIES[2];
[1]Pharmacol. and Pharmaceut. Sci., [2]Titus Family Dept. of Clin. Pharm., [3]Neurol., USC, Los Angeles, CA

Abstract: Purinergic P2X4 receptors (P2X4Rs) are adenosine-5'-triphosphate (ATP) activated ion channels. They are expressed on multiple types of cells across the central nervous system (CNS). Previous findings have linked P2X4Rs to neuropathic pain, neuroendocrine effects and hippocampal synaptic plasticity. Recently, findings from our group, using gene knockout and pharmacological strategies have identified potential roles for P2X4Rs in sensorimotor gating, social behavior and ethanol drinking behavior. We found deficits of prepulse inhibition (PPI) of acoustic startle reflex, (an operational measure of sensorimotor gating) in P2X4R knockout (KO) mice as well as disruption of PPI function by ivermectin (IVM), a positive modulator of P2X4Rs. Moreover, IVM did not disrupt PPI function in P2X4R KO mice, indicating that IVM is modulating this function via P2X4Rs. We found significant increases in dopamine (DA) receptors including D1 and D2 receptors (D1Rs & D2Rs) in P2X4R KO mouse ventral striatum, a brain region critical for PPI modulation. Additionally, the PPI deficits in P2X4R KO mice were rescued by D1R antagonist, SCH-23390 and D2R antagonist, raclopride, which links DA dysregulation with sensorimotor gating deficits. On basis of our initial observation that there might be a link between P2X4R function and DA neurotransmission, we hypothesized that IVM disrupts PPI function via activation of DA system in wildtype (WT) C57BL/6 mice. This was accomplished by testing the effects of IVM (10 mg/kg) on PPI function in the presence of DA receptor antagonists, SCH-23390 (1 mg/kg) and raclopride (3 mg/kg). We found that the PPI disruptive effects of IVM were significantly blocked by SCH-23390, but not by raclopride, indicating a role for D1Rs in interacting with P2X4Rs in disrupting PPI. IVM is reported to increase phosphorylation of dopamine and cyclic-AMP regulated phosphoprotein of 32kDa (DARPP-32) and extracellular regulated kinase-1/2 (ERK 1/2). This molecular explanation is reminiscent of signaling cascades activated by non-selective DA agonists that are known to disrupt PPI, suggesting that IVM might be mediating its PPI disruptive effects via activation of these signaling molecules. Future studies will investigate the molecular mechanism by which SCH-23390 blocks the PPI disruptive effects of IVM. Sensorimotor gating deficits has major implications in wide spectrum of neuropsychiatric diseases including schizophrenia, bipolar disorder, attention deficit hyperactivity and these findings will be instrumental in elucidating a novel drug target for treatment of sensorimotor gating deficits linked to psychiatric illnesses.

Disclosures: S. Khoja: None. **L. Asatryan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Liana Asatryan is on a patent for the use of IVM for treatment of alcohol use disorders. **M.W. Jakowec:** None. **D.L. Davies:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Daryl L. Davies is an inventor on a patent for the use of IVM for treatment of alcohol use disorders.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: NIH Grant U19MH82441

Title: Efficacy of beta-arrestin biased dopamine D2 receptor compounds as preclinical treatment for schizophrenia-like behaviors

Authors: *W. C. WETSEL[1], R. M. RODRIGUIZ[2], V. M. POGORELOV[2], S. PARK[2], C. M. SCHMERBERG[2], M. G. CARON[3], J. JIN[4];
[1]Dept Psychiat & Behav Sci., Duke Univ. Dept. of Psychiatry and Behavioral Sci., Durham, NC; [2]Psychiatry and Behavioral Sci., [3]Cell Biol., Duke Univ. Med. Ctr., Durham, NC; [4]Structural and Chem. Biology, Oncological Sciences, and Pharmacol. and Systems, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The dopamine D2 receptor (D2R) is a member of the G protein-coupled receptor family and is the primary target for antipsychotic drugs. D2Rs are coupled to the inhibition of adenylyl cyclase. Besides affecting G protein-dependent signaling, antipsychotic drugs also influence the recruitment of β-arrestin 2 (βArr2) to the receptor. In D2R-containing neurons, βArr2 serves as a scaffolding protein for certain kinases and phosphatases. Recent results have shown that the G protein-dependent and this G protein independent pathway can be modulated differentially. This property is termed functional selectivity. However, antipsychotic drugs to the D2R were not developed with this property in mind. The purpose of these studies was to determine whether βArr-biased compounds would show preclinical efficacy in a hyperdopaminergic genetic model of schizophrenia-like behaviors. Responses to the D2R βArr-biased compounds, UNC9975 and UNC9994, were examined in the dopamine transporter knockout (DAT-KO) mice. Both compounds suppressed open field hyperlocomotion in these mutants. By contrast, prepulse inhibition (PPI) was not restored in the DAT-KO mice. However, both compounds rescued acute amphetamine-disruption of PPI with C57BL/6 mice. Both sociability and novel object recognition memory were restored in the DAT-KO mice. Nevertheless, in a test for motivation both compounds reduced the breakpoint levels in C57BL/6 mice. In a test for extrapyramidal side-effects, cataleptic responses to the βArr-biased compounds were very low in C57BL/6 mice compared to that for haloperidol. Collectively, both UNC9975 and UNC9994 show considerable preclinical efficacy in alleviating several symptom domains of schizophrenia-like responses in the chronically hyperdopaminergic mice. These findings indicate that functionally selective compounds for the D2R may provide a novel

approach for developing antipsychotic drugs to treat schizophrenia and other related disorders in patients.

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Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Title: Diencephalic D2 dopamine receptor expression in a rat model for tardive dyskinesia

Authors: *S. E. BACHUS;
Psychology, Univ. of Maryland Baltimore County, Baltimore, MD

Abstract: While striatal D2 dopamine receptors are upregulated by chronic haloperidol (HAL), we have previously found striatal D2 mRNA to not be correlated with vacuous chewing movements (VCM), a rat model for tardive dyskinesia (Bachus et al., Behav. Brain Res. 231:323, 2012). We have also found upregulation of the dopamine-synthetic enzyme tyrosine hydroxylase, by chronic HAL, in the hypothalamus, in this model (Stanton et al., SfN Abstr., 2003), though this was not correlated with VCM. Thus, we have extended our survey of D2 receptor expression in this cohort to the diencephalon.
Group housed male Long-Evans rats, with starting body weights of 90-165g, were treated for 24 weeks with HAL (28.5mg/kg/ml, i.m.: n=43) or vehicle (sesame oil: n=21) injections every 3 weeks. VCM were counted for each rat for 2 minutes weekly, for 7 weeks prior to HAL treatment and then throughout HAL treatment, by observers blinded to treatment group. Over the final 2 weeks, 4 samples were rated under both quiet (only ambient air-conditioning) and noisy (constant loud music and key-rattling) conditions. Cryostat-cut sections from fresh-frozen brains, containing zona incerta (ZI) and paraventricular hypothalamus (PVN), or ventromedial hypothalamus (VMH), were assayed by in situ hybridization histochemistry with oligonucleotide probes complementary to D2 mRNA, or a mis-sense control probe.

Consistent with extensive literature implicating VMH in aggression, a significant relationship was found, in the controls, between VMH D2 mRNA and aggression that was noted incidentally during the treatment period ($t=2.63$, $p<.02$), indicating that these diencephalic D2 mRNA measurements were accurate. Moreover, PVN D2 mRNA was significantly positively correlated with previously measured PVN oxytocin mRNA among the controls ($r=.50$, $p=.02$). However, no D2 mRNA upregulation was found to occur after chronic HAL in any of these regions. Interestingly, ZI D2 mRNA was strongly correlated with VCM under noisy conditions among the controls ($r=.75$, $p<.00005$), but not among the HAL-treated rats.
The dissociation between the relationship found between VMH D2 mRNA and aggression, but the lack of relationship to HAL-induced VCM, argues that the negative finding for VCM is a believable one. Diencephalic D2 receptors appear to respond differently to chronic neuroleptic exposure, compared to D2 receptors in the basal ganglia. These data suggest that individual differences in subthalamic D2 receptor function may contribute to spontaneous dyskinesia, though not, apparently, to tardive dyskinesia.

Disclosures: S.E. Bachus: None.

Poster

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Topic: H.03. Schizophrenia

Support: CIHR Grant 125984

Title: Effects of sodium nitroprusside on MK-801-induced impairments in the trial-unique, delayed nonmatching-to-location task

Authors: *J. HURTUBISE[1], W. N. MARKS[1], D. A. DAVIES[1], J. K. CATTON[1], G. B. BAKER[2], J. G. HOWLAND[1];
[1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: The cognitive symptoms observed in schizophrenia are highly prevalent and predictive of patient functional outcome but are not usually alleviated by conventional antipsychotics. In a recent pilot study, sodium nitroprusside (SNP), a nitric oxide donor, was identified as a promising adjunct treatment to reduce the working memory impairments experienced by schizophrenia patients. Adjunctive SNP has also been reported to decrease the positive and negative symptoms experienced by patients for weeks following a single administration. The mechanisms underlying these changes and the areas of cognition affected

remain largely unknown. Therefore, it is of interest to examine the effects of SNP using a rodent model of schizophrenia that has demonstrated predictive validity. The aim of the present experiment was to explore the effects of SNP on the acute MK-801 rodent model of schizophrenia using a highly translatable task in order to establish its validity. Working memory and pattern separation were measured using the trial-unique, delayed nonmatching-to-location (TUNL) task in touchscreen-equipped operant conditioning chambers. Acute MK-801 (0.05 or 0.1 mg/kg) administration 25 minutes prior to task initiation impaired both areas of cognition. When SNP (2 mg/kg; i.p.) and MK-801 (0.1 mg/kg; i.p.) were administered within 5 minutes of each other, no interaction was observed. Interestingly, SNP improved performance on trials with difficult to discriminate patterns (p=.058). Previous rodent studies using the ketamine model of schizophrenia and the novel object preference task observed a preventative effect of SNP administration. When we administered SNP (5.0 mg/kg) nearly 4 hours prior to MK-801 (0.05 mg/kg) no cognitive improvements were observed. Our results suggest that SNP may have intrinsic cognitive enhancing properties but is not capable of reducing MK-801-induced working memory and pattern separation impairments in the TUNL task. This study failed to mirror the results of the human pilot study that observed improved working memory following SNP administration. Further, it did not replicate previous animal studies using ketamine. Ultimately, the findings suggest that the effects of MK-801 in the TUNL task may not hold the predictive validity needed for its use in the study of SNP. In order to advance the understanding of SNP, future studies should investigate other translatable paradigms to establish validity.

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Topic: H.03. Schizophrenia

Support: The Ohio State University

Title: Accumbens-prefrontal interactions in the regulation of multiple transmitter systems: implications for cognitive deficits in schizophrenia

Authors: ***V. VALENTINI**[1,2], J. D. SCHUMACHER[2], D. PHENIS[2], D. BORTZ[2], J. P. BRUNO[2];
[1]Univ. of Cagliari-Dept. Biomed. Sci., Cagliari, Italy; [2]Dept of Psychology, Ohio State Univ., Columbus, OH

Abstract: Interactions among cortical and subcortical regions are necessary for expression of complex elements of cognition. We have been studying the neurochemical bases for top-down regulation as it is critical for the mediation of cognitive control (i.e. response inhibition, selective attention, working memory) in normal rats and in animal models of schizophrenia - a condition exhibiting deficits in top-down controls. Our previous research demonstrated that enhancing NMDA receptor activity in the nucleus accumbens (NAc) shell stimulated ACh and glutamate release in ipsilateral prefrontal cortex (PFC). Moreover, NMDA infusions facilitated the animals' ability to perform a sustained attention task when faced with distracting cues - an example of top-down regulation. We further analyzed this functional system by measuring the reciprocal neurochemical consequences of increasing prefrontal glutamate and ACh on levels in ipsilateral NAc shell. In all animals microdialysis probes were implanted in PFC. In half the group a second probe was implanted into the shell. In the other half of animals a glutamate-sensitive biosensor was implanted into shell. Cortical activation was achieved through reverse dialysis of S-ESBA, a KAT-II inhibitor. This perfusion decreased levels of kynurenic acid (KYNA), the endogenous negative modulator of the alpha7 nicotinic receptor. Elevated KYNA levels are seen in the PFC of patients with SZ and such increases produce cognitive deficits in animal models of SZ. Thus, KAT-II inhibitors are seen as potential novel therapeutics with cognition-enhancing properties. Transmitter levels were measured using ultra-HPLC with all analytes being measured from the same animals at 20 min intervals. In some cases, NAc glutamate was determined on a sec-by-sec scale using a selective biosensor. Local perfusion with S-ESBA (5 mM) increased PFC glutamate (130% above baseline), ACh (60%), DA (70%), and 5-HT (30%) levels. This broad cortical activation increased in NAc glutamate (50%), ACh (20%), and DA (20%) levels. Collectively, the results demonstrate that endogenous levels of KYNA tonically inhibit the release of a wide range of prefrontal transmitters. Activation of one or more of these cortical systems is sufficient to stimulate glutamatergic, cholinergic, and dopaminergic transmission in the NAc shell. While these reciprocal effects are seen in resting animals, the effects of reducing cortical KYNA levels in trained, task-performing rats (intact and models of SZ) will also be presented. Such reciprocal relations between NAc shell and PFC are speculated to form the basis of a neural system that mediates top-down regulation and cognitive control.

Disclosures: V. valentini: None. **J.D. Schumacher:** None. **D. Phenis:** None. **D. Bortz:** None. **J.P. Bruno:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Program#/Poster#: 364.24/MMM27

Topic: H.03. Schizophrenia

Support: MH57440

CNPq/Brazil grant 200606/2015-8

Title: Investigation of α-7 nicotinic receptor modulation effects on VTA dopamine neuron activity in the MAM animal model of schizophrenia

Authors: *G. A. NEVES, A. A. GRACE;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cholinergic neurotransmission has been implicated in the pathophysiology of several neuropsychiatric disorders. Several post-mortem, genetic and epidemiologic data link specifically the α7 nicotinic receptors to schizophrenia. Although the role of this subtype of acetylcholine receptor in the development of schizophrenia symptoms is not fully understood, the potential use of α7 modulators as an alternative or add-on treatment strategy for cognitive impairments in schizophrenia is an active field of research. However, there is a lack of studies about the effects of α7 ligands in neurodevelopmental models of schizophrenia, as well as the necessity of a deeper assessment of the differences between orthosteric and allosteric modulators examined in vivo. Taking these into account, the objective of our study is to investigate the effects of α7 activation or modulation on dopamine neuron activity in the ventral tegmental area (VTA), a brain area involved in schizophrenia symptoms development, in the methylazoxymethanol acetate (MAM) animal model of schizophrenia. Sprague-Dawley pregnant dams were treated with MAM or saline (NaCl 0.9%) on gestational day 17. Recordings of VTA dopamine neuron activity are performed on the male offspring at adulthood, after acute i.v. treatment with one of the following drugs: PNU 282987 (full α7 agonist), SSR 180711 (partial agonist) NS-1738 (type 1 positive allosteric modulator) or PNU 120596 (type 2 positive allosteric modulator). Neuronal activity is recorded with open filter settings (low pass = 10 Hz; high pass = 10 kHz) while making 6-9 vertical passes through the VTA separated by 200 μm. The number of spontaneously active dopamine neurons per track, as well as mean firing frequency and the percentage of spikes in bursts is then accessed. All the experimental procedures are being conducted according to NIH Guide for the Care and Use of Laboratory Animals and were previously approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Previous results of our group demonstrated that MAM treated animals present an increase in the number of active dopamine cells in the VTA, with no changes in its firing rate or in its burst firing pattern (Lodge and Grace, 2007). We hypothesize that acute activation of α7 receptors in control rats would increase dopamine cells activity and that the effects in MAM treated rats may not necessarily be the same, since this animals seems to have an increase in cholinergic tone.

Disclosures: G.A. Neves: None. **A.A. Grace:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: CONACyT Grant 575264

CONACyT Grant 252808

Title: Risperidone ameliorates behavioral and morphological changes induced by neonatal ventral hippocampus lesion in rat

Authors: *H. TENDILLA, S. MENESES-PRADO, R. A. VÁZQUEZ-ROQUE, G. FLORES; Inst. De Fisiología, Benemérita Univ. Autónoma De Puebla, Puebla, Pue. CP 72570, Mexico

Abstract: Risperidone (RISP) is an atypical antipsychotic widely used in the treatment of schizophrenia (SCHZ), however its mechanism of action has not been fully described yet. Besides, neonatal ventral hippocampus lesion (NVHL) in the rat is considered SCHZ-related neurodevelopmental model and one of the most effective to reproduce qualitative, quantitative and temporal way the anatomical-functional, behavioral and biochemical characteristics correlated with psychopathology of this disorder. Previous reports of our group had demonstrated hyperresponsiveness to novel environment, dendritic retraction and spine loss in limbic regions in rats with NVHL. In this study we aimed to evaluate the effects of subchronic RISP treatment (0.25 mg/kg/day for 21 days) at post-pubertal age on behavioral and neuronal abnormalities due to NVHL. The behavioral analysis included locomotor activity; on the other hand the morphological evaluation included dendritic analysis by using the Golgi-Cox method in prefrontal cortex, nucleus accumbens and dorsal hippocampus. Behavioral data showed a reduction in the hyperresponsiveness in the RISP-treated NVHL rats. Moreover histological analysis of the limbic regions showed that RISP ameliorates dendritic atrophy and neuronal loss induced by NVHL. Our results supports the functionality of NVHL as a model of SCHZ-related behavior and suggest that RISP reverses behavioral deficits in these animals by modulating neuronal reorganization in the brain.

Disclosures: H. Tendilla: None. **S. Meneses-Prado:** None. **R.A. Vázquez-Roque:** None. **G. Flores:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: CIHR; MOP 246144

NSERC

Title: Involvement of prefrontal GABAergic transmission in schizophrenia-like behaviour induced by chronic adolescent THC exposure

Authors: *J. RENARD, C. KRAMAR, H. HSZKUDLAREK, L. G. ROSEN, W. J. RUSHLOW, S. R. LAVIOLETTE;
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Abstract: Marijuana is the most widely used illicit drug among adolescents. We have previously shown that chronic treatment with the psychoactive component of cannabis, tetrahydrocannabinol (THC) during adolescence in rats, is associated with a schizophrenia-like phenotype in adulthood involving molecular adaptations in the prefrontal cortex (PFC) and a hyperactive sub-cortical dopamine (DA) system (Renard et al; 2016). Adolescence is a vulnerable period for brain development, where the inhibitory GABAergic system plays a pivotal role during central nervous system maturation. GABAergic hypofunction has been observed in the PFC of post-mortem brains of schizophrenia patients, and may be a mechanism by which the PFC loses its ability to modulate sub-cortical DA signals which may ultimately lead to schizophrenia-like neuropsychopathology. In the present study, we hypothesized that the ability of adolescent THC exposure to induce a schizophrenia-like phenotype in later adulthood (Renard et al; 2016) may be associated with PFC GABAergic hypofunction and a resulting dysregulation of sub-cortical DA transmission. We exposed adolescent rats (postnatal day (PND) 35 to 45) to THC (i.p. injections, twice daily with THC from 2.5 to 10 mg/kg). At adulthood (PND75), we studied the functionality of PFC GABAergic neurotransmission using molecular analyses, behavioural tasks and *in-vivo* electrophysiological recordings of DA neurons in the mesolimbic pathway. Our results show that (1) the GABAergic marker GAD 67 is profoundly reduced in the PFC of adult rats exposed to THC during adolescence and (2) the behavioural alterations observed following adolescent THC exposure in social interactions/recognition, anxiety levels and motivation (Renard et al;2016) are reversed by infusions of a GABA-A receptor selective agonist muscimol in the adult PFC. Furthermore, activation of adult PFC GABA-A receptors normalizes the spontaneous firing of VTA dopaminergic neurons. We are currently performing electrophysiological neuronal recordings to analyze cortical oscillations and neuronal activity dynamics in the PFC. Our findings demonstrate that hypofunction of inhibitory PFC GABAergic

neurotransmission plays a crucial role in the schizophrenia-like behaviours observed following adolescent THC exposure. These findings improve our knowledge of the precise neurobiological mechanisms leading to long-term deleterious effects of THC exposure during adolescence in the onset of schizophrenia in adulthood and suggest that restoring GABAergic cortical function may ameliorate these pathological effects.

Disclosures: J. Renard: None. **C. Kramar:** None. **H. hszkudlarek:** None. **L.G. Rosen:** None. **W.J. Rushlow:** None. **S.R. Laviolette:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

Location: Halls B-H

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Topic: H.03. Schizophrenia

Support: CONACyT grants No. 575264

CONACyT grants No.252808

Title: Chronic risperidone administration attenuates neuronal abnormalities in the basolateral amygdala induced by an animal model of schizophrenia in the rat

Authors: *R. A. VAZQUEZ, SR, H. TENDILLA-BELTRAN, S. MENESES-PRADO, G. FLORES;
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Abstract: The neonatal ventral hippocampal lesion (nVHL) has emerged as a model of schizophrenia-related behavior in the rat. These rats exhibit behavioral changes that manifest mainly after puberty. Together with the behavioral alterations, neural morphological changes have been reported in this model. Besides, it has been reported that Risperidone (RISP) is an atypical antipsychotic widely used in the treatment of Schizophrenia (SCHZ), nevertheless, the mechanisms how Risperidone works in this disorder it is not really clear. We recently demonstrated that nVHL animals exhibit dendritic atrophy and spine loss in the basolateral amygdala (BLA). This study aimed to determine whether RISP treatment was capable of reducing BLA neuronal alterations observed in nVHL rats. Besides, we evaluated social interaction in these animals. The morphological evaluation included examination of dendrites using the Golgi-Cox procedure and stereology to quantify the total cell number in BLA. Golgi-Cox staining revealed that nVHL induced dendritic retraction and spine loss in BLA pyramidal neurons. Stereological analysis demonstrated nVHL also produced a reduction in cells in BLA.

Interestingly, repeated RISP treatment ameliorated dendritic pathology and neuronal loss in the BLA of the nVHL rats. Our data show that RISP may foster recovery of BLA damage in post-pubertal nVHL rats and suggests that the use of neuroleptic agents for the management of some schizophrenia-related symptoms may help to understand the amygdala alterations pathways in these disorders.

Disclosures: R.A. Vazquez: None. **H. Tendilla-Beltran:** None. **S. Meneses-Prado:** None. **G. Flores:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Title: Repeated ropinirole treatment resulting in recovery of sensorimotor gating induces ΔFosB in mouse nucleus accumbens neurons that co-express D1 and D3 dopamine receptors, but not D2 receptors

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[1]Interdisciplinary Grad. Program in Neurosci., Arizona State Univ., Tempe, AZ; [2]Basic Med. Sci., [3]Basic Med. Sciences, Psychiatry, [4]BMS, Pharmacology, Psychiatry, Univ. of Arizona Col. of Med., Phoenix, AZ; [5]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Prepulse inhibition of the acoustic startle response (PPI) is a cross-species operational measure of sensorimotor gating that is disrupted in patients with schizophrenia. Acute treatment with ropinirole, a D2-like dopamine receptor agonist with 10-fold greater affinity for the D3 receptor, disrupts PPI in rodents. We showed previously that daily ropinirole treatment for 28

days induces PPI recovery, mediated by CREB phosphorylation in nucleus accumbens (NAc) neurons. Repeated ropinirole also increases expression in the NAc of ΔFosB, a stable transcription factor that produces different behavioral effects when expressed in D1 vs. D2 receptor-expressing NAc neurons. In order to determine which subtype of NAc neurons express ΔFosB after repeated ropinirole treatment, the present study examined co-localization of ΔFosB with D1 (D1R), D2 (D2R), and D3 (D3R) dopamine receptors in the mouse striatum. Adult male BAC transgenic mice that express tdTomato in striatal neurons containing D1R (D1R-tdTomato) or enhanced green fluorescence protein (eGFP) in neurons containing D2R (D2R-eGFP) were treated daily with sterile saline vehicle or ropinirole HCl (0.1 mg/kg, ip) for 28 days. Brains were removed 7 days after termination of treatment, and sections were immunolabeled using antisera directed against FosB and/or D3R, followed by fluorescent secondary antisera. Labeled cells were quantified using a modified stereological approach (Image J; http://imagej.nih.gov/ij). D3R expression was significantly greater in the NAc than in the caudatoputamen (CP), while D2R density was higher in CP than in NAc, consistent with previous reports. Repeated ropinirole treatment selectively induced ΔFosB in NAc neurons, but not in CP neurons in both lines. Furthermore, this effect was present in NAc neurons expressing D3R in both lines, and in NAc neurons expressing D1R in D1R-tdTomato brain tissue, but not in NAc neurons expressing D2R in D2R-eGFP brain tissue. Approximately 30-40% of D1R-expressing neurons in the NAc core also expressed D3R, and the largest effect of ropinirole treatment on ΔFosB expression occurred in neurons that co-express D1 and D3R in the NAc core. These data suggest that ropinirole-induced ΔFosB expression is associated with D1 and D3R function rather than D2R activity in NAc neurons. These NAc neurons could contain heterodimeric D1 and D3R whose repeated activation by ropinirole drives D1R-coupled signaling, which could underlie enhanced CREB phosphorylation and ΔFosB expression. Thus, a subset of NAc neurons altered by repeated ropinirole treatment might affect sensorimotor gating symptoms in schizophrenia.

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Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: Clasado Ltd.

Title: Gender specific interactions between the prebiotic B-GOS and the antipsychotic olanzapine: an analysis of central NMDA receptor subunits and weight gain

Authors: *A. KAO, B. LENNOX, P. W. J. BURNET;
Univ. Dept. of Psychiatry, Univ. of Oxford, Oxfordshire, United Kingdom

Abstract: The antipsychotic, olanzapine (OLZ), is an effective treatment for schizophrenia, but often leads to secondary health concerns such as metabolic syndrome and weight gain. Studies in rats have shown that chronic OLZ administration induces weight gain and reduces glutamate N-methyl-D-Aspartate receptor (NMDAR) levels in the hippocampus. This latter effect may hinder the recovery of cognitive function in schizophrenia patients receiving OLZ. We have shown that dietary augmentation of beneficial gut bacteria using the prebiotic Bimuno® galacto-oligosaccharides (B-GOS) increases central levels of NMDAR subunits in rodents. The aim of this study was to determine whether the effects of OLZ on central NMDAR subunit levels and body weight in rats are influenced by B-GOS intake. Adult male (n = 24) and female Sprague-Dawley (n = 24) rats were orally administered with B-GOS (0.5g/day) or water for 7 days, before receiving a single injection of OLZ (10mg/kg/day; i.p.), or saline, for 14 days. Weight gain and water intake were monitored daily, and levels of enteric *bifidobacteria* from faecal pellets were evaluated weekly. The levels of NMDAR subunit proteins (GluN1, GluN2A, GluN2B) were measured in the frontal cortex and hippocampus with immunoblotting. Two-way ANOVAs were used to analyse all data, with repeated measures included to explore weight gain throughout the study. There was a significant B-GOS x OLZ interaction ($p < 0.05$) on GluN2B levels in the frontal cortex of male rats: B-GOS-fed rats injected with saline had 25% greater levels of cortical GluN2B than water-fed controls ($p = 0.008$). However, OLZ administration significantly elevated hippocampal GluN2B expression in the absence of B-GOS ($p = 0.046$). A B-GOS x OLZ interaction was not observed for central NMDAR subunit levels in female rats. However, OLZ treatment overall, significantly increased cortical GluN1 ($p = 0.002$) and GluN2A ($p = 0.036$). This was driven by the significant difference (16% increase) between B-GOS/saline and B-GOS/OLZ groups ($p = 0.003$). Levels of subunit mRNAs are currently underway. In female, but not male rats, OLZ induced significant weight gain ($p < 0.001$) compared to saline-controls, which was not observed in OLZ-injected animals on the B-GOS diet. Our data indicate, therefore, that there are gender-specific effects of the prebiotic B-GOS and antipsychotic OLZ on central NMDAR subunits and metabolism in the rat. Some of these interactions suggest that inclusion of B-GOS as an adjunct to OLZ treatment in schizophrenia may have benefits to NMDAR dependent cognitive function, as well as preventing weight gain.

Disclosures: **A. Kao:** None. **B. Lennox:** None. **P.W.J. Burnet:** None.

Poster

364. Animal Models of Schizophrenia and Pharmacological Treatments

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Topic: H.03. Schizophrenia

Support: NIMH Grant F31MH109238

NIMH Grant R01MH107491

Title: Behavioral dissection of the dot-pattern expectancy task (DPX) in non-human primates

Authors: *A. L. DENICOLA, M. V. CHAFEE;
Univ. of Minnesota, Minneapolis, MN

Abstract: Schizophrenia patients suffer a wide range of cognitive impairments that are poorly treated by available therapeutics and strongly predict their functional outcome. One such cognitive deficit can be isolated with the Dot-Pattern Expectancy Task (DPX). The first (cue) stimulus provides contextual information about the current trial that is needed to respond to the second (probe) stimulus. Cue information must be maintained during a delay before the presented probe can trigger a response. The four categories of 'Cue-Probe' trials are 'AX', 'AY', 'BX', and 'BY'. 'AX' trials, 67% of all the trials presented, require a target response, while the three other trials require a non-target response. Patients with schizophrenia perform worse on 'BX' trials compared to controls. Previous work in our lab has demonstrated that monkeys performing DPX will also have a specific 'BX' impairment when given an injection of Ketamine, an N-methyl-D-aspartate receptor antagonist. There are a number of cognitive processes that may play a differential role in production of the 'BX' error. The current study attempts to dissect these potential processes. First, the error may emerge due to an inability to encode the cue identity. To address this, we use a backward masking paradigm of the DPX task. At variable times into the presentation of the cue a mask is presented, blocking the cue's identity during the rest of the cue period. Second, the error could be due to poor working memory, causing the subject to lose the ability to maintain the cue identity across the delay period. We adjust the delay period ranging from 0.5 seconds to 4.5 seconds. Under normal performance conditions for each DPX task modification, the monkey's performance on the 'AY' and 'BY' trial types is resistant to changes in mask length and delay length, potentially due to the 'Y' probe always requiring a non-target response. 'AX' and 'BX' performance decreases as the mask or the delay lengths are increased because the contextual information of the cue is either impaired (by mask) or working memory is taxed (by the delays). In both the masking and the delay paradigms, the interaction between trial type and the second predictor variable (mask length or delay length) significantly predicts error rates. These results indicate that specific mask lengths and delay lengths affect behavioral performance on the four trial types differentially.

Future experiments determining how Ketamine affects the performance on the masking and random delay DPX paradigms will give insight into the effects of NMDAR antagonism on individual cognitive control aspects underlying the emergence of the 'BX' error seen in patients with Schizophrenia.

Disclosures: A.L. Denicola: None. **M.V. Chafee:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 365.01/MMM34

Topic: I.03. Anatomical Methods

Title: Quantitative, real-time live-cell analysis method and reagents for evaluation of cell health in neuronal cultures

Authors: J. N. RAUCH[1], M. L. BOWE[1], L. OUPICKA[1], D. M. APPLEDORN[2], *D. M. ROCK[2];
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Abstract: Monitoring cell health in long term culture is critical for the evaluation of compound treatments that affect survival of neurons and culture conditions that support neuronal growth. Many traditional approaches rely on endpoint assays and imaging techniques that require immunochemical staining, approaches that limit obtaining real-time kinetic information. Here we present data with a quantitative, live-cell imaging approach that can be used to evaluate cellular viability in neuronal cultures over days/weeks in culture. To exemplify our approach, we evaluated glutamate-induced toxicity in primary rat forebrain neurons using red and green cell impermeable DNA dyes. Glutamate produced a concentration- and time-dependent increase in red or green object count (indicating cell death) over an evaluation period of 72 hours post addition. At a 48 hour post addition time point, the EC_{50} for glutamate was in the mid μM range, similar for both reagents. The NMDA receptor antagonist MK-801 blocked the glutamate-induced increase in object count with IC_{50}s in the low nM range. Glutamate produced a similar effect when measured with Annexin V reagents. These data demonstrate how our approach allows for real-time continuous evaluation of cell health supporting quantitative analysis for viability and compound effects on neurons.

Disclosures: J.N. Rauch: A. Employment/Salary (full or part-time): Essen BioScience. **M.L. Bowe:** A. Employment/Salary (full or part-time): Essen BioScience. **L. Oupicka:** A. Employment/Salary (full or part-time): Essen BioScience. **D.M. Appledorn:** A.

Employment/Salary (full or part-time): Essen BioScience. **D.M. Rock:** A. Employment/Salary (full or part-time): Essen BioScience.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 365.02/MMM35

Topic: I.03. Anatomical Methods

Title: A signal amplification system for fluorescent microscopy

Authors: *R. LIN, Q. FENG, Y. SHI, M. LUO;
Natl. Inst. of Biol. Sciences, Beijing, Beijing, China

Abstract: Immunohistochemical staining remains the most powerful methods for detecting protein expression and other signaling molecules. Amplifying the target antigen signals often requires the use of enzymes to catalyze the generation of colored reaction products. However, current methods perform poorly in producing fluorescent signals and often generate high background. The widespread use of fluorescent microscopy calls for an effective method that can sensitively detect antigen levels using fluorescent reporters. Here we report the development of a signal amplification system that dramatically increases the fluorescent signals of immunohistochemical reactions. This method—so-called immunoNA—combines immunohistochemistry with nucleotide amplification (NA). Fluorescent substrates are conjugated to small nucleotides. Antibodies serve as the linkers between fluorescent nucleotides and target molecules. The immunoNA system uses the commonly available materials, requires mild reaction condition, and produces high amplification rate. We first tested the amplification system by incorporating NA into classic immunodetection methods. We observed up to 100-fold increase of fluorescent intensity in Western blotting and immunohistochemical staining of proteins in cultured cells and brain sections. We also demonstrated that immunoNA is compatible with tissue clearing methods, such as iDISCO. To facilitate rapid labeling in large issue samples, we further developed a method that combines protein tags which has small chemical substrates with NA. Modified fluorescent nucleotides directly bind to the protein tag and initiate the reaction without requiring other molecules as linkers. Delivering the protein tag with AAV vectors results in rapid and strong labeling of neuronal morphology, including distal terminals. Thus, the immunoNA system allows sensitive fluorescent detection of target antigens, and should find widespread applications in immunohistochemistry, western blotting, and neuronal tract tracing.

Disclosures: **R. Lin:** None. **Q. Feng:** None. **Y. Shi:** None. **M. Luo:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Title: Validated Antibody Database: a curated database of antibodies validated in literature

Authors: *H. XIE;
Synatom Res., Princeton, NJ

Abstract: In clinical and research laboratories, antibodies are an indispensable tool for detecting, quantitating, and isolating proteins and other moieties in cells, tissues, and body fluids. While antibodies used for therapeutics and clinical diagnosis are subject to stringent regulations by health authorities throughout the world, there are no standards or third-party quality controls for research antibodies. Hundreds of biotech companies offer research antibodies, which are either polyclonal, monoclonal, or more recently, recombinant. However, the specificities of these antibody reagents do not always match scientists' expectations. An antibody might bind non-specific targets and might not be suitable for a specific application. Utilization of such an antibody often leads to false positive results. These misleading results nullify important discoveries, discredit a research team and impede scientific progress. Academic journals have withdrawn articles presenting data based on non-specific antibodies, and poor antibody quality has been attributed as one of the causes for the irreproducibility of published discoveries. In order to help alleviate this antibody quality and specificity problem, Labome organizes antibody applications cited in formal publications and has developed Validated Antibody Database (VAD). VAD, a manually curated database, compiles commercial and non-commercial antibodies whose specificities and applications have been independently reported in published results from formal articles. One of the benefits of our curation effort is the identification of cross-reactive species and novel applications for antibodies, which are often developed for human/mouse proteins and tested by suppliers for a limited number of applications. Labome registers many antibodies having cross-reactivities with model organisms such as flies, worms, zebrafish, and frogs and with novel applications from the literature. VAD version 2.2 contains 143357 entries of antibody applications from 38430 articles, covering 35146 antibody reagents from 110 suppliers. The database is updated every other month. VAD is freely accessible for online browsing.

Disclosures: H. Xie: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Synatom Research.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Support: NSF Grant 1353757

Title: Miniature picosecond diode laser system for two-photon fluorescence imaging of the mouse brain

Authors: R. D. NIEDERRITER[1], B. N. OZBAY[3], G. L. FUTIA[3], *D. RESTREPO[4], E. A. GIBSON[3], J. T. GOPINATH[1,2];
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Abstract: Two-photon excitation fluorescence microscopy (TPFM) is a powerful technique that allows imaging deep into brain tissue. TPFM requires a pulsed laser source with greater than 100 W peak power. Mode-locked Ti:sapphire lasers can provide this power but are expensive and require large amounts of space. In contrast, an attractive alternative is offered by compact and efficient semiconductor lasers. Pulsed laser sources based on diode lasers have the potential to simplify and miniaturize TPFM. In addition, the two-photon action cross sections of important fluorescent molecules such as eGFP peak near 976 nm, where high power semiconductor lasers are readily available. We demonstrate TPFM imaging of brain tissue using a compact and efficient picosecond light source. Our source is based on a modulated diode laser, fiber amplifiers, and a dispersive pulse compressor. The average laser power is 30 mW, the pulse duration is 3 ps, and the wavelength is 976 nm. The repetition rate is electronically variable from kHz to 10 MHz. The compact source is coupled with a laser scanning microscope for real time imaging of cell structures. The maximum imaging depth is greater than 200 microns, limited by the available laser power. Increased penetration depth is expected to be possible with improved laser design to increase the average power. Our compact and lightweight laser system will enable new biological imaging studies, especially combined with fiber-coupled and miniature microscope systems. In the future, this could be further miniaturized to include all components on a single semiconductor chip.

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Poster

365. Anatomical Techniques: Circuit Tracing and Staining

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 365.05/MMM38

Topic: I.03. Anatomical Methods

Support: NIH Grant 2RO1NS05863905/08

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Whole slide imaging was performed in the Digital Histology Shared Resource at Vanderbilt University Medical Center (www.mc.vanderbilt.edu/dhsr)

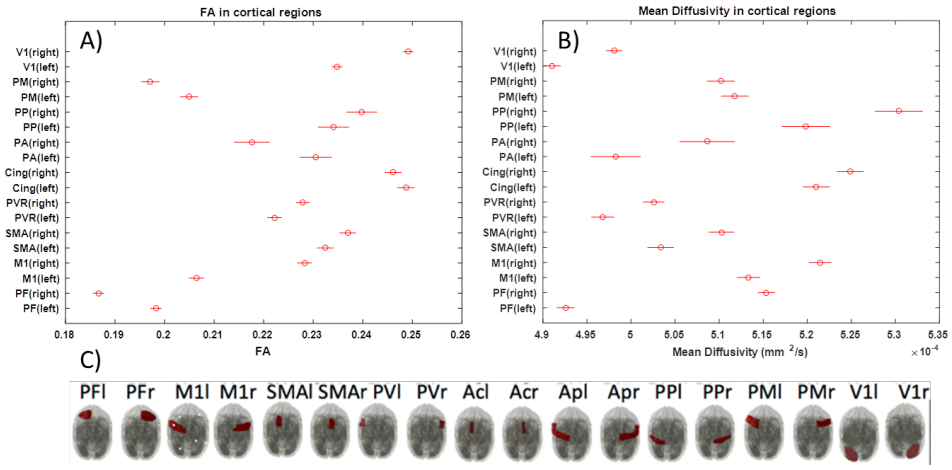
Confocal microscopy was performed at Cell Imaging Shared Resource at Vanderbilt University

Title: Determining the contributions of cell and myelin densities to diffusion magnetic resonance imaging (DMRI) parameters in the cortex of squirrel monkey using quantitative histology

Authors: *V. JANVE[1], K. SCHILLING[2], Y. GAO[2], B. A. LANDMAN[2], I. STEPNIEWSKA[3], A. W. ANDERSON[2];
[1]Biomed. Engin., [2]Dept. of Biomed. Engin., [3]Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: DMRI methods have shown potential to investigate the *in-vivo* tissue micro-structure non-invasively. Recent DMRI studies have reported systematic diffusion patterns within the cortex (McNab, J.A. et al. NeuroImage, 2013; Kleinnijenhuis M. et al. Cortex, 2013). Tissue microstructural inferences from DMRI methods such as DTI are based on mathematical models of water diffusion in tissue and thus need histological validation. Thus we carried out a systematic quantitative histological validation to determine the contributions of various tissue microstructural elements such as cell and myelin densities to the DMRI parameters.
High resolution DMRI measurements at 9.4T were performed on three healthy squirrel monkeys. Diffusion weighted scans were performed using a PGSE multi-shot spinwarp imaging sequence (TR=4.6s, TE=42ms, 32 gradient directions, b≈1000s/mm2, 300μm voxel, 192x128x115 matrix). The data used in this study were acquired for a larger project (Choe et al. Magn Reson Imaging, 2011). The fractional anisotropy (FA), mean diffusivity (MD), and principal eigenvectors (PEV) were computed using log-linear tensor fitting. The extracted brains were frozen and serially sectioned into 50 μm coronal sections (block photographed every third sectioned). Every sixth section was stained for Nissl substance (Thionine) and adjacent for myelin (Gallyas silver). Nissl and myelin stained slides were imaged at 20x magnification with

0.5 µm/pixel resolution and registered to MRI. 18 cortical grey matter ROIs and five white matter tracts were marked based on histology. The preliminary results indicate significant differences ($p < 0.05$) between DMRI parameters (FA, MD) derived from DTI model across different cortical regions. Fig. (A) and, (B) shows the mean FA and MD values in different cortical regions along with their 95% confidence intervals, respectively. Fig. (C) Shows the corresponding anatomical regions in the cortex. Further analyses will investigate the correlations between DMRI parameters (e.g. FA, MD, axial diffusivity, radial diffusivity) and quantitative histology.



Disclosures: **V. Janve:** None. **K. Schilling:** None. **Y. Gao:** None. **B.A. Landman:** None. **I. Stepniewska:** None. **A.W. Anderson:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

Location: Halls B-H

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Topic: I.03. Anatomical Methods

Support: 973 project No. 2015CB755602

NSFC Grant 91432105, 91432116, 91232000

the director fund of the WNLO

Title: A whole-brain imaging platform for fast identifying molecular phenotype in specific neural circuit

Authors: *J. YUAN, T. JIANG, B. LONG, T. XU, Q. LUO, H. GONG;
BRITTON CHANCE CENTER FOR BIOMEDICAL PHOTONICS, HUAZHONG UNIVERSITY OF SCIENCE AND TENCHNOLOGY, HUBEI, China

Abstract: Identifying cell types in specific neural circuits is crucial to understanding our brain and conquering brain diseases. As one of important cellular indications, molecular phenotype plays a vital role in classifying cell type. However, identifying molecular phenotype in specific neural circuits needs massive whole-brain screening. It's so laborious that it is urgent to achieve a technical breakthrough. Here, we propose a novel idea of fast identification of cell types in specific neural circuit: embed sample in agar, a safe argent to antigen; acquire whole-brain projection of specific neural circuit and save all slices sequentially by sectioning-based fast whole-brain optical imaging; choose slices of target regions according to imaging result and then perform immumohistochemical staining, and finally identify cell types in the circuit. So, we develop a fast whole-brain optical imaging system with automated slice collection: accelerate acquiring structural information of neural circuits and collect high-quality slices of soft samples. Using these technologies, we plan to classify molecular phenotype of type-specific neuron outputs. This study can potentially provide a routine platform for structural and functional study of specific neural circuits in the brain.

Disclosures: J. Yuan: None. **T. jiang:** None. **B. long:** None. **T. xu:** None. **Q. luo:** None. **H. gong:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Support: Arizona Biomedical Research Commission Grant ADHS14-082991

Undergraduate Biology Research Program with BIO5 Support

Title: Semiautomated quantification of Toxoplasma - CNS host cell interactions

Authors: *C. J. POTTER[1], O. A. MENDEZ[2], T. BELLO[1], M. VALDEZ[1], E. G. FERNANDEZ[1], T. P. TROUARD[3], A. A. KOSHY[4];
[2]Neurosci. GIDP, [3]Dept. of Biomed. Engin., [4]Dept. of Neurology, Dept. of Immunobiology, [1]Univ. of Arizona, Tucson, AZ

Abstract: *Toxoplasma gondii* is an obligate intracellular parasite that establishes a chronic infection in the central nervous system (CNS) of many mammals, including humans and mice. While asymptomatic in most, the persistent CNS infection can be fatal in immunocompromised patients and is present in up to a third of the world's population. Despite this prevalence, *Toxoplasma* - CNS interactions are poorly understood. Recent studies have indicated that cysts localize to specific regions of the brain, and behavioral outcomes have been connected with these localizations. In an effort to further investigate the *Toxoplasma* - CNS interaction, we took advantage of our *Toxoplasma*-Cre system, which allows us to permanently mark and identify CNS cells that have directly interacted with parasites. In this system, we infect Cre reporter mice with parasites that have been engineered to secrete a Toxoplasma:Cre recombinase fusion protein into host cells, thus only cells that have been injected with *Toxoplasma* protein will undergo Cre-mediated recombination and express ZsGreen, a fluorescent protein. This system offers a novel method to visualize specific cells that have interacted with *Toxoplasma* and investigate behavioral outcomes of this interaction. To quantify regions of interaction, we immunohistochemically label the ZsGreen positive cells (ZsG^+), then detect and map the neuroanatomic location of ZsG^+ cells with a custom MATLAB-based semiautomated computer program. To determine if ZsG^+ cell location varies between different *Toxoplasma* strains, we infected mice with either of two strains (type II or type III), and analyzed infected brain tissue at 3 and 8 weeks post-infection. Our preliminary data suggests that, in CNS infection with either strain, *Toxoplasma* parasites primarily interact with cortical neurons. This cortical preference is seen both in the absolute number of ZsG^+ cells and when normalized based on regional area to account for random distribution. Within the cortex, we found that the somatosensory, motor, and visual cortices contained the highest normalized distribution of ZsG^+ cells. These data indicate that *Toxoplasma* may have a higher affinity for specific brain regions and potentially for specific neuronal subtypes. Experiments investigating regional vascular and cell-density variations still need to be conducted to confirm these results. Future investigation using co-localization of ZsG^+ cells with neuronal subtype markers will aid in elucidating the nature of *Toxoplasma* - CNS host cell interactions, and may help us understand how *Toxoplasma* - CNS interactions affect functional outcomes.

Disclosures: **C.J. Potter:** None. **O.A. Mendez:** None. **T. Bello:** None. **M. Valdez:** None. **E.G. Fernandez:** None. **T.P. Trouard:** None. **A.A. Koshy:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Support: NIH R01 MH101178

NIH ES102805

Title: Axonal divergence of noradrenergic locus coeruleus neurons that innervate discrete terminal fields

Authors: *D. J. CHANDLER[1], N. W. PLUMMER[2], B. D. WATERHOUSE[3], P. JENSEN[2]; [1]Drexel Univ. Col. of Med., Philadelphia, PA; [2]Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [3]Dept. of Cell Biol., Rowan Univ. Sch. of Med., Stratford, NJ

Abstract: Conventional anatomical tract tracing methods, retrograde and anterograde, have been used to identify the afferent inputs and efferent projections of individual neurons within targeted brain regions. However, these traditional approaches are impractical for revealing the full extent of axon collaterals from broadly projecting neurons. The noradrenergic locus coeruleus (LC) is a compact brainstem nucleus that sends projections to the entire central nervous system. While it is clear that LC neurons send collaterals to diverse terminal fields, it has not been possible to identify all of the structures co-innervated by subsets of LC cells. Injecting multiple retrograde tracers into discrete terminal fields to identify all structures co-innervated by specific subsets of LC cells is not practical, and because of the small size and non-topographic organization of the LC, anterograde tracers cannot be targeted to specific cell types within it to determine if they project to unique terminal fields. We have previously shown that LC cells projecting to the medial prefrontal cortex (mPFC) are anatomically distinct from those that innervate primary motor cortex (M1), but it is unclear what other brain regions receive input from these neurons. We have now developed a recombinase-dependent intersectional strategy in transgenic mice to identify axon collaterals arising from cells that project to a particular terminal field. Expression of a recombinase-dependent fluorescent indicator allele, in combination with $En1^{Dre}$ and Dbh^{Flpo} recombinase driver alleles, exclusively labels LC neurons. Subsequent recombination by Cre recombinase delivered via a retrogradely transported canine adenovirus (CAV2-Cre) restricts eGFP expression to those LC neurons projecting to the virus injection site, allowing visualization of their axonal networks throughout the brain. As an initial experiment, we have injected CAV2-cre into mPFC, M1, hippocampus, or ventral tegmental area of these mice. Preliminary data show that LC cells projecting to mPFC are also the source of fibers observed in the lateral hypothalamus, septal nuclei, and piriform and parietal cortices. Labeled axons were notably absent in the hippocampus, sensory thalamus, and cerebellum, areas that receive major inputs from LC. These findings suggest that the LC contains subsets of neurons whose axonal projection networks are limited to specified terminal fields rather than indiscriminately distributed via highly divergent axon networks to randomly organized targets within the LC efferent domain.

Disclosures: D.J. Chandler: None. **N.W. Plummer:** None. **B.D. Waterhouse:** None. **P. Jensen:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Title: *Ex vivo* retrograde transport in rat cortex: a model for human cortical connectivity

Authors: *A. KSENDZOVSKY[1], A. S. TOLPYGO[2], S. WALBRIDGE[1], J. S. DIAMOND[3], D. D. FERRANTE[2], A. CUMMINS[3], J. D. HEISS[1], J. KAPUR[4], P. P. MITRA[2], K. A. ZAGHLOUL[1];
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Abstract: Introduction
There are currently no molecular techniques to visualize human cortical connectivity, which limits our understanding of cortical structure. Several *in-vivo* methods (axonal tracing) have been developed to define connectivity in animals but these have not been applied to humans due to ethical limitations. Resected human cortical tissue from epileptic patients, however, is potentially amenable to these techniques and can be used to study cortical connectivity. In this study we describe a rat model of *ex-vivo* retrograde transport (RT) used to characterize cortical connectivity. This model serves as the basis for axonal tracing in resected human cortical specimens.
Methods
Wild-type Sprague Dawley rats were sacrificed and their brains immediately harvested, cut into 4.5mm blocks and placed into carbogenated aCSF (cACSF). The motor cortex was injected with 50nL, 100nL, and 250nL of 0.1 and 1% fluorescent cholera-toxin subunit-b (CTB). The injected blocks were maintained at 25°, 30°, and 35°C on a patch-clamp stage with continuously circulating cACSF for 4 and 24hrs. Tissue blocks were analyzed for axonal transport using CLARITY and tissue sectioning with antibody amplification. *In-vivo* rat studies with the above injection parameters were used to corroborate axonal transport.
Results
Resected tissue was successfully kept alive *ex vivo*, which allowed transport of CTB to occur. CTB transport was blocked by retro-2, a RT inhibitor, confirming RT as the primary mechanism of CTB travel through the tissue. CTB was visualized in cell bodies with fluorescent microscopy while axons were elaborated using secondary antibody amplification. High injection volumes (250nL) showed non-specific cortical distribution while lower volumes (50nL) showed neurons directly connected to the injection site. Higher CTB concentration (1%) allowed for increased CTB uptake and increasing incubation time showed distal connections. The ideal stage

temperature for transport was 30°C. Z-stack registration of serial sections allowed for axonal tracing in a 3D plane. CLARITY was used to confirm axonal transport and allowed for direct visualization of intra-neuronal and intra-axonal CTB. The above parameters were recreated *in-vivo*, which showed similar axonal transport. Ideal *ex-vivo* parameters for connectivity studies were determined to be higher CTB concentrations, lower CTB volumes, 30°C, and longer aCSF incubation times.

Conclusions

In this study we present a novel *ex-vivo* method of axonal tracing in rat cortical tissue. Optimized tissue parameters were described and this method is being utilized for direct translation into human cortical specimens.

Disclosures: **A. Ksendzovsky:** None. **A.S. Tolpygo:** None. **S. Walbridge:** None. **J.S. Diamond:** None. **D.D. Ferrante:** None. **A. Cummins:** None. **J.D. Heiss:** None. **J. Kapur:** None. **P.P. Mitra:** None. **K.A. Zaghloul:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Support: NIH Grant 5R01NS092875-02

NYS SCIRP DOH01-Fellow-2015-00012

Title: Adeno-associated virus injection to rat motor cortex for fluorescent tracing of the corticospinal tract: effects of survival time and delivery method.

Authors: *H. PARK[1], J. B. CARMEL[1,2];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Dept. of Neurol. and Pediatrics and Brain Mind Res. Inst., Weil Cornell Med., New York, NY

Abstract: We aimed to improve the efficacy of corticospinal tract (CST) tracing with viruses encoding fluorescent proteins by varying the rat survival time after injection into motor cortex and also by comparing delivery by pressure injection versus iontopheresis. The CST is critical for skilled movement in people, and the CST in rodents has been used extensively to study neural plasticity.. Current anterograde tracers include the dextran amines and viruses encoding fluorescent proteins. Viruses encoding fluorescent proteins have the advantage of being able to amplify the tracer. Adeno-associated viruses (AAVs) have compared favorably to dextran

amines in mice, but delivery in rats has not been optimized, which was the goal of the current experiments. First, we tested the time that takes to get CST axons labeled in the cervical spinal cord gray matter after a single pressure injection of AAV1-EGFP or AAV1-tdTomato into motor cortex. Animals survived 4, 6, or 8 weeks after injection and were perfused and the brain and spinal cord cryosectioned for fluorescence microscopy. After 4 week, there was strong fluorescent labeling of CST neurons in the motor cortex and axons in the brain. At 6 weeks, the main CST in the spinal cord dorsal column was strongly labeled. However, axons in the cervical spinal cord gray matter were visible and countable only at 8 weeks after injection. Second, we tested current durations of iontophoretic injection to obtain the correlation between current duration and infection efficiency. We tested 5, 10, 20 and 40 min of current with the same setting of 5 microAmps current and 7 seconds on/ 7 seconds off pulses. The area of infection and number of infected neurons in the infected sections was increased in duration-dependent manner. Finally, labeled axons in the cervical spinal cord gray matter compares favorably between pressure injection and iontophoresis at 8 weeks after delivery. Thus, AAV1 viral tracing with iontophoretic injection allows tight control of the injection area and high labeling efficiency of the rat CST.

Disclosures: H. Park: None. **J.B. Carmel:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Topic: I.03. Anatomical Methods

Support: NIH Grant NS086960

Lions Eye Foundation

Research to Prevent Blindness

NIH Grant NS045855

NIH Grant NS057558

NIH Grant AG025894

Title: Mapping neocortical visual circuits by labeling neurons with unique hues using HSV-1 vectors expressing Brainbow

Authors: *A. I. GELLER, G.-R. ZHANG, O. CHAO, H. ZHAO, H. CAO, X. LI;
LSUHSC, New Orleans, LA

Abstract: A critical problem in neuroscience is elucidating the connectome. It is desirable to label specific neurons with unique tags, as mammalian brains contain many neurons. A new technology, Brainbow, labels neurons with hundreds of hues by combinatorial expression of multiple fluorescent proteins (FPs). Nonetheless, in Brainbow mice, many neurons contain the same hue, as labeled neurons far exceed the number of hues. In contrast, we labeled small numbers of neurons with unique hues, by expressing Brainbow from a HSV-1 vector.
The vector uses the vesicular glutamate transporter-1 promoter, which is glutamatergic-specific, to express a Brainbow cassette with four FPs. We used monomeric FPs with separable spectra, and favorable protein stabilities and quantum properties, enhanced blue FP-2, emerald green FP, mOrange2, and LSSmKate2 (red). Each FP was targeted to axons by the GAP-43 axon-targeting domain.
Helper-virus free HSV-1 vector packaging, with a Cre plasmid, produced arrays of Brainbow cassettes and supported Brainbow recombination. Rolling circle DNA replication produces concatamers; a HSV-1 genome-sized (~152 kb) DNA is packaged; as pVGLUT1brainbow is ~20 kb, 7 or 8 Brainbow are packaged into a vector particle. A PCR assay showed that each FP was in a position to be expressed from the VGLUT1 promoter, in different Brainbow cassettes. pVGLUT1brainbow labeled specific neurons with different, often unique, hues. Rats were sacrificed 8 days after gene transfer into postrhinal (POR) cortex. Neurons in POR cortex contained specific hues representing multiple FPs; 100 to 200 hues were observed, similar to Brainbow mice.
Also, an area that receives projections from POR cortex, perirhinal (PER) cortex, contained axons with different hues. Of note, based on hue, specific axons in PER cortex were matched to specific cell bodies in POR cortex.
We are now using HSV-Brainbow to enumerate classes of neurons in POR cortex, based on their projections to multiple neocortical areas. We previously showed that these neurons encode essential information for specific visual object discriminations. Thus, the present studies will map the circuit that encodes these discriminations.

Disclosures: **A.I. Geller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkermes Inc.. **G. Zhang:** None. **O. Chao:** None. **H. Zhao:** None. **H. Cao:** None. **X. Li:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 365.12/MMM45

Topic: I.03. Anatomical Methods

Title: Investigating small intestine neuromuscular anatomy using optical imaging

Authors: *M. AHMED, Y. BAI, J. GOMES, J. C. RAMELLA-ROMAN, R. JUNG;
Dept. of Biomed. Engin., Florida Intl. Univ., Miami, FL

Abstract: The innervation of the gut is comprised of an extrinsic (autonomic) nervous system and an intrinsic nervous system. Intestinal tract electrical stimulation can influence gastric motility. In order to target specific layers of the intestinal mucosa and understand neural control mechanisms, *in-vitro* preparations of excised rat intestine have been used. For *in-vivo* stimulation in intact preparations, novel approaches will be required to identify the neuroanatomical targets for electrode placement. We used two optical imaging approaches to identify the different layers of rat small intestine and cross-validated the images with conventional histology. Duodenum and jejunum-ileum intestine samples were obtained from euthanized male Sprague-Dawley rats weighing 300 gm and 6-8 weeks old. 10μm paraffin embedded sections were stained with Hematoxylin & Eosin (H&E) while 10μm cryostat sections were H&E and Nissl stained. Muscle layers were clearly identifiable and the thickness of muscle layers (longitudinal muscle layer and circular muscle layer) and neuron layers (myenteric plexus and submucosal plexus) was measured and compared in the duodenum and jejunum-ileum.
The same sections were also examined using a custom-made nonlinear optical microscope with a field of view of about 200 μm. This microscope combines two-photon fluorescence (where the sample is excited by low energetic photons (800 nm) and high energetic photons (520 nm) are emitted by fluorescence) and second harmonic generation (where the emission wavelength is half the excitation wavelength (800 nm)). The optical images obtained using two-photon microcopy were co-registered with the stained images. Although the optical imaging could not identify neurons in plexus layers the serosa, longitudinal and circular muscle layers can be clearly identified. From the location of the muscle layers, location of the myenteric plexus neuronal layer can be interpolated since it is sandwiched between the longitudinal and circular muscle layers providing a target for electrical stimulation.

Disclosures: M. Ahmed: None. **Y. Bai:** None. **J. Gomes:** None. **J. C. Ramella-Roman:** None. **R. Jung:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Program#/Poster#: 365.13/MMM46

Topic: I.03. Anatomical Methods

Support: Neuroscience Associates Contract for initial counts

Title: Stereology of the substantia nigra pars compacta comparing totalnumbers of tyrosine hydroxylase negative neurons to tyrosine hydroxylasepositive neurons in 3 different mouse models

Authors: *S. O. AHMAD[1], E. SCHLEIF[2];
[1]Doisy Hlth. Sciences: Office of Occup. Therapy, [2]Occup. Sci. and Occup. Therapy, St. Louis Univ., Saint Louis, MO

Abstract: This was an exercise in comparing the total number of neurons to TH+ neurons in the SNPC of different mice types. There has been quite a bit of debate of whether the total number of neurons in the SNPC is the most significant factor or TH+ neurons alone in Parkinsons disease. This looks at the proof of concept of double staining with Nissl and Tyrosene Hydroxylase. In this study, brains of mice bred with the alpha-synuclein gene were observed. With this gene, mice present with a severe motor phenotype similar to that of Parkinson's. Observations in this study were conducted on heterozygous, knockout, and wild-type mice. These mice were injected either with alpha-synuclein pre-formed fibrils or PBS control. The brains of the mice were then divided into right and left sides and neurons were counted using TH+ and Nissl+ stains. The average numbers of neurons for mice injected with alpha-synuclein PFF were 27834.5213 for heterozygous, 14287.4674 for knockout, and 11750.5014 for wild-type using the TH+ stain. Using the Nissl+ stain, the numbers were as follows for the mice injected with alpha-synuclein PFF; 8449.98565 for heterozygous, 7045.68873 for knockout, and 7352.27876 for wild-type mice. The average numbers of neurons for mice injected with PBS, the control, using the TH+ stain were 15235.4638 for heterozygous, 16207.1899 for knockout, and 14068.181 for wild-type mice. Using the Nissl+ stain on the same mice injected with PBS, the average numbers are 7677.3414 for heterozygous, 7515.281 for knockout, and 7121.8675 for wild-type mice. There was no significant difference between the number of neurons in the Nissl+ stain in the mice with alpha-synuclein PFF and the number of neurons with PBS in any of the genotypes. There also was no significant difference between the overall number of neurons found in the TH+ stain in the alpha-synuclein PFF mice and the PBS mice. However, there was a significant difference of 0.029 with a p-value of 0.05 between the alpha-synuclein PFF knockout and wild-type mice when compared to the PBS knockout and wild-type mice.

Disclosures: S.O. Ahmad: None. **E. Schleif:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

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Program#/Poster#: 365.14/MMM47

Topic: I.03. Anatomical Methods

Support: NIMH Intramural Research Program

Title: *In vivo* MRI overestimates amygdala damage following ibotenic acid lesions in rhesus monkeys

Authors: *B. M. BASILE, E. C. FIUZAT, C. L. KARASKIEWICZ, E. A. MURRAY;
Lab. of Neuropsychology, Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: Selective, fiber-sparing excitotoxic lesions are a state-of-the-art tool for determining the causal contributions of different brain areas to behavior. For nonhuman primates especially, it is advantageous to keep subjects with high-quality lesions alive and contributing to science for many years. However, this requires the ability to estimate lesion extent accurately. Previous research has shown that in vivo T2-weighted magnetic resonance imaging (MRI) accurately estimates damage following selective ibotenic acid lesions of the hippocampus. Here, we show that the same does not apply to lesions of the amygdala. Across 19 hemispheres from 13 rhesus monkeys, MRI assessment consistently overestimated actual amygdala damage as assessed by microscopic examination of Nissl-stained histological material. Two outliers suggested that near-complete MRI-estimated damage may predict actual damage of at least 45%, but more data from incomplete lesions are necessary to evaluate this hypothesis. Nevertheless, ibotenic acid injections routinely produced extensive damage (median = 82%) that correlated with total injection volume, validating the general success of the technique. The field will benefit from more research into in vivo assessment techniques, and additional evaluation of the accuracy of MRI assessment in different brain areas. For now, in vivo MRI assessment of ibotenic acid lesions of the amygdala can be used to confirm successful injections, but MRI estimates of lesion extent should be interpreted with caution.

Disclosures: **B.M. Basile:** None. **E.C. Fiuzat:** None. **C.L. Karaskiewicz:** None. **E.A. Murray:** None.

Poster

365. Anatomical Techniques: Circuit Tracing and Staining

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 365.15/MMM48

Topic: F.04. Stress and the Brain

Title: Room temperature subdissection of rodent brain possible following heat inactivation

Authors: *K. SKOLD[1], L. SEGERSTRÖM[2], M. BORÉN[1], I. NYLANDER[2];
[1]Denator AB, Uppsala, Sweden; [2]Dept. of Pharmaceut. Biosciences/Pharmacology/Neuropharmacology, Addiction & Behavior, Uppsala, Sweden

Abstract: Introduction and Objectives: Brain tissue is exceptionally susceptible to post-sampling change. Loss of blood flow and subsequent cellular signaling and enzymatic reactions cause substantial changes to levels of peptides, protein phosphorylations, metabolites and other molecules. The severity of change increase with time after sampling and the aim is normally to keep the time between sampling and snap freezing as short as possible. However when substructures of the brain needs to be isolated, the time before freezing can easily reach 20 min depending on the number of structures of interest. During this time substantial changes will have occurred, making analytical results difficult to interprets and potentially erroneous. Heat based inactivation of enzymes in whole excised rodent brains limits the post-sampling changes and enable dissection at room temperature without further post-sampling change during the dissection time. The objective of this study have been to show the possibility to sub-dissect a rodent brain after heat stabilization. Methods: Mouse brains heat stabilized and dissected into sub-regions both with and without the aid of a matrix. Levels of peptides in the sub-regions with different time after heat stabilization were measured using RIA. Results and Discussion: The excised rodent brain can be heat stabilized with minimal deformation. Subsequently it can be sub-dissected into regions of interest. RIA analysis of dynorphin levels in samples with increasing time at room temperature after heat stabilization show unchanged levels of dynorphin indicating no enzymatic activity in the samples after heat stabilization. Heat based inactivation of enzymatic activity preserve levels of analytes from post-sampling changes giving more relevant analytical results. Conclusion:The result of the present study show that sub-dissection is possible and that levels of analytes remain constant at room temperature over the time period needed to perform the sub-dissection. General enzyme inactivation using heat denaturation is a promising approach to address enzyme driven change post-sampling.

Disclosures: **K. Skold:** A. Employment/Salary (full or part-time): Head of Research. **L. Segerström:** None. **M. Borén:** A. Employment/Salary (full or part-time): Head of Development. **I. Nylander:** None.

Poster

366. Transsynaptic Tracing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 366.01/MMM49

Topic: I.03. Anatomical Methods

Title: Wiring transmission in the serotonergic system

Authors: *A. BERTERO[1,2], A. BIFONE[2], M. PASQUALETTI[1,2];
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Abstract: Serotonergic neurons are part of one of the most widely distributed systems of the mammalian brain. Indeed, serotonin is involved in a wide range of physiological processes, including the control of appetite, sleep, memory, mood, stress and sexual behavior. The raphe nuclei (B1-9) of the brain stem are the origin of serotonergic projections to the whole central nervous system. In the last years, several studies have unraveled the heterogeneity of serotonergic neurons, in terms of developmental programs, molecular and electrophysiological properties. Recently, a map of the complex topographical organization of the serotonergic fibers has been drawn using intersectional fate mapping strategy, as well as retrograde or anterograde tracing (Bang et al, 2012; Fernandez et al. 2015; Muzerelle et al, 2014). Serotonergic neurons have un-myelinated fiber varicosities, where the transmitter is synthesized, stored and released in a “volume transmission” (VT) mode (Agnati et al, 1995). To a lesser extent, serotonergic fibers also present synapse-like specializations where synaptic contacts are established by 5-HT terminals with specific neuronal targets acting in a conventional “wiring transmission” (WT) mode. However, experimental strategies used to map serotonergic projections so far where not selective for VT versus WT, and the organization of WT is still the object of investigation. Taking advantage of the properties of the rabies virus, whose envelope can drive the infection of neurons exclusively through their presynaptic terminals, we have selectively mapped the serotonergic WT system originating in the raphe nuclei. We injected recombinant G-deleted rabies virus in several brain regions of Tph2::GFP knock-in mice, in which serotonergic neurons were clearly labeled by the expression of GFP (Migliarini et al, 2013). We also used monosynaptic tracing, coupling pseudotyped recombinant rabies virus with a helper adeno-associated virus (Wall et al, 2010). This experimental approach revealed that each brain district hereby investigated receives WT from a relatively small and region-specific number of serotonergic neurons, thus making it possible to establish a correlation map between specific serotonergic neurons in the raphe nuclei and distinct brain areas. Altogether, this study sheds new light on communication properties of serotonergic system, and may help understand the selective role of serotonergic WT in health and disease.

Disclosures: **A. Bertero:** None. **A. Bifone:** None. **M. Pasqualetti:** None.

Poster

366. Transsynaptic Tracing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 366.02/DP10 (Dynamic Poster)

Topic: I.03. Anatomical Methods

Support: NIH Grant 1R01MH100635

Title: High resolution and high field diffusion MRI in the visual system of primates

Authors: ***J. R. KORENBERG**[1], O. ABDULLAH[2], L. DAI[3], J. TIPPETS[2], M. NAVAS-MORENO[4], M. BURBACK[5], A. ANGELUCCI[6], E. HSU[2], S. JOSHI[2];
[1]Brain Institute, Pediatrics, Univ. of Utah, Salt Lake Cty, UT; [2]Bioengineering, [3]Dept. of Pediatrics, Sch. of Med., [4]Brain Inst., [5]Univ. of Utah, Salt Lake City, UT; [6]Ophthalmology Res., Univ. of Utah/Moran Eye Ctr., Salt Lake City, UT

Abstract: Establishing a primate multiscale genetic brain network linking key microstructural brain components to social behavior remains an elusive goal. Diffusion MRI, which quantifies the magnitude and anisotropy of water diffusion in brain tissues, offers unparalleled opportunity to link the macroconnectome (resolution of ~0.5mm) to histological-based microconnectome at synaptic resolution. We tested the hypothesis that the simplest (and most clinically-used) reconstruction technique (known as diffusion tensor imaging, DTI) will yield similar brain connectivity patterns in the visual system (from optic chiasm to visual cortex) compared to more sophisticated and accurate reconstruction methods including diffusion spectrum imaging (DSI), q-ball imaging (QBI), and generalized q-sampling imaging. We obtained high resolution diffusion MRI data on *ex vivo* brain from Macaca fascicularis: MRI 7T, resolution 0.5 mm isotropic, 515 diffusion volumes up to b-value (aka diffusion sensitivity) of 40,000 s/mm^2 with scan time ~100 hrs. Tractography results show that despite the limited ability of DTI to resolve crossing fibers at the optic chiasm, DTI-based tracts mapped to the known projections of layers in lateral geniculate nucleus and to the primary visual cortex. The other reconstructions were superior in localized regions for resolving crossing regions. In conclusion, despite its simplifying assumptions, DTI-based fiber tractography can be used to generate accurate brain connectivity maps that conform to established neuroanatomical features in the visual system.

Disclosures: **J.R. Korenberg:** None. **O. Abdullah:** None. **L. Dai:** None. **J. Tippets:** None. **M. Navas-Moreno:** None. **M. Burback:** None. **A. Angelucci:** None. **E. Hsu:** None. **S. Joshi:** None.

Poster

366. Transsynaptic Tracing

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 366.03/MMM50

Topic: I.03. Anatomical Methods

Support: Clinical Research Fund, University Hospitals Leuven

Title: Non-human primate white matter anatomy: Klingler fiber dissection study

Authors: *T. DECRAMER[1], J. VAN LOON[2], P. JANSSEN[1], T. THEYS[2];
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Abstract: Introduction: Brain connectivity has been extensively studied in non-human primates (NHPs) using tracer techniques. Imaging studies using diffusion tensor imaging in NHPs are scarce but suggest similar white matter connections compared to the human brain. Our group recently studied commissural motor fibers in the human brain (Naets et al., 2015) and showed that leg motor connections are running in the posterior part of the corpus callosum. To our knowledge, post-mortem anatomical dissection studies of white matter tracts in the NHP brain have not been performed. In this study we discuss the course and topography of the different white matter tracts in the NHP. Methods: 8 hemispheres were dissected according to Klingler's fiber dissection technique (Klingler et al., 1935). Results: Major white matter tracts could be demonstrated using both a mesial and lateral dissection. These fibers include the corona radiata, the uncinate fasciculus, the anterior commissure, the corpus callosum, the stratum sagittale, the cingulum and the fornix. Conclusion: The major white matter tracts in NHPs were found to be similar to human white matter tract anatomy. In particular, callosal fibers connecting the primary motor cortex invariably ran through the posterior corpus callosum, which contrasts to older anatomical studies (Pandya et al., 1971).

Disclosures: T. Decramer: None. **J. van Loon:** None. **P. Janssen:** None. **T. Theys:** None.

Poster

366. Transsynaptic Tracing

Location: Halls B-H

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Program#/Poster#: 366.04/MMM51

Topic: I.03. Anatomical Methods

Support: KL2TR001103

Haggerty Foundation

Texas Institute of Brain Injury and Repair

Title: High throughput imaging of motor system connectivity in the mouse brain

Authors: *S. GOKHALE, K. POINSATTE, S. MIRZA, D. M. RAMIREZ, X. KONG, E. J. PLAUTZ, M. P. GOLDBERG;
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Abstract: Stroke results in profound alterations to architecture in the brain, particularly the corticospinal tract (CST). Some plasticity in the brain may contribute to functional recovery following ischemic injury, while other changes may be maladaptive. Studies of connectivity in the CST have been limited by standard imaging methods which do not allow for visualization and analysis of global axonal connectivity in the whole brain. We employed two novel methods of imaging: whole slide imaging and serial two-photon tomography (STPT) in order to fully visualize axonal projections to distal forelimb musculature. First, a pseudorabies viral (PRV) vector carrying green fluorescent protein (GFP) was injected into the left forelimb flexor in naïve 10-12 week-old C57 mice. PRV was transported retrogradely and transynaptically, resulting in labeled neurons in the motor cortex and other regions of interest (i.e. red nucleus, zona incerta, hypothalamus). Whole slide imaging was performed using an automated slide scanner (Nanozoomer, Hamamatsu Photonics K.K., Hamamatsu City, Japan) to produce whole slide images of serial coronal sections, allowing for visualization of multiple levels of the brain in a single slide image. In addition to whole slide imaging, STPT was performed. Brains were processed by the UT Southwestern Medical Center's Whole Brain Microscopy Facility (WBMF) using a TissueCyte 1000 imaging system (Tissue Vision, Somerville, MA). The TissueCyte uniquely performs automated sectioning and fluorescent imaging of the brain in order to produce 3-dimensional images with micron-level resolution. This machine allows for unprecedented visualization of axonal connectivity in the whole brain. We have imaged motor systems in the brains of uninjured mice using two different methods, each with distinct benefits. Whole slide imaging allows for quantification of regions of interest in the brain on a single slide, while STPT produces a highly detailed image that improves our understanding of motor systems in 3-D space. Future directions will investigate changes in connectivity following stroke injury and

during recovery, allowing for a greater understanding of the complexity of plasticity and how it contributes to beneficial and pathological circuit remodeling after injury.

Disclosures: **S. Gokhale:** None. **K. Poinsatte:** None. **S. Mirza:** None. **D.M. Ramirez:** None. **X. Kong:** None. **E.J. Plautz:** None. **M.P. Goldberg:** None.

Poster

366. Transsynaptic Tracing

Location: Halls B-H

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Program#/Poster#: 366.05/MMM52

Topic: I.03. Anatomical Methods

Support: NSF EAGER 1547967

NIH U01 NS094288

Title: An improved viral method to overcome viral tropisms for retrograde labelling of neurons

Authors: ***S.-J. LI**, A. VAUGHAN, A. KEPECS;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Deciphering how neural circuits are anatomically organized is crucial in understanding how the brain processes information. A number of different retrograde viral strategies have been used to understand the architectural principles of connectivity between different brain regions. Compared to classical retrograde tracers, such as the Fluoro-Gold, retrograde viral vectors have the advantage of encoding flexible and relative large cargos within their genome, enabling the combination of anatomical analysis with genetic labelling, optogenetic tagging and recording. Unfortunately, all existing retrograde viruses are limited by their tropism, which make viruses fail to infect some cell types due to the lack of receptors on cell surface for virus internalization and axonal transport. This variable tropism presents a serious problem for viral technologies to reveal the ground truth in connectivity.
Here we developed a novel receptor complementation strategy to overcome tropisms of a commonly used retrograde virus, canine adenovirus type 2 (CAV-2). We designed an AAV construct that expresses the coxsackievirus and adenovirus receptor (CAR), a conserved cell adhesion molecule facilitating the internalization and axonal transport of CAV-2. The expression of CAR greatly increased the ability of neurons to take up CAV-2 and enhanced the overall retrograde labelling rate. Both human and mouse homologs of CAR were able to promote the infection rates of CAV2, indicating a conserved function. These effects were also tested in multiple long-range projecting neural circuits in mice and rats. We also combined the advantage

of CAR complementation with multiple CRE-dependent optogetic tools such as ChR2, making it a useful tool for anatomical labelling of neurons during recording.
Taken together, these results present a novel strategy to minimize the effects of viral tropism, improve on existing retrograde tracing techniques and provide reagents for the optogenetic control of specific projection-neurons.

Disclosures: S. Li: None. **A. Vaughan:** None. **A. Kepecs:** None.

Poster

366. Transsynaptic Tracing

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Topic: I.03. Anatomical Methods

Support: NIH Grant P01NS057228

Title: Transgenic expression of rabies glycoprotein in mouse hind limb muscle increases the efficiency of motor pool infection by SADB19dG rabies virus

Authors: *L. GOMEZ PEREZ, R. W. GRIFFITH, F. J. ALVAREZ;
Physiol., Emory Univ., Atlanta, GA

Abstract: Monosynaptic retrograde tracing of neural connections using modified rabies viruses (RV) has become a widely used tool by neuroscientists, particularly the SADB19dG attenuated RV in which the gene for the glycoprotein (RV-G) necessary for transsynaptic infection has been replaced by a fluorescent protein (FP) gene (mCherry or EGFP). Therefore only neurons monosynaptically connected to "starter" pools of neurons expressing SADB19dG trans-complemented with RV-G are specifically labeled with FPs. In the case of mapping premotor circuits in the spinal cord, SADB19dG is injected into specific muscles and is taken up by motor neurons (MN) innervating these muscles. From these MN RV transsynaptically infects presynaptic interneurons if MN also express RV-G. RV-G expression is induced in MN by either adeno-associated virus (AAV1-G) transfection or using cre-lox conditional expression in transgenic mice. One problem is the low number of "starter" MN infected when injections are kept small to restrict labeling to just one specific muscle. In addition, the number of infected MN decreases further when moving from neonates to adults. Particularly low numbers are obtained when only SADB19dG RV is injected in muscle. We found that animals receiving co-injections or sequential injections of a SADB19dG-mCherry and AAV1-G into the mouse lateral gastrocnemius (LG) had 2.5 - 4-fold more mCherry labeled MN than animals injected with SADB19dG-mCherry alone, even when RV-G was highly expressed in MN in Chat-cre mice

carrying cre-dependent RV-G expressing alleles (Chat-cre; RGTV mice). This led us to investigate whether muscle infection and production of G-coated RV particles by muscle is necessary for effective infection of large numbers of MN. We first confirmed that both AAV1 and RV SADB19dG effectively infect muscle allowing trans-complementation of SADB19dG with RV-G inside muscle cells after co-injections. Then we tested animals in which cre is driven by the human alpha-skeletal actin (ACTA1) promoter to direct RV-G expression specifically to muscle and found a 3-fold increase in labeled spinal MN after SADB19dG-mCherry injection into the LG. Furthermore, we used AAV1-EGFP viruses to confirm that the increased labeling efficiency after virus co-injection is not due to cooperative effects between RVs and AAVs. These data demonstrate improved number of "starter" MN by expressing G-coated SADB19dG in the muscle. In essence muscle becomes a source of G-coated RV virus that transsynaptically enters the MN pool. In this situation lower titers of SADB19dG can be injected in muscle with little effect on the final number of mCherry-labeled MN.

Disclosures: **L. Gomez Perez:** None. **R.W. Griffith:** None. **F.J. Alvarez:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.01/MMM54

Topic: I.03. Anatomical Methods

Support: This work was supported by the National Institute on Drug Abuse Intramural Research Program

Title: Development of methods for longitudinal imaging of DREADDs *In vivo*

Authors: *J. L. GOMEZ, L. A. RODRIGUEZ, R. ELLIS, M. MICHAELIDES;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Chemogenetics comprises an emerging neuromodulation technology which relies on in vivo administration of selective pharmacological actuators that can remotely and transiently modulate mutated receptors expressed in specific cell types thus leading to changes in cell signaling and corresponding changes in behavior and physiology. The most widely used chemogenetic system is called DREADD (Designer Receptor Exclusively Activated by Designer Drug) which uses the actuator clozapine n-oxide (CNO) and the DREADD receptors hM3Dq, hM4Di and others. At present there are numerous studies showing that DREADDs can alter behaviors associated with many types of disease states. However, there is a lack of information about how exactly CNO engages DREADDs in vivo. To examine this we are developing

radiometric methods for imaging engagement of DREADDs in vitro, and in vivo via the use of positron emission tomography (PET). Our goal is to map distribution and kinetics of CNO in vivo, develop occupancy assays for screening novel DREADD actuators in vivo, and to be able to non-invasively track longitudinal expression of DREADDs in experimental subjects. We expect that the findings from this work will be relevant not only for rodent DREADD studies but particularly for non-human primates studies using DREADDs where expression of the specific DREADD can be determined non-invasively.

Disclosures: J.L. Gomez: None. **L.A. Rodriguez:** None. **R. Ellis:** None. **M. Michaelides:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

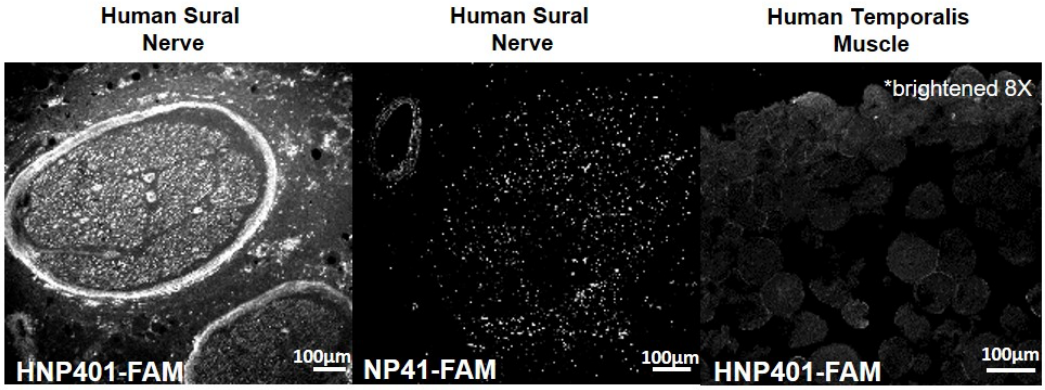
Support: NIH Grant EB014929

Title: Novel human nerve binding peptides for fluorescence guided surgery.

Authors: *D. V. HINGORANI;
Surgery, Univ. of California San Diego, LA Jolla, CA

Abstract: Introduction: Surgical instrumentation has advanced with the now routine use of laparoscopic, endoscopic and robotic techniques. However, surgeons still typically use standard white light reflectance illumination to visualize the surgical field despite emerging efforts with fluorescence contrast agents [1]. In reflectance, nerves can be particularly difficult to distinguish from adjacent tissue, which can result in inadvertent injury. To generate novel fluorescent probes for nerve detection, we previously employed phage display technology to find peptides that could fluorescently label rodent peripheral nerve [2]. Here we ask if phage display methods can also identify peptides that specifically target human nerve, as this would have greater translational value. Methods: Phage selection identified peptides that bind human nerves *in vitro*. Fluorescently tagged versions of the nerve-screened peptides were applied to flash frozen and unfixed tissue sections of human and rodent nerve. Results: In tissue sections of human nerve, we compared the binding of our most promising new peptide, HNP401 to that of the original rodent-nerve targeted peptide, NP41. HNP401-FAM produced prominent fluorescent labeling of human sural nerve compared to topically applied NP41-FAM. HNP401-FAM binding in human temporalis muscle were much dimmer (see Figure). We seek to characterize whether HNP401

can light up autonomic innervation in the genitourinary system including the cavernosal nerves, which can be damaged during prostatectomies. Conclusions: HNP401 is a novel peptide with improved binding to human nerve relative to our original phage-screened peptide NP41. 1. Nguyen QT, Tsien RY. Nat Rev Cancer, 2013, 13(9), p653-62. 2. Whitney MA, et al. Nat Biotechnol, 2011, 29(4), p352-6



Disclosures: D.V. Hingorani: None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.03/MMM56

Topic: I.03. Anatomical Methods

Support: DARPA Grant N66001-11-1-4013

Title: Characterization of a transgenic mouse expressing fluorophores in neurons, microglia, astrocytes, and oligodendrocytes

Authors: J. GAIRE[1], H. C. LEE[2], S. CURRLIN[1], *K. J. OTTO[1];
[1]Univ. of Florida, Gainesville, FL; [2]Purdue Univ., West Lafayette, IN

Abstract: Whole brain imaging has been a hot topic for past 4-5 years. The ability to visualize deeper into the brain and other organs to glean more information is imperative to understand the structural and molecular details. Optical visualization of whole brains and thicker tissues has become possible especially with the recent advances in tissue clearing techniques. Various clearing techniques use refractive index matching solvents to remove the lipids, facilitating optical clarity and antibody penetration. Regardless of the type of clearing method used, the

sample has to be immunostained with antibodies of interest for fluorescent imaging. Immunostaining especially in thick tissues has many drawbacks which include: limited penetration depth and the need for exposure to high temperature and strong detergent concentrations. To address this, especially for nervous tissue, we developed a transgenic mouse model expressing fluorophores in four major brain cell types. We crossed mice expressing green fluorescent protein (488 nm) in microglia and Prism mice expressing DsRed (543 nm) in astrocytes, yellow fluorescent protein (514 nm) in neurons, and cerulean fluorescent protein (458 nm) in oligodendrocytes . These mice were purchased from Jackson Laboratories and a colony of each mouse line was established. These two lines of mice were then crossed to generate a transgenic line that express fluorophores in microglia, astrocytes, neurons and oligodendrocytes. Genotypically validated mice from different litters were confirmed by evaluating phenotypic expression in brain slices using confocal microscopy. Sequential imaging using different laser lines was utilized to avoid cross talk between closely spaced fluorophores. Furthermore, co-localization experiments carried out using anti-Iba1 for microglia, anti-MBP for oligodendrocytes, anti-NeuN for neurons, and anti-GFAP for astrocytes followed by respective Alexa-fluor conjugate 633 confirmed that the fluorophores in transgenic mice were expressed in the correct cell types. Experiments investigating the presence of fluorophores in peripheral organs utilizing various clearing techniques and high resolution microscopy are also being conducted. In addition, we are utilizing this model system to study the cellular response to brain implanted devices both *in situ* and *in vivo*. This novel transgenic mouse model in tandem with high resolution *in vivo* imaging techniques will help to further our understanding of the interplay of different cell types in normal and diseased states.

References:
[1] Jung S et al., MCB, June 2000, p. 4106-4114.
[2] Dougherty JD et al., PLoS ONE 7(7), 2012

Disclosures: **J. Gaire:** None. **H.C. Lee:** None. **S. Currlin:** None. **K.J. Otto:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.04/MMM57

Topic: I.03. Anatomical Methods

Support: NIH Grant R21 NS092019

Kaufman Foundation

Title: Fluorogen-activating peptide tagged neuroligin (FAP-NL), a powerful new tool for high-throughput synapse identification and connectomics

Authors: *D. A. KULJIS[1], M. T. MATSUSHITA[1], T. A. SPIX[1], C. A. TELMER[1,2,3], M. P. BRUCHEZ[1,2,3], A. L. BARTH[1];
[1]Dept. of Biol. Sci., [2]Dept. of Chem., [3]Mol. Biosensors and Imaging Ctr., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The formation and maintenance of synaptic connections is fundamental to the function of neuronal networks in the brain. High-throughput methods for mapping these connections will be essential for advancing our understanding of brain function and dysfunction, but efforts in this field have had limited success. To address shortcomings of current fluorescence-based imaging approaches for synapse visualization, we developed a new technology using genetically-encoded, fluorogen activating peptide (FAP)-tagged neuroligin (NL; FAP-NL). Advantages of our approach include unprecedented signal-to-noise, fluorescence in a far-red channel distinct from commonly used fluorophores (ie. CFP, GFP, YFP, RFPs), and single-step sample preparation for use in fixed or live tissue. Viral transduction of FAP-NL in somatosensory cortex sparsely labels multiple neuronal cell types, and application of malachite green (MG) derivative dyes produces punctate fluorescent signal on spines and shafts of transduced cells consistent with synaptic localization. Our initial characterization focuses on structural and functional analysis of pyramidal and GABAergic neurons of rodent somatosensory cortex. Utilizing 3D reconstructions of transfected neurons, we evaluated synapse densities and FAP-NL colocalization with synaptic markers. Whole-cell patch clamp recordings were performed to evaluate whether FAP-NL expression alters functional parameters of synaptic connectivity. Compared to untransfected cells, no significant differences in miniature excitatory and inhibitory postsynaptic current frequencies and amplitudes were detected. Overall, FAP-NL proves to be a powerful tool for quantitative synaptic visualization in brain tissue that does not appear to alter synaptic communication. Used in combination with cell type-specific reporter lines, this method will enable high-throughput automated analysis of synapse density, distribution, and input-specific synaptic partners for molecularly distinct neurons.

Disclosures: D.A. Kuljis: None. **M.T. Matsushita:** None. **T.A. Spix:** None. **C.A. Telmer:** None. **M.P. Bruchez:** None. **A.L. Barth:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Topic: I.03. Anatomical Methods

Support: JSPS KAKENHI Grant Number 15K09904

Title: Development of a PET probe targeting P2X7 receptor for imaging neuroinflammation

Authors: *M. SHUKURI[1,2], K. KATO[2], T. KUMAMOTO[3], N. IHARA[4,2], T. HANAKAWA[2]; [1]Lab. of Physical Chem., Showa Pharmaceut. Univ., Machida,Tokyo, Japan; [2]Dept. of Advanced Neuroimaging, Integrative Brain Imaging Ctr., Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Tokyo, Japan; [3]Dept. of Pharm., Musashino Univ., Nishitokyo, Tokyo, Japan; [4]Grad. Sch. of Pharmaceut. Sci., Univ. of Tokyo, Tokyo, Japan

Abstract: P2X7 purinoreceptor (P2X7R) is sensitive to extracellular adenosine triphosphate (ATP) and plays an important role in inflammatory processes, including neuroinflammation and pain sensation. Actually, upregulation of P2X7R has been reported in a variety of neurological and neurodegenerative diseases such as Alzheimer's disease, spinal cord injury, and sensory neuropathy. Thus, P2X7R is a potential target for the diagnosis and therapy for those pathological neuroinflammatory processes. Positron emission tomography (PET) may provide a noninvasive *in vivo* imaging method to monitor P2X7R expression and/or activity, and could become a valuable tool for investigating the role of the P2X7R in the neuropathological conditions. Although there are several reports on radioligands based on P2X7R inhibitors, *in vivo* detection of activated P2X7R in neuroinflammation has not yet been successful [1, 2)]. In the present study, we synthesized ^{11}C labeled pyroglutamic acid amide derivatives (PGAA1 and PGAA2 by GSK) as P2X7R antagonists [3)]. To evaluate the usefulness of PET probes based on PGAA1 and PGAA2 as imaging agents for detecting neuroinflammation, we performed PET and *ex vivo* autoradiography (ARG) studies in rats treated with intrastriatal injection of lipopolysaccharide (LPS). In the PET studies, we observed increased radioactivity of ^{11}C-PGAA1 and ^{11}C-PGAA2 in the treated hemisphere on the day 3 after LPS injection. Kinetic analysis of PET data revealed that brain uptakes of ^{11}C-PGAA1 were better than those of ^{11}C-PGAA2, but the uptake values of ^{11}C-PGAA1 were not so high from a quantitative point of view. In contrast, the *ex vivo* ARG study clearly revealed increased accumulation of both ^{11}C-PGAA1 and ^{11}C-PGAA2 in the treated hemisphere. In a confirmatory immunohistochemical study, we found the activated microglia expressing increment of P2X7R in the brain regions where ^{11}C-PGAA1 and ^{11}C-PGAA2 showed high radioactivity. These results suggest the possibility of P2X7R as an imaging target specific to activated microglia during neuroinflammatory processes. Although further studies to improve brain uptake are required, ^{11}C labeled pyroglutamic acid amide derivatives showed some adequate properties for imaging P2X7R in neuroinflammation with PET.

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3) Abdi, M. H. et. al. Bioorg Med Chem Lett. 20(17):5080-5084, 2010.

Disclosures: **M. Shukuri:** None. **K. Kato:** None. **T. Kumamoto:** None. **N. Ihara:** None. **T. Hanakawa:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.06/MMM59

Topic: I.03. Anatomical Methods

Support: R24 NS092986

R01 EB018464

R01 NS091230

R01 EB021018

R01 CA194596

Title: High-performance probes for two-photon phosphorescence lifetime microscopy (2PLM) of oxygen

Authors: *S. VINOGRADOV[1], T. ESIPOVA[2], M. BARRETT[3], B. WEBER[3];
[1]Dept. of Biochem. and Biophysics, [2]Biochem. and Biophysics, Univ. of Pennsylvania, Philadelphia, PA; [3]Inst. of Pharmacol. & Toxicology, Univ. of Zurich, Zurich, Switzerland

Abstract: The ability to quantify oxygen *in vivo* in 3D with high spatial and temporal resolution is much needed in many areas of biological research. Our laboratory has been developing the *phosphorescence quenching* technique for biological oximetry - an optical method that possesses intrinsic microscopic capability. In the past we have developed dendritically protected oxygen probes for quantitative imaging of oxygen in tissue. More recently we expanded our design on special two-photon enhanced phosphorescent probes. These molecules brought about first demonstrations of the two-photon phosphorescence lifetime microscopy (2PLM) of oxygen *in vivo*, providing new information for neouroscience and stem cell biology. However, current two-photon oxygen probes suffer from a number of limitations, such as sub-optimal brightness and high cost of synthesis, which dramatically reduce imaging performance and limit usability of the method. In this paper we address the principles of 2PLM, probe chemistry, photophysics and their connection with imaging speed and resolution. We then present a new approach to brightly phosporescent chromophores with internally enhanced two-photon absorption cross-sections, which pave a way to a new generation of 2PLM probes.

Disclosures: **S. Vinogradov:** None. **T. Esipova:** None. **M. Barrett:** None. **B. Weber:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Program#/Poster#: 367.07/MMM60

Topic: I.03. Anatomical Methods

Support: NIH Grant NS093866

NIH Grant DC014858

Title: Identification and characterization of hippocampal neurons that encode novel environments using a genetically-encoded optical voltage sensor

Authors: *Y. MA[1], P. O. BAYGUINOV[2], M. B. JACKSON[2];
[1]Physiol. Grad. Training Program, [2]Neurosci., Univ. of Wisconsin Madison, Madison, WI

Abstract: The hippocampus plays an essential role in encoding and storing information about the environment. The encoding is thought to entail activation of specific subsets of neurons, and the storage is thought to entail strengthening of synapses between the encoding cells. This study aims to identify the neurons in the hippocampus that are activated by novel environments, and to investigate the changes in their excitability and functional connectivity. We utilized a genetically-encoded hybrid Voltage Sensor (hVOS) to label recently activated neurons, and to image their electrical activity. hVOS employs a fluorescent protein tethered to the cell membrane, and an anionic small molecule, dipicrylamine (DPA). Membrane potential changes alter the distance between DPA and the fluorescent protein, leading to changes in FRET between these two components and transducing voltage to fluorescence intensity. Using *Cre*-lox technology, we expressed hVOS probe under the control of a tamoxifen (TAM)-inducible immediate early gene promoter, *c-fos*. With TAM injection, exposure to a novel environment led to hVOS probe labeling of neurons active in that environment. Hippocampal slices from these animals showed sparse labeling of granule cells and CA1 pyramidal cells. We imaged responses of the labeled granule cells and CA1 pyramidal cells evoked by electrical stimulation, and analyzed signal propagation in subcellular compartments of these neurons. Our results revealed the functional activity within hippocampal neuronal populations that were activated by a novel environment. The hVOS probe targeting and imaging strategy developed in this study has broad application in the investigation of neurons active during experience and defined by unique sensory inputs. Funding: NIH grants NS093866 and DC014858.

Disclosures: **Y. Ma:** None. **P.O. Bayguinov:** None. **M.B. Jackson:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.08/MMM61

Topic: I.03. Anatomical Methods

Support: NIH grant NS093866

NIH grant DC014858

Title: Functional innervation of hilar mossy cells revealed using a genetically-encoded hybrid optical voltage sensor

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Abstract: Hilar mossy cells of the dentate gyrus (DG) innervate both granule cells and interneurons which inhibit granule cells. Understanding how the distinct circuits interact and how specific cell types function as a unit is paramount in elucidating the function of the dentate gyrus. Circuit anomalies have been implicated in mossy cell dysfunction, which may contribute to disease states such as Alzheimer's disease and epilepsy. Imaging voltage changes in targeted populations of neurons with a genetically-encoded voltage sensor offers a powerful approach to the study of neural circuits. One such voltage-sensing probe is the hybrid optical voltage sensor (hVOS), which utilizes a genetically-encoded fluorescent protein and an anionic hydrophilic molecule (dipicrylamine) that interact via FRET to report voltage changes with sub-millisecond response times. To investigate the spatiotemporal dynamics of mossy cell activation we have used a *Cre* reporter line of mice expressing hVOS, and have crossed it with *Cre* drivers for calbindin 2 (calretinin) and calcitonin receptor-like receptor, two specific markers of hilar mossy cells. Morphological analyses revealed hVOS expression in cells in the hilus with 2-4 proximal mossy dendrites. Electrical stimulation of the perforant path evoked hVOS responses in the hilus and the inner molecular layer (IML). Temporal analysis revealed initial responses occurred deep within the hilus, close to the pyramidal cell layer (PCL) of the CA3 region. From there a depolarizing wavefront propagated outward through the hilus and on to the IML. Mossy cells are also known to be innervated by the pyramidal cells of the CA3 region. Electrical stimulation of the PCL and fimbria generated responses that could be detected in individual hilar mossy cells, and the responses were substantially larger in mossy cells of the infrapyramidal blade of the DG. This is consistent with previous reports that have suggested that the lower blade may have stronger hilar innervation. By targeting a genetically-encoded voltage sensor to hilar mossy cells, these imaging studies confirmed the established circuitry in the DG, and offer the possibility of exploring the spatiotemporal dynamics of activation of specific cell types in the DG network. Funded by NIH grants NS093866 and DC014858.

Disclosures: **P. Bayguinov:** None. **Y. Ma:** None. **M.B. Jackson:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.09/MMM62

Topic: I.03. Anatomical Methods

Support: Wellcome Trust Grant 108726

Title: Brain cell type mapping by *In situ* sequencing

Authors: ***X. QIAN**[1], T. HAULING[1], L. MAGNO[2], A. MUÑOZ MANCHADO[3], P. LÖNNERBERG[3], N. SKENE[3], M. PACHITARIU[2], N. KESSARIS[2], S. LINNARSSON[3], J. HJERLING-LEFFLER[3], K. D. HARRIS[2], M. NILSSON[1];
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Abstract: The brain is composed of phenotypically heterogeneous cell types and such heterogeneity is important for its function. With recent advances in single-cell sequencing, an increasing number of molecularly defined cell types are being discovered and described. However in this approach, information about the original tissue context and spatial location of a cell is lost. Here, we demonstrate how *in situ* sequencing (1) can be used to build a cell type map of the brain.
In situ sequencing enables highly multiplexed RNA expression profiling directly on whole tissue sections, with up to subcellular resolution. By using barcoded padlock probes and rolling circle amplification, it generates amplicons containing the probe sequence *in situ* and sequences the barcodes with sequencing by ligation chemistry, using widefield fluorescence imaging as readout. With our error-detecting scheme designed to minimize false positives arising from sequencing error, four-base barcodes can encode up to 64 transcripts. The whole process requires little hands-on time and takes 1-2 weeks depending on tissue size, which directly affects the imaging time.
We selected well-known markers for cell types in mouse brain together with novel markers derived from single-cell RNA-sequencing data (2). 10um-thick fresh-frozen brain tissue sections were used to perform *in situ* sequencing. The spatial distribution of individual markers matched the *in situ* hybridization images from Allen Brain Atlas in most cases. Using unsupervised clustering, we could recapitulate the cell types described in single-cell RNA-sequencing, validating once again the *in situ* sequencing data. In addition, NeuN staining was performed on *in situ* sequenced sections to help better define cell boundaries.

In situ sequencing is scalable in the number of cells and targets analyzed, thus making it an ideal tool for high-throughput cell type mapping. The feasibility to perform immunofluorescence or hematoxylin and eosin (H&E) staining afterwards not only adds versatility to the method but also allows straightforward validation.

1. Ke *et al.*, Nature Methods 10:857-60, 2013.
2. Zeisel *et al.*, Science 347:1138-42, 2015.

Disclosures: **X. Qian:** None. **T. Hauling:** None. **L. Magno:** None. **A. Muñoz Manchado:** None. **P. Lönnerberg:** None. **N. Skene:** None. **M. Pachitariu:** None. **N. Kessaris:** None. **S. Linnarsson:** None. **J. Hjerling-Leffler:** None. **K.D. Harris:** None. **M. Nilsson:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Program#/Poster#: 367.10/NNN1

Topic: I.03. Anatomical Methods

Support: Wellcome Trust Grant 108726

Title: Molecular classification of CA1 interneurons by single-cell and in-situ RNA sequencing

Authors: ***K. D. HARRIS**[1], X. QIAN[2], T. HAULING[2], L. MAGNO[1], P. LONNERBERG[3], A. MUNOZ MANCHADO[3], N. SKENE[3], M. PACHITARIU[1], N. KESSARIS[1], S. LINNARSSON[3], J. HJERLING-LEFFLER[3], M. NILSSON[2];
[1]Univ. Col. London, London, United Kingdom; [2]SciLifeLab, Stockholm, Sweden; [3]Karolinska Institutet, Stockholm, Sweden

Abstract: GABAergic interneurons are key regulators of hippocampal circuits, but our understanding of the diversity and classification of these cells remains controversial. Interneurons are distinguished by molecular markers, but single molecules are not sufficient to identify individual cell classes, which requires combinatorial analysis of multiple markers simultaneously.
To understand the molecular organization of CA1 area interneurons, we applied two new methods that are capable of characterizing large numbers of genes simultaneously: single-cell RNA sequencing (1), which can characterize RNA expression across the entire genome of dissociated cells, but cannot identify their spatial location in tissue prior to dissociation; and in situ RNA sequencing using the padlock probe method (2), which can provide subcellular spatial localization of individual RNA molecules, but is limited to identification of tens, rather than thousands of genes.

Applying a novel clustering algorithm to a single-cell RNA-seq database of CA1 interneurons revealed a hierarchy of cell types (3), including a novel cells class that we term CGE-*Reln* cells identified by combinatorial expression of several markers including *Cxcl14, Rgs12, Cpne5*, and *Reln.* The classification also made several other predictions, such as the expression of *Npy* in *Cck*+ interneurons. Analysis using immunohistochemistry and *in situ* hybridization confirmed these predictions, and analysis in Lhx6-Cre mice confirmed the novel class of interneurons is not derived from MGE.
To further test these predictions we applied *in situ* sequencing to slices of mouse hippocampus. Padlock probes were designed to target genes that were identified by single-cell sequencing as having strongly divergent expression between cell classes. This analysis confirmed the major predictions of the single-cell classification, and identified the likely laminar locations for the major cell classes it identified.
1. Zeisel et al, Science 347:1138-42, 2015
2. Ke et al, Nature Methods 10:857-60, 2013
3. Harris et al, bioRxiv http://biorxiv.org/content/early/2015/12/16/034595, 2015

Disclosures: **K.D. Harris:** None. **X. Qian:** None. **T. Hauling:** None. **L. Magno:** None. **P. Lonnerberg:** None. **A. Munoz Manchado:** None. **N. Skene:** None. **M. Pachitariu:** None. **N. Kessaris:** None. **S. Linnarsson:** None. **J. Hjerling-Leffler:** None. **M. Nilsson:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Brain Research Foundation BRF-SIA-2014-03

Simons Foundation 382793/SIMONS

PhD felowship from the Boehringer Ingelheim Fonds

PhD fellowship from the Genentch Foundation

Title: Mapping brain-wide corticocortical projections at single-cell resolution by sequencing of barcoded RNA

Authors: *L. HUANG, J. M. KEBSCHULL, A. M. ZADOR;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: In the mammalian neocortex, excitatory neurons in different layers send long-range axonal projections to different cortical and sub-cortical targets. These distinct patterns may function to route different streams of information to appropriate targets. Even within a given cortical layer different neuronal classes may have characteristic projection patterns, but it is not known how many distinct projection classes there are. To date, there has been no comprehensive survey of the long-range connectivity of the mammalian cortex at single neuron resolution. We have recently developed MAPseq (Multiplexed Analysis of Projections by sequencing), a method that exploits high-throughput DNA sequencing for efficiently mapping the inter-areal projections of many individual neurons at single cell resolution. In MAPseq, we uniquely label each neuron in a population with a random RNA sequence ("barcode") by infecting with a viral library. The virus also encodes a protein engineered to transport the barcode to the presynaptic terminals. We then dissect projection targets of interest, extract the barcode RNA from each target, and quantify the abundance of each barcode sequence present in each area. The abundance of a particular barcode sequence in each target represents a measure of the strength of the projection of the barcoded neuron to the target. We are now scaling up MAPseq to label neurons distributed throughout an entire cortical hemisphere, and thereby determine the full cortico-cortical projection map of an individual mouse in a single experiment. After infecting the neurons in one cortical hemisphere with a MAPseq barcode viral library, we dissect the cortex into several hundred ~1 mm x 1 mm x 300 m cuboids. We then sequence the barcodes in each cuboid and determine the location as well as the projection targets of each neuron. This brain-wide MAPseq has the potential to uncover the entire cortico-cortical projection structure at single cell resolution, and will provide insight into the principles that underlie the organization of cortical circuits.

Disclosures: L. Huang: None. **J.M. Kebschull:** None. **A.M. Zador:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Simons Foundation 382793/SIMONS

PhD felowship from the Boehringer Ingelheim Fonds

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Title: High-throughput mapping of single neuron projections by sequencing of barcoded RNA

Authors: *J. M. KEBSCHULL, L. HUANG, P. GARCIA DA SILVA, A. P. REID, I. D. PEIKON, D. F. ALBEANU, A. M. ZADOR;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Long-range connections have been mapped out systematically in the mouse by large-scale efforts such as the Allen Mouse Brain Connectivity Atlas. However, these brain-wide studies rely on bulk labeling techniques only, and are therefore blind to the diversity of single neuron projections arising from intermingled heterogeneous populations critically limiting the insight that can be gained into the brain's computations.
We have recently developed MAPseq (Multiplexed Analysis of Projections by sequencing), a novel approach to map single neuron projection patterns throughout the brain in high throughput. Using MAPseq, we can map the projections of thousands and potentially millions of individual neurons in one or many brain areas per animal in under seven days. To do so, we virally introduce random nucleotide sequences ("barcodes") into neurons to convert projection-mapping into a form that exploits high-throughput sequencing. With this method, the complexity and throughput of single neuron tracing becomes comparable to that of conventional bulk mapping techniques.
In a proof-of-principle experiment, we applied MAPseq to locus coeruleus (LC), a small noradrenergic nucleus that projects throughout the brain. Our single neuron resolution analysis uncovers idiosyncratic projection patterns for individual LC neurons with strong innervation of one or more preferred targets in cortex or olfactory bulb and weak but broad innervation of the rest of cortex. Our data thus reconcile seemingly conflicting data in the literature, and open the possibility of differential control of LC over cortical areas.
MAPseq is not limited to tracing projections from one area, but scales to map the projections of neurons from many brain areas in a single animal. Currently, we are using MAPseq to determine the entire cortico-cortical mesoscale connectome at single cell resolution in a single experiment. We envision that, in the future, MAPseq will be used to obtain a whole brain projection map at single neuron resolution from a single mouse.

Disclosures: **J.M. Kebschull:** None. **L. Huang:** None. **P. Garcia da Silva:** None. **A.P. Reid:** None. **I.D. Peikon:** None. **D.F. Albeanu:** None. **A.M. Zador:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.13/NNN4

Topic: I.03. Anatomical Methods

Support: Eunice Kennedy Shriver NICHD intramural research program

Title: Cellular analysis of ErbB4 isoforms in CNS neurons using a next generation *In situ* hybridization technology for single exon detection

Authors: *L. M. ERBEN[1,2], M.-X. HE[3], M. XIAO-MING[3], E. PARK[3], A. BUONANNO[1]; [1]Section on Mol. Neurobiology, NICHD, NIH, Bethesda, MD; [2]Inst. of Mol. Psychiatry, Univ. of Bonn, Bonn, Germany; [3]Advanced Cell Diagnostics, Newark, CA

Abstract: The Neuregulins, a family of neuronal factors characterized by an EGF-like domain, and its cognate neuronal receptor ErbB4 have been associated with a risk for schizophrenia and different types of cancers. Four distinct ErbB4 isoforms generated by alternate splicing at two loci, which regulate receptor proteolytic processing and downstream signaling, have been identified. In its extracellular juxtamembrane region alternatively splicing of single exons results in JMa (69 bp) and JMb (39 bp) variants, whereas differential inclusion or exclusion of a 48 bp exon mapping to the receptor cytoplasmic tail gives rise to ErbB4 Cyt-1 or Cyt-2 isoforms, respectively. Of note, the ratio of JMa/JMb and Cyt-1/Cyt-2 ErbB4 transcripts have been reported to be altered in postmortem brains of schizophrenia patients, as well as in different brain cancers (e.g. medulloblastoma, astrocytic glioma). In order to analyze at a single-neuron level the expression pattern of distinct ErbB4 JMa/JMb and Cyt-1/Cyt-2 variants, we resorted to the use of a next-generation RNAscope *in situ* hybridization technique. As shown here, we find that the next-generation RNAscope, which utilizes a single "ZZ probe", provides sufficient sensitivity to visualize ErbB4 transcripts that vary by a single exon. The design of ZZ probes that map onto exon-to-exon boundaries circumvent potential hybridization to genomic DNA. After proving the specificity of the system by using single exon mutant mice, we analyzed the distribution of ErbB4 isoforms in the mouse brain by using either chromogenic DAB staining or a FastRed dye that allows for signal detection using light or fluorescence microscopy.

Disclosures: **L.M. Erben:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Probes were provided by ACD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACD holds the patent. **M. He:** A. Employment/Salary (full or part-time): Employed by ACD. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Probes were provided in kind by ACD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

ACD hold the patent. **M. Xiao-Ming:** A. Employment/Salary (full or part-time): Employed by ACD. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Probes were provided in kind by ACD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACD holds the patent. **E. Park:** A. Employment/Salary (full or part-time): employed by ACD. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Probes were provided in kind by ACD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACD holds the patent. **A. Buonanno:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Probes were provided in kind by ACD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACD holds the patent.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.14/NNN5

Topic: I.03. Anatomical Methods

Title: Vascular neuroanatomy of the short-tailed fruit bat, Carollia perspicillata

Authors: *R. ORMAN[1], T. RAGAN[3], R. KOLLMAR[2], M. STEWART[1];
[1]Physiology&Pharmacology, [2]Cell Biology, Otolaryngology, SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Tissue Vision, Inc., Somerville, MA

Abstract: Neurovascular coupling (the relation of blood flow to neuronal activity) is a fundamental feature of brain function that is widely exploited in brain-imaging technologies. Techniques have been developed to image the brain vasculature of small animals in exquisite detail, affording the opportunity to assess the structural baseline of the brain vasculature. To establish the neurovasculature anatomy of the bat as a complement to our forebrain atlas (Scalia et al., 2013), we used a fluorescence imaging/reconstruction method developed by Tissue Vision (www.tissuevision.com) to map the brain vasculature of the short-tailed fruit bat, Carollia perspicillata. Animals were anesthetized with urethane and transcardially perfused with 4% wt/vol paraformaldehyde followed by 2% wt/vol gelatin coupled to rhodamine B isothiocyanate. Brains were imaged with an in-plane resolution of 1.2 micrometers per pixel, and coronal sections were spaced 50 micrometers throughout the brain. The resulting full reconstruction of the brain vasculature will be used to compare the bat brain to the brains of other species. These data are essential for identifying targets for various experiments dependent on manipulating brain

blood flow.
Reference: Scalia, F., Rasweiler, J., Scalia, J., Orman, R., and Stewart, M. Forebrain atlas of the short-tailed fruit bat, Carollia perspicillata. Springer, New York, 2013

Disclosures: R. Orman: None. **T. Ragan:** None. **R. Kollmar:** None. **M. Stewart:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Program#/Poster#: 367.15/NNN6

Topic: I.03. Anatomical Methods

Support: European Research Council/313481

Stichting Parkinson Fonds

Dutch Brain Foundation

Title: Crossing the Styx: An integrative pipeline translating post mortem findings to *In vivo* MRI space.

Authors: *N. JUDD[1], A. ALKEMADE[1], M. KEUKEN[1], G. DE HOLLANDER[1], R. BALESAR[2], D. SWAAB[3], B. FORSTMANN[1];
[1]Developmental Psychology, [2]Univ. of Amsterdam, Amsterdam, Netherlands; [3]Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Using high-field MR technology (7 Tesla), detailed visualization of individual brain structures can now be achieved. Yet regardless of the field strength, the detail obtained is generally well above the cellular level, necessitating the fine-grained anatomical resolution offered by post-mortem staining methods. The goal of the studies presented was to develop a pipeline that allows the integration of post-mortem histological analyses with ultra-high field Magnetic Resonance Imaging (MRI). For proof of concept we studied the human subthalamic nucleus, a small biconvex nucleus that functionally is part of the basal ganglia. Human *post mortem* brain specimens were collected and formalin fixed, embedded in paraffin and cut in 6 micrometer sections while performing blockface imaging with 300 micrometer intervals. Consecutive sections were sampled with 300 micrometer intervals and subjected to immunohistochemical staining using various markers including GABA-ergic, glutamatergic, dopaminergic, and serotonergic signaling. Sections were digitally imaged using a Roche Ventana Slide Scanner and the resulting images were analyzed using histogram derived thresholding procedures available in the ImageJ software. 3D staining patterns were remodeled to individual

MRI space using linear and non-linear registration. These 3D reconstructions were used for cluster analyses to investigate the staining patterns in a quantitative fashion.
Here, we will present successful examples of 3D reconstruction of histological staining patterns and their quantitative analyses. Additionally, technical challenges including fixation techniques tailored to allow both MRI scanning and, at the same time ensuring staining quality, will be discussed, as well as the inherent limitations of working with human *post mortem* human brain tissue.
In conclusion, the presented method can be applied to any brain tissue, from a variety of species. The pipeline is flexible and modular, and can be implemented in a single interdisciplinary lab, or the different modules can be implemented by separate research groups providing the required complimentary expertise. The pipeline offers therefore a wide applicability, and provides a novel method for the translation of human *post mortem* data into *in vivo* MRI space.

Disclosures: **N. Judd:** None. **A. Alkemade:** None. **M. Keuken:** None. **G. de Hollander:** None. **R. Balesar:** None. **D. Swaab:** None. **B. Forstmann:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.16/NNN7

Topic: I.03. Anatomical Methods

Title: Methods for automated neuroanatomical annotations from zebrafish gene expression data

Authors: *S. PAJEVIC[1], G. D. MARQUART[1], K. M. TABOR[1], D. E. DALLE NOGARE[1], T. MUELLER[2], H. A. BURGESS[1];
[1]NIH, Bethesda, MD; [2]Kansas State Univ., Manhattan, KS

Abstract: Transgenic tools are increasingly used to visualize, stimulate and manipulate the nervous system. The widespread availability of transgenic animals enables *in vivo* imaging of gene expression patterns, which can then be used to identify distinct cell types and segment anatomic regions. This information can then be used to create annotated anatomical brain maps. While manual labeling is commonly used with histological stains for this, it is a laborious and subjective process that is prone to persistent biases and errors driven by misconceptions. This is particularly true at early developmental stages where many brain regions lack conspicuous nuclear organization. In this work, we implement an automated computational procedure aiming to segment distinct neuroanatomical regions using a large gene expression dataset obtained from 150 transgenic zebrafish lines. For each line 3-10 larvae were imaged at 6 days post-fertilization and co-registered to the same reference brain. After affine and elastic registration, images were

averaged and normalized to yield a 3D representation of transgene expression in each line. We used several machine learning methods and developed novel strategies for obtaining relevant neuroanatomical maps and also for assessing their performance. Our sample space consisted of individual image voxels whose primary features were the 150 distinct gene expression values. These were combined with voxel coordinates and voxel adjacency information to create optimized discrimination and distance functions. In our implementation we were able to perform clustering on many millions of voxels. To validate our results we used a set of 15 well characterized and conservatively outlined neuro-anatomical regions, identified by a human expert, and used various performance metrics (e.g., Fowlkes-Mallows index) to optimize and validate our algorithms. We used both agglomerative and divisive clustering strategies, and developed a novel criterion for splitting/merging the clusters, which in addition to between- and within-cluster variability takes into account the shape and morphology of the resulting clusters. Performance metrics of this procedure were very high and better than other commonly used criterions (pseudo F-statistic and cubic clustering criterion). The main advantages of automated methodology are (1) the accurate and unbiased segmentation of neuroanatomical larval brain regions, and (2) identification of regions previously unannotated. We anticipate that as more transgenic lines are generated, this method will allow the production of maps with increasingly fine-grained segmentation of distinct neuronal cell types.

Disclosures: **S. Pajevic:** None. **G.D. Marquart:** None. **K.M. Tabor:** None. **D.E. Dalle Nogare:** None. **T. Mueller:** None. **H.A. Burgess:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Program#/Poster#: 367.17/NNN8

Topic: I.03. Anatomical Methods

Support: NIMH/NIH Grant 1R01MH100635

Title: Predictive sub-voxel fiber tractography of the primate limbic system using high-resolution confocal to diffusion-tensor MRI maps

Authors: ***A. VAN HOEK**[1], L. DAI[2], I. YEUNG[3], O. ABDULLAH[4], E. HSU[4], S. JOSHI[4], J. R. KORENBERG[5];
[1]Neurol., [2]Dept. of Pediatrics, Sch. of Med., [4]Bioengineering, [5]Pediatrics, Med. Genet., [3]Univ. of Utah, Salt Lake City, UT

Abstract: Homeobox gene transcription factors are required for longitudinal axis and specific organ development, including inner ear and hypothalamus. We found that anti Homeobox family antibodies specifically target longitudinal axonal-like structures, devoid of myelin, associated with the striae of Lancisi and indusium griseum, representing structures running along the corpus callosum. Triple labeling of coronal sections of the macaque monkey was performed with DAPI, anti-Homeobox antibodies and antibodies targeted to neuronal/axonal components (MBP, Olig2, Smi31, Smi32). Confocal microscopy was carried out using a diode laser to excite DAPI and 80 MHz pulsed lasers (Leica white-light laser) to excite Alexa-488 and Alexa-568 labeled secondary antibodies. Additionally, differential interference contrast (DIC) images in the focal planes were recorded simultaneously. In DIC mode, longitudinal structures, lateral to the longitudinal anti-Homeobox stained bundle axons were detected, which sparingly colocalized with anti-MBP antibodies. The nature of these DIC bundles is unknown. DIC inspection provided detailed morphological entities that are difficult to detect in bright field. Blending of blue, green and red channels with DIC (white) proved to be advantageous for aligning 3D reconstructed confocal images with the associated block-face and MRI imaging data. Using sub-voxel seeding positions in DSI Studio software, Fiber tractography may predict a continuum of the longitudinal axonal-like structures with the cingular bundle, dentate gyrus, fornix, and parts of the habenula, because areas in these were also labeled with anti-Homeobox antibodies. Thus, mapping antibody-specific high-resolution data to diffusion-tensor MRI will be a valuable and viable asset in the unraveling of complex fiber tracts.

Disclosures: **A. Van Hoek:** None. **L. Dai:** None. **I. Yeung:** None. **O. Abdullah:** None. **E. Hsu:** None. **S. Joshi:** None. **J.R. Korenberg:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Program#/Poster#: 367.18/NNN9

Topic: I.03. Anatomical Methods

Support: VA Merit Review

Title: Estimation of stage-specific progression in Th17 cell-induced adoptive transfer experimental autoimmune encephalomyelitis (EAE) using *In vivo* fluorescence imaging analysis.

Authors: *S. LEE, H. CHO, Y. SHIN, H. SALAPA, M. LEVIN;
Univ. of Tennessee, Memphis, TN

Abstract: Diagnosis of central nervous system (CNS) damage in early stages of neurodegeneration is vital to impede the progression of disease. In this regard, we applied near-infrared fluorescent (NIRF) optical imaging techniques in the EAE mouse model of multiple sclerosis (MS). MS is an inflammatory and demyelinating condition of the CNS, characterized by perivascular infiltrates composed largely of T lymphocytes and macrophages. However, they are not the only immune components contributing to the pathophysiology of MS. B-cell and antibody activation are also believed to contribute. The exact cause of MS remains undetermined, but evidence indicates connections to an autoimmune process arising from viral components, environmental factors, or genetic deposition. Numerous avenues of research support the hypothesis that autoimmune mechanisms play a major role in the development of the disease. Pathologically similar lesions to those seen in MS can be induced in laboratory rodents by immunization with CNS-derived antigens. This form of disease induction, broadly termed EAE, is frequently the starting point in MS research with respect to studying pathogenesis and creating novel treatments. MS can develop due to various mechanisms, but one of the main contributors is the production of matrix metalloproteinases (MMPs) from various brain and immune cells, enzymes involved in blood-brain barrier (BBB) breakdown, demyelination and axonal injury. MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 might have an important role in MS because they enhance demyelination and axonal injury in the neurodegenerative process. We present a novel NIRF imaging strategy that can used to determine the activity of MMPs and CNS damage simultaneously by detection of exposed the RNA binding protein heterogeneous nuclear ribonuclear protein A1 (hnRNP A1) in the brain and spinal cord in mice with EAE. In this study, retro-orbital injection of MMPSense 750 FAST (MMP750) dye and Alexa Fluor® 680 conjugated monoclonal mouse antibodies specific to hnRNP A1 (hnRNP A1_680F) were administered in mice with EAE. Both dyes were detected with intensities proportional to the degree of CNS damage in the animals. Thus, our dual fluorescence imaging method can be used to detect CNS damage, which correlates with clinical grading of EAE in mice.

Disclosures: **S. Lee:** None. **H. Cho:** None. **Y. Shin:** None. **H. Salapa:** None. **M. Levin:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.19/NNN10

Topic: I.03. Anatomical Methods

Title: Novel method for quantitative assessment of cortical lesion location and size

Authors: *J. A. MASIS[1], M. JÖSCH[2], D. MANKUS[2], D. D. COX[2];
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Abstract: Lesion quantification has traditionally relied heavily on estimation: brains are sectioned, stained and imaged. ROIs over lesions are hand-drawn on a computer and volumes are estimated based on the distance between slices. Lesion location, especially in cortex, is heavily estimated as well because it is based on approximate antero-posterior coordinates that correspond to the skull and not the brain. Particularly when studying regions that lack clear anatomical demarcating boundaries, such as cortex, accurate lesion size and location quantification is paramount. Here we propose a novel method for cortical lesion size and location quantification leveraging staining techniques traditional in electron microscopy with the use of a MicroCT machine. We stain whole rat brains in osmium tetroxide until we achieve sufficient osmium penetration; we then embed the brains in resin and scan the entire brain in a MicroCT machine. From the scan we compare the 3D reconstruction of a lesioned brain to an average reference brain and semi-automatically calculate lesion size and location relative to the reference brain using the freely available ANTs software package traditionally used for comparing human brains in MRI datasets. Not only does this method provide much more accurate estimates for lesion size and location, it is arguably also less time-consuming than traditional sectioning methods, more reliable, and produces samples that can survive for much longer than the limited lifetime of brain sections.

Disclosures: **J.A. Masis:** None. **M. Jösch:** None. **D. Mankus:** None. **D.D. Cox:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.20/NNN11

Topic: I.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research B 25293058

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ACCEL, JST

AMED-CREST, AMED

Nakatani Foundation

Takeda Science Foundation

Title: Real-time *In vivo* electrochemical measurement of local drug concentrations by using diamond microelectrode

Authors: *G. OGATA[1,2], Y. ISHII[3], K. ASAI[3], Y. SANO[4], F. NIN[1,2], T. YOSHIDA[1,6], T. HIGUCHI[1], K. HORI[1], K. MAEDA[4], S. KOMUNE[6,7], M. TAKAI[5], H. KUSUHARA[4], Y. EINAGA[3,8], H. HIBINO[1,2,9];
[1]Dept. of Mol. Physiol., Niigata Univ. Sch. of Med., Niigata, Japan; [2]Ctr. for Transdisciplinary Res., Niigata Univ., Niigata, Japan; [3]Dept. of Chemistry, Fac. of Sci. and Technol., Keio Univ., Yokohama, Japan; [4]Lab. of Mol. Pharmacokinetics, Grad. Sch. of Pharmaceut. Sci., [5]Dept. of Bioengineering, Grad. Sch. of Engin., The Univ. of Tokyo, Tokyo, Japan; [6]Dept. of Otorhinolaryngology, Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan; [7]Div. of Otolaryngology-Head and Neck Surgery, Yuaikai Oda Hosp., Kashima, Saga, Japan; [8]JST-ACCEL, Yokohama, Japan; [9]AMED-CREST, AMED, Niigata, Japan

Abstract: For pharmacological and physiological studies in neuroscience, it is crucial to analyze the real-time scale of local concentrations of systemically administered drugs *in vivo* in various organs and tissues. Conventional methods do not accomplish this task because they require considerable analyte quantities and have low sampling rates. They also cannot address how change of drug concentrations correlates with target cell or tissue functions over time. Here we described a system equipped with two different sensors. One is a boron-doped diamond microelectrode, which can electrochemically measure compounds. The other is a classical glass microelectrode that pursues electrical signals in extracelluar and intracellular compartments. We tested bumetanide, a loop diuretic that can suppress the epilepsy but have an ototoxic effect, as a model analyte. In the guinea-pig cochlea, the change of bumetanide concentration was longitudinally detected with a time resolution of 5 sec while the extracellular potential underlie hearing was continuously monitored. Furthermore, in the rat brain, the drug behavior as well as the response of the local filed potential representing neural activity were simultaneously measured over time. This multi-sensing system can monitor other drugs and their physiological relevance in different tissues and organs and has applications in life science research and medicine.

Disclosures: **G. Ogata:** None. **Y. Ishii:** None. **K. Asai:** None. **Y. Sano:** None. **F. Nin:** None. **T. Yoshida:** None. **T. Higuchi:** None. **K. Hori:** None. **K. Maeda:** None. **S. Komune:** None. **M. Takai:** None. **H. Kusuhara:** None. **Y. Einaga:** None. **H. HIbino:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.21/NNN12

Topic: I.04. Physiological Methods

Title: Physiological activity monitoring with conductive polymer based silk electrode

Authors: Y. TAKIZAWA, H. TAKAHASHI, M. NISIZAWA, *K. TORIMITSU;
Tohoku Univ., Sendai, Japan

Abstract: Conductive and flexible fiber/textile is one of suitable candidates for electrodes of various wearable devices. We reported already that fiber/textile electrode modified with poly(3, 4-ethylene-dioxythiophene)-poly(styrenesulfonate) is highly flexible, biocompatible, and suitable for wide variety for physiological measurements. Here we report the formation of flexible electrode using silk fiber/textile for implantable or wearable electrode in physiological measurements. Polymerization of the conductive polymer with the silk allowed us for long-term in vivo or transdermal measurement. The silk electrode also has silk's original softness and smoothness, and stretchability even after polymerized. Through transdermal ECG and EMG measurement and in vivo muscle EMG measurement of mouse and chick, signals were successfully obtained with this electrode. Flexible and biocompatible characteristics of the electrodes enabled us to monitor these activities stably for the long-term. As this silk electrode is flexible and biocompatible, stable measurement could be achieved for variety of physiological activities. In future, we would like to reduce the resistance value and noise of the electrodes, optimize them for the practical measurement, and conduct stimulation of muscles.

Disclosures: Y. Takizawa: None. **H. Takahashi:** None. **M. Nisizawa:** None. **K. Torimitsu:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.22/NNN13

Topic: I.04. Physiological Methods

Support: NIH

Title: *In vivo* zinc imaging in mouse brain using two-photon microscopy

Authors: Z. NANNAN[1], *S. DING[2];
[1]Dalton Res. Ctr., [2]Univ. of Missouri, Columbia, MO

Abstract: Zinc is the second abundant essential trace element and cofactor for many enzymes and transcription factors in mammalian cells. A growing body of evidence indicates that Zinc plays a dynamic role in the physiology and pathology in the CNS. Zinc is critical for immunity, growth and development. Exogenous Zn^{2+} can modulate the activity of glutamate, $GABA_A$ and glycine receptor ion channels. Vesicular Zn^{2+} can promote presynaptic and inhibit postsynaptic long-term potentiation. Zinc homeostasis is a key player in aging; neuronal deficiency may play a role in the onset and progression of cognitive deficit s in normal aging and Alzheimer's disease. Zinc is also a potent neurontoxin in various pathological conditions including ischemia, epilepsy and traumatic brain injury. Although mobile zinc imaging has been reported in culture cells and brain slice preparations using fluorescence microscopy, there appears no report on *in vivo* imaging of intracellular Zinc in the brains of live animals. In current study we used two-photon (2-P) microscopy to imaging intracellular Zinc in astrocyte and neurons in live mice. Using surface incubation of the dye over a dura-removed cranial window, we found that the AM form of Zinc fluorescence dye Newport Green (NG) is preferentially loaded in astrocytes in the cortex of mouse brain, while the dye can be loaded in both astrocytes and neurons by pressure injection. Time lapse imaging shows that there are no transient Zn^{2+} increases in astrocytes and neurons both in Zinc free and 0.3 mM ZnCl2 containing extracellular ACSF solutions. Application of 0.3 mM ZnCl2 containing ACSF solution to astrocytes induce increase in NG fluorescence, indicating that extracellular Zn^{2+} can enter astrocytes regardless of the mechanism. We also examined whether glutamate and high K^+ can induce NG fluorescence increase. In summary, our approach will be useful be for imaging Zinc mobilization or entry in astrocyte and neurons *in vivo* under physiological can pathological conditions.

Disclosures: Z. Nannan: None. **S. Ding:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

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Topic: I.04. Physiological Methods

Support: NIH Grant R01 NS084975

The Grainger Foundation

Title: Nonlinear regression of *In vitro* fast scan cyclic voltammetry data for extraction of neurochemical biomarkers

Authors: *H. PARK, B. S. PAEK, J. K. TREVATHAN, K. A. LUDWIG, J. L. LUJAN, K. H. LEE;
Mayo Clin., Rochester, MN

Abstract: Background:
Fast scan cyclic voltammetry (FSCV) via carbon fiber microelectrodes (CFM) is an established technique that allows detection of electro-active molecules, and as such, has been proposed as a method for investigating neurochemical involvement in multiple disease mechanisms and therapeutic responses.[1,2] However, variability in neurochemical detection sensitivity resulting from CFM fabrication, the heterogeneity of the medium around the sensing electrode, and the dynamic nature of the brain's chemical environment confound the recorded signal, preventing the characterization of the individual neurochemical effects as well as the accurate measurement of neurotransmitter concentrations.[3] In this study, we present a method to characterize the nonlinear relationship between actual concentration of neurotransmitters and their measured FSCV signals using nonlinear regression techniques. Here, we focus the application of this technique to the measurement of dopamine, adenosine, and changes in pH.
Methods:
In vitro FSCV data were collected for different combinations and concentrations of dopamine, adenosine, and pH changes mixed in tris buffer, saline, ascorbic acid, and artificial cerebrospinal fluid (ACSF) solutions. Data collection for each sample was repeated with five different CFMs. An artificial neural network was trained for nonlinear regression analysis on the analyte concentrations (outputs) and principal components (inputs) extracted from FSCV data. The trained model was then used for the validation and test data set to predict the actual concentration from novel data.
Results:
The regression model trained on the *in vitro* data identified the nonlinear FSCV features describing the presence of target analytes. Additionally, the nonlinear relationship between *in vitro* FSCV measurement and actual analyte concentration was identified with small prediction errors, despite the presence of other analytes, pH change, and background drift due to CFMs.
Conclusions:
These results suggest that dopamine and adenosine concentration, as well as changes in pH can be accurately identified from solutions with multiple analytes present in different concentrations. This has potential applications for *in vivo* identification and quantification of analytes of interest in an effort to better understand neurochemical activity in brain. Further validation of algorithm performance needs to be performed on larger data sets involving the presence of additional neurotransmitters such as serotonin and histamine.

Disclosures: H. Park: None. **B.S. Paek:** None. **J.K. Trevathan:** None. **K.A. Ludwig:** None. **J.L. Lujan:** None. **K.H. Lee:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

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Program#/Poster#: 367.24/NNN15

Topic: I.04. Physiological Methods

Support: R21 AG045691

R01 AG042513

P01 NS074969

P50 AG568132

Knight ADRC

Title: Development of a micro-immunoelectrode for rapid detection of alpha-synuclein *In vivo*.

Authors: *C. M. YUEDE[1], H. LEE[1], H. M. EDWARDS[1], M. XIONG[1], C.-Z. LI[2], J. R. CIRRITO[1];
[1]Neurol., Washington Univ., Saint Louis, MO; [2]Florida Intl. Univ., Miami, FL

Abstract: Alpha-synuclein (α-syn) aggregates to form insoluble fibrils (Lewy bodies) in Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and 60% of Alzheimer's disease (AD) cases. Oligomeric forms of α-syn have been shown to increase synaptic transmission, impair LTP and memory. However knockout of a-syn is also associated with impaired synaptic transmission and memory formation. Tools for investigating the characteristics of α-syn production, release and clearance are highly sought to advance our knowledge of the mechanisms underlying the normal physiological function and pathophysiology involvement of this protein in disease. We have previously developed a micro-immunoelectrode (MIE) to measure rapid changes in Aβ in the AD mouse brain, and have now extended this technique to measure changes in α-syn levels in vivo. Murine α-syn contains 5 tyrosine residues, which can be oxidized and detected using carbon-based electrodes. Anti-α-syn antibodies were attached to the surface of a carbon fiber microelectrode to provide specificity for α-syn over other electroactive molecules. Tyrosine residues, present in the α-syn bound to the electrode, were shown to oxidize at ~0.65V using square wave voltammetry. The magnitude of the oxidation peak is proportional to the amount of α-syn bound to the antibodies on the electrode surface. We screened multiple antibodies to determine response characteristics of each antibody attached to the MIE. We have optimized many parameters for detecting α-syn using these MIEs in vitro, and show rapid changes in α-syn levels in the brain extracellular fluid, or interstitial fluid (ISF), in response to increased synaptic activity in the mouse brain.

Disclosures: **C.M. Yuede:** None. **H. Lee:** None. **H.M. Edwards:** None. **M. Xiong:** None. **C. Li:** None. **J.R. Cirrito:** None.

Poster

367. Staining, Tracing, and Imaging Techniques: Novel Probes

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 367.25/NNN16

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

Support: SIR project RBSI148TJD

Title: A new reporter for copper (II) detects specifically the lysosome compartment in live cells

Authors: *M. GIUFFRIDA[1], G. TRUSSO[2], C. SATRIANO[2], S. ZIMBONE[1], A. COPANI[3], G. TOMASELLI[2], E. RIZZARELLI[2];
[1]institute of biostructures and bioimaging (IBB), Natl. Council of Res. (CNR), Catania, Italy; [2]Dept. of Chem. Sci., [3]Dept. of Drug Sci., Univ. of Catania, Catania, Italy

Abstract: Copper is an essential biometal in the CNS, where it is highly concentrated. Chaperones and transportes regulate tighly the copper homeostasis of Cu(I) by diverting the ion within different subcellular compartments, including lysosome (Ohrvik, 2013).
Noteworthy, lysosomal dysfunction are associated with common neurodegenerative diseases including Alzheimer's disease (Fraldi, 2016)
Here we show a novel lysosome-targeting fluorescent sensor with high sensitivity and selectivity for Cu(II), bearing a naphthalimide scaffold for ratiometric response.
The spectroscopic properties of Lyso-CS2 (Lysosomal Copper Sensor 2) were evaluated in HEPES buffer (10 mM) at pH 5 in order to reproduce the lysosomal microenvironment. At the same pH, we also tested the selectivity of Lyso-CS2 for divalent copper with respect to other biologically relevant metal ions like Zn^{++} or Ca^{++}.
Laser scanning microscopy (LSM) analysis demonstrated that Lyso-CS2 is rapidly internalized by cells and sensitive to copper level variations. Moreover, the designed sensor perfectly fulfills the expectation of lysosomial co-localization.
The development of effective fluorescent probes directed to particular subcellular localization is relevant for the understanding of copper distribution in physiological and pathological conditions. The ability of Lyso-CS2 to detect Cu(II) in the lysosomal compartment may represent a powerful diagnostic tool for the study of early changes in its metallostasis, typical of several disease.

Disclosures: **M. Giuffrida:** None. **G. Trusso:** None. **C. Satriano:** None. **S. Zimbone:** None. **A. Copani:** None. **G. Tomaselli:** None. **E. Rizzarelli:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.01/NNN17

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

Support: NIH Grant U01 GM104604

NIBIB Grant P41 EB001978

ONR Grant N00014-13-1-0211

Title: Consequences of sparse activity in the ento-dentate-CA3 pathway: Investigations using a large-scale, biologically realistic, computational model of the hippocampus

Authors: ***G. J. YU**, T. W. BERGER, D. SONG;
USC, Los Angeles, CA

Abstract: Brain regions communicate via spatio-temporal patterns of spiking activity that are received, transformed, and transmitted to afferent regions. The hippocampus is organized in a relatively feedforward architecture which allows the study of these spatio-temporal transformations, which is interesting in the hippocampal context to understand how it is able to convert short-term memory into long-term memory. We incorporate key properties in a large-scale, biologically-realistic, structural, computational model of the hippocampus to allow the study of the propagation and transformation of spatio-temporal patterns within the hippocampal subfields. Detailed, compartmental models of individual neurons with intricate and unique morphologies are arranged according to the anatomical structure of the hippocampus at numbers approaching the realistic population sizes. The distribution of axons and convergence and divergence values are used to constrain the synaptic connectivity among the neurons. Classic theories on hippocampal function use the characteristic of sparse granule cell activity in the dentate gyrus to explain the purpose of the dentate gyrus, most commonly to perform an operation called pattern separation. Within a network consisting of the entorhinal cortex, the dentate gyrus, and the CA3, the consequences of this sparse activity are explored using behaviorally relevant grid cell input. The local circuitry of the dentate gyrus is manipulated to affect the sparseness of activity, and the ramifications of the sparsity is evaluated in the CA3.

Disclosures: **G.J. Yu:** None. **T.W. Berger:** None. **D. Song:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

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Program#/Poster#: 368.02/NNN18

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

Support: NIH Grant U01 GM104604

NIH Grant P41 EB001978

Title: Simulated effects of acetylcholinesterase inhibitors on hippocampal cell network activity

Authors: *A. MERGENTHAL, J.-M. C. BOUTEILLER, E. HU, T. W. BERGER;
USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is a degenerative neurological condition that impairs a patient's memory ultimately compromising the patient's ability to live independently. Current treatments can only slow the progress of symptoms and do not address the underlying cause. A hallmark of AD is the disruption of cholinergic input to the hippocampus from the basal forebrain (BF). Currently the primary method of treatment for AD focuses on inhibiting the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) to compensate for this dysfunction. The present study aims to simulate a neuronal network in the CA1 region of the hippocampus in three broad states (healthy, diseased, and diseased with AChE inhibited). The model includes various hippocampal neuron types that are likely affected by fluctuations in ACh concentrations including the primary pyramidal cell, cholecystokinin positive (CCK+) basket cells, and oriens lacunosum-moleculare (OLM) cells. By simulating such a network under various concentrations of ACh, not only can the model recreate a diseased state but it can also simulate the effects of treatments by acetylcholinesterase inhibitors (AChEI). The proposed model also includes mechanisms for creating long term potentiation (LTP), thereby allowing changes in long term dynamics within the network. Given these time dependent dynamics, the divergence between the states' behavior provides a way to measure the effectiveness of medication to compensate for the diseased state and allows the exploration of novel therapeutic strategies for treating the disease.

Disclosures: **A. Mergenthal:** None. **J.C. Bouteiller:** None. **E. Hu:** None. **T.W. Berger:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

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

Support: NIH U01 GM104604

NBIB P41 EB001978-24

Title: A model of axonal branching for medium and long range fibers in a multi-scale model of hippocampal tissue

Authors: *C. S. BINGHAM, J.-M. BOUTEILLER, D. SONG, T. W. BERGER;
Biomed. Engin., USC, Los Angeles, CA

Abstract: In the construction of large networks of detailed neuronal models with interconnected populations of cells some experimental goals require explicit reconstructions of cell morphology. One important example includes the use of detailed neuronal models to approximate the response of cells to extracellular electrical potentials. Full complexity morphologies, including accurately diverging axonal arbors, may be critical to meaningful prediction of network activation in response to suprathreshold stimulation. While principles of construction that have been used to generate accurate and esthetically pleasing dendritic arbors can be partially co-opted for creation of axonal arbors, in many principle cell types the axon trunk path and length prior to primary bifurcation as well as the arbor orientation are highly variable and are not captured by previously published models. This work proposes a general solution which allows modelers the flexibility to generate a broad variety of axonal arbor renderings. By extending the TREES Matlab toolbox, sections of fiber that follow an arbitrary path are prepended to arbors that are generated by the toolbox in a modular fashion. The resulting structures can then be output in a format compatible with prominent simulation environments, such as NEURON, where they may be simulated in parallel as components of a large network of cells. This work represents an important new feature of a multi-scale detailed neuronal-admittance model of hippocampal tissue response to extracellular stimulation. This greater modeling platform is designed for the purpose of optimizing electrode geometry, array geometry, and stimulation protocol for use in the efficient design of neuroprostheses.

Disclosures: C.S. Bingham: None. **J. Bouteiller:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

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

Support: U01 GM104604

P41 EB001978

Title: A detailed computational model of mechanisms underlyingcalcium regulation and dysregulations in glutamatergic postsynaptic spines

Authors: *E. Y. HU, A. MERGENTHAL, J.-M. C. BOUTEILLER, D. SONG, T. W. BERGER; Biomed. Engin., USC, Los Angeles, CA

Abstract: Experimental studies on calcium dynamics within spines of hippocampal pyramidal neurons have repeatedly shown that calcium regulation is of crucial importance to normal processes such as learning and memory or pathological ones like neurodegeneration; however, a complete picture of the mechanisms and pathways has yet to be established due to the complexity of the system and the physical constraints that limit experimental observations. Consequently, computational efforts have been undertaken to help better understand postsynaptic calcium dynamics. The present study proposes to integrate number of established detailed computational models that participate in shaping postsynaptic calcium dynamics in the spine. Models are first validated individually with respect to their initial calibration source, and then together as an ensemble within the platform to ensure proper compatibility with other models. We then use this platform to simulate the postsynaptic calcium transients observed experimentally in response to presynaptic activation as well as those observed in response to backpropagating action potentials. We also present data on other important factors that can either influence or be influenced by calcium - important factors such as channel activation properties and key players in long term potentiation. Longer term goals for this research include adaptation of the model to large scale systems using input-output, data-driven modeling methodology, to allow for the characterization of the calcium dynamics and learning in a large scale neuronal network model.

Disclosures: **E.Y. Hu:** None. **A. Mergenthal:** None. **J.C. Bouteiller:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

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Support: U.S. Office of Naval Research N00014-13-1-0211

U.S. NIBIB P41 EB001978

U.S. NIH 1U01GM104604

Title: A closed-loop multi-scale simulation paradigm for accurate modeling of electrical stimulation in hippocampus

Authors: *P. HENDRICKSON[1], K. LOIZOS[2], A. GILBERT[2], D. SONG[1], G. LAZZI[2], T. W. BERGER[1];
[1]USC, Los Angeles, CA; [2]Electrical Engin., Univ. of Utah, Salt Lake City, UT

Abstract: In order to accurately model the pattern of activation due to electrical stimulation of the hippocampus, a multi-scale computational approach is necessary. At the system level, the Admittance Method (ADM) is used to calculate the extracellular voltages created by a stimulating electrode. At the network and cellular levels, a large-scale multi-compartmental neuron network is used to calculate cellular activation. Work has been previously presented showing an open-loop coupling strategy between the two methodologies, where voltages in 3D space predicted by the ADM model are used as extracellular inputs to cells in the large-scale neuronal network. This modeling methodology, however, doesn't allow for membrane currents generated by individual neurons to affect the electric field in the surrounding 3D space. A more biologically realistic modeling paradigm would be for closed-loop, bi-directional communication to occur between NEURON and the ADM model, as extracellular voltages are dependent on both the current generated by an extracellular electrode and currents generated by the cells themselves. This work demonstrates functional bi-directional communication between parallel NEURON and the ADM solver using a small multi-compartmental neuronal network and an Admittance Method model with parameters similar to those measured in bulk neural tissue. At each time step of the simulation, each NEURON process sends the membrane current in each compartment to an intermediary process, which collects all membrane currents and passes them to the ADM solver. Once the ADM solver receives the membrane currents, it applies each one to the nearest node in the Admittance Method model and calculates voltages in the bulk tissue. The voltages at the nearest nodes to each compartment are then interpolated, and the full set of voltages is sent back to the intermediary process, which properly distributes them back to individual NEURON processes. NEURON solves for the next time step, and the data-sharing

loop resets. In this way, bi-directional communication between the processes in this multi-scale model creates a coupled closed-loop simulation methodology. In addition to demonstrating this bi-directional communication, we present an analysis of the performance and scaling of the model, and the effect of the closed-loop paradigm vs. open-loop on neural activity. This bi-directional communication paradigm will allow for models that study a variety of phenomena, including the patterns of activation in large-scale neural networks in response to extracellular stimulation, and the effects of ephaptic coupling on local cellular activity.

Disclosures: P. Hendrickson: None. **K. Loizos:** None. **A. Gilbert:** None. **D. Song:** None. **G. Lazzi:** None. **T.W. Berger:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

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Title: Extracellular potentials generated by axon bundles

Authors: *T. MCCOLGAN[1], P. T. KUOKKANEN[1], J. LIU[2], H. WAGNER[3], C. E. CARR[2], R. KEMPTER[1];
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Abstract: Many experimental methods used to understand the brain are based on extracellular field potentials (EFPs). These include the Local Field Potential(LFP), Current Source Density (CSD), Multiunit Activity (MUA), the Electroencephalogram (EEG), the Electrocorticogram (ECoG) and the auditory brainstem response (ABR). The origins of these field potentials are often unclear, and were long thought to lie primarily in synaptic currents and somatic spikes. As a consequence, many modeling studies and interpretations of the EFP focus solely on extracellular potentials induced by synaptic currents on the dendrites and by spikes at the soma of a postsynaptic neuron.

Based on previous recordings of field potentials in the auditory brainstem, we here present a model of extracellular potentials from axon fiber bundles. The aim of the model is toshow how a wide array of field potentials may be explained by the axons' anatomical features. In particular, we show that the branchings and terminations of axons in a typical projection area lead to a dipolar EFP structure. Dipoles have a farther spatial reach than the quadrupolar potentials traditionally associated with axons. The model predicts strong contributions of nerve tracts to the extracellular potential, which are currently neglected by other models.
Along with the theoretical description, our multichannel electrode recordings from the barn owl auditory brainstem show several features observed in the model. We recorded responses in the nucleus laminaris (NL) to tones, clicks, and white noise stimuli. The low-frequency (<1kHz) component of the EFP response to auditory click stimulation in NL shows the polarity reversal predicted by the model. The low-frequency component has a dipolar structure and extends spatially for at least several millimeters from the nucleus.
Our work suggests that both modellers and experimentalists should consider possible axonal contributions to extracellular recordings. We propose a novel way to relate anatomy, spiking activity, and the spatial structure of the extracellular potential.

Disclosures: **T. McColgan:** None. **P.T. Kuokkanen:** None. **J. Liu:** None. **H. Wagner:** None. **C.E. Carr:** None. **R. Kempter:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.07/NNN23

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

Support: NIH US-German Collaborative Research in Computational Neuroscience 1R01EB014641-01

Title: Model-based control of spreading depression by applied electric field in spatially extended neuron-glia model

Authors: ***S. VAN WERT**, S. J. SCHIFF;
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Abstract: Spreading depression is a wave-like neural phenomenon that occurs in the cortex of the human brain and is linked to disorders such as migraine, epilepsy, and stroke. For use in a proposed model-based control scheme to treat such disorders, we present a spatially extended cellular model of the brain that replicates the key dynamics of these pathological phenomena. To

allow for a physiological means of control in the cellular environment, we include electrodiffusion currents which can be directly modulated by applied electric fields. Investigating this model and its use in a model-based control scheme allows us to determine best practices for understanding and treating—or preventing—the disorders that are associated with SD in real brain tissue.

The presented model builds upon and spatially extends the one-compartment model in [1] which unified various neuron dynamics in a model that accounted for tracking of ion flux, ion concentration changes, energy use, and volume change. In the presented model we add an active-membrane glia compartment and extend the model in space to a soma-dendrite axis, which allows control to be applied in the direction of that axis. This applied control modulates the electrodiffusion fluxes in the cell environment, which in some conditions can mitigate, reverse, or prevent any pathological dynamics.

In search of viable control schemes for potential use in treatment, we demonstrate the plausibility and effectiveness of different control schemes, such as proportional control or Kalman-filter controllers, to alter the neural dynamics of SD and the related behaviors of seizure and stroke as well. The implemented controllers provide novel approaches for universal treatment of these and potentially other pathologies in neural tissue through avoidance of excessive demands on energy or significant disruptions to the local ionic environment. This application of model-based control lays a foundation for medical technology that monitors critical features of brain dynamics and supplies an appropriate and safe treatment stimulus.

References

1. Wei Y, Ullah G, Schiff SJ: Unification of neuronal spikes, seizures, and spreading depression. *J Neurosci* 2014, 34(35):11733-11743

Disclosures: S. Van Wert: None. **S.J. Schiff:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.08/NNN24

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

Support: OIST Graduate University

Title: Impact of dendritic morphology on functional subunits in dendrites

Authors: *S. HONG, A. TAKASHIMA, E. DE SCHUTTER;
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Abstract: Studies have shown that active computation in dendrites is highly compartmentalized (Mel and Häusser, Curr Opin Neurobiol, 2003), even up to the level of individual dendritic branches (Branco and Häusser, Curr Opin Neurobiol, 2010). This suggested that dendrites form local computational 'subunits', which make single neurons (especially pyramidal neurons) act like two-layer neural networks (Poirazi et al., Neuron 2003). Evidence supporting these hypotheses is largely based on active membrane mechanisms in dendrites that give rise to their rich computational capabilities (e.g., Branco et al., Science, 2010) and independent operations (Behabadi and Mel, PNAS, 2014). However, morphology is known to play a significant role in dendritic signaling: For example, even when mechanisms supporting active signal propagation are artificially embedded in simulations, morphological properties of cerebellar Purkinje cells still prevent spike backpropagation into their dendrites (Vetter et al., J Neurophys, 2001). This suggests that morphology can have large impacts also in determining functional compartmentalization of dendrites.

Here we investigated how the morphological structure can determine compartmentalization of dendrite-to-dendrite signaling and formation of subunits, by combining a data-driven statistical analysis and computational modeling. We simulated central neurons of diverse morphological types with the passive membranes where localized inputs were injected. Response patterns in the dendritic membrane were collected as 'features' corresponding to the input sites. Then, we grouped all input locations into clusters via dimensionality reduction and clustering. We found that those clusters in pyramidal neurons usually consist of a few nearby branches, containing 2.07±0.09 dendritic terminals per cluster (mean±SEM), while nearly a half of clusters s were single dendritic branches particularly in cortical pyramidal neuron. On the other hand, clusters tended to consist of multiple branchlets in cerebellar Purkinje cells (12.9±0.82 terminals). We also simulated models that have active mechanisms to generate dendritic spikes, and found that spreading of a dendritic spike largely overlaps with clusters.

Our work demonstrates the importance of dendritic morphology in defining functional units in dendritic trees of single neurons. Our method can be a useful tool for understanding neuronal computation in terms of computational subunits and constructing simplified functional models (e.g., Poirazi et al., Neuron, 2003).

Disclosures: **S. Hong:** None. **A. Takashima:** None. **E. De Schutter:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.09/NNN25

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

Title: Explicitly incorporating dendritic spines of different morphological classes into a multi-compartment model of a pyramidal neuron

Authors: *S. E. MOTLEY[1], T. HOANG-TRONG[2], J. KOZLOSKI[2], J. H. MORRISON[3,4], R. KERR[5];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Computat. Biol. Center, IBM Res. Division, IBM T. J. Watson Res. Ctr., Yorktown Heights, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Univ. of California Davis, Davis, CA; [5]IBM Res. Australia, Carlton, Australia

Abstract: On average, each neuron in the brain receives roughly 10,000 synaptic inputs. For principal neurons, the majority of these are made onto protrusions from the dendrite, called spines, rather than the dendritic shaft itself. These spines act as individual compartments, which are chemically (and possibly electrically) isolated from the rest cell. Spine compartmentalization likely plays a role in shaping synaptic plasticity, and there is evidence suggesting it may also shape how dendrites integrate their inputs. Here, we create the first multi-compartment model of a neuron in which a realistic number of spines (~8,000) are modeled explicitly as individual compartments. Simulations of this model neuron are run efficiently using a parallel computing infrastructure. We discuss how experimental data is used to constrain the geometry of each spine compartment as a function of spine morphological class. Similarly, experimental data is then used to distribute these different classes of spines in a statistically realistic manner along the dendrite. We show how spine neck resistance may be calibrated to match experimental results, and how this type of model may be used to understand how changes in spines such as occur in certain cells during aging can affect both the behavior of neurons and their synaptic plasticity.

Disclosures: S.E. Motley: None. **T. Hoang-Trong:** None. **J. Kozloski:** None. **J.H. Morrison:** None. **R. Kerr:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.10/NNN26

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

Title: Atomistic study of the interaction PPAR -gamma and PPAR-beta/delta with its agonists: A computational study

Authors: *A. MORALES[1,2], F. PÉREZ-SEVERIANO[1], A. ZAMORANO- CARRILLO[2]; [1]THE NATIONAL INSTITUTE OF NEUROLOGY AND NEUROSURGERY, Mexico, Mexico; [2]Lab. of Biochem. and Computat. Biophysics, ENMH-IPN, Mexico, Mexico

Abstract: PPARs (Peroxisome Proliferator Activated Receptors) are members of the nuclear receptor family that can be activated by endogenous (fatty acids) or exogenous (thiazolidinediones, fibrates) agonists. There are three isotypes of PPARs (PPARα, PPARβ/δ, and PPARɣ) studied as therapeutic targets in different diseases, mainly those characterized by high oxidative stress levels like the neurodegenerative. PPARβ/δ and PPARɣ are highly expressed in brain, and their activation by ligands could increase the level of protecting molecules against tissular damage, but the isoform ɣ is the most studied in neurodegenerative models comparing to β/δ. Therefore, the aim of this work was to explore de atomic interactions and structure changes in PPARs with agonist union. Herein, we atomistically describe the affinity of PPARɣ and PPARβ/δ, with important endogenous (Eicosapentaenoic and Oleic acids -EPA, OA) and exogenous ligands (GW1929, GW501516, L-165041) by computational simulations. In particular, a docking was performed in AutodockVina and the results were analyzed by MOE software, following a refinement by molecular dynamics simulations. Nine conformers were obtained for each docking, and the top affinity was -8.3 Kcal/mol for GW1929 interacting with four polar residues of PPAR-ɣ arm I (Ser289, His323, His449, and Tyr473) and PPAR-β/δ arm I (Ser289, His449, and Tyr473). Thus, the interactions have resulted in hydrogen bonds might be provided by polar residues and the carboxylate groups.

Disclosures: A. Morales: None. **F. Pérez-Severiano:** None. **A. Zamorano- Carrillo:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.11/NNN27

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

Support: National Natural Science Foundation of China under Grant No. 61450004

National Natural Science Foundation of China under Grant No. 81471633

National Natural Science Foundation of China under Grant No. 91330103

Title: The substances transportation in the brain extracellular space can be modulated with External Stimulation

Authors: *Z. TENG, X. GUAN, Q. HE, Y. FU, H. HAN;
Peking Univ. Third Hosp., Beijing City, China

Abstract: The transportation of substances in the brain extracellular space (ECS) is crucial for both the maintenance of brain homeostasis and many new coming therapeutic approaches, like stem cell implants, brain tissue engineering. We here report a method to modulate the flow or diffusion speed of the molecules in the ECS. The half-life of the probes was calculated to represents the clearance speed of the probe molecules in the brain ECS. and the half-life in the selected brain ECS division was compared before and after the corresponding external stimulation with the monitoring of the electrophysiological recording. The flow or diffusion speed of the probe molecules in the specific brain ECS divisions can be modulated with the corresponding external simulation model. The ISF flow in the thalamus could be modulated using a painful stimulation model and the ISF flow in the amygdala can be modulated with eugenol. We thereafter established the link between the neuronal activities and the substance transportation in the brain ECS. The changes of in the brain ECS falls in the time scale of hours and was not accompanied with any structural changes. This observation suggests a potential, non-invasive way to modulate the brain function.

Disclosures: Z. Teng: None. **X. Guan:** None. **Q. He:** None. **Y. Fu:** None. **H. Han:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.12/NNN28

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

Support: BBSRC grant

Innovate UK grant

Title: A novel method to create an *In vitro* dynamic blood brain barrier model

Authors: P. MIRANDA-AZPIAZU[1], G. JOSE[1], S. PANAGIOTOU[2], *S. SAHA[1];
[1]Univ. of Leeds, Leeds, United Kingdom; [2]Univ. of Liverpool, Liverpool, United Kingdom

Abstract: The Blood-Brain Barrier (BBB) is critical for preventing entry of toxic substances into the brain and BBB leakage is linked to several diseases including stroke, neuroinfectous diseases and schizophrenia. Animal BBB models, while informative, are expensive and lack consistent predictive value for humans[1]. Current *in vitro* models primarily use static two dimensional cultures, which do not mimic the *in vivo* situation. Endothelial cells *in vivo* continuously

experience shear stress generated by the blood flow, so dynamic cell cultures would correspond to a scenario closer to the real situation. To date, human BBB models show very low transendothelial resistance (TEER) values, a classic method of determining the tightness of the barrier. The present study aims to establish, optimize and characterize a human dynamic *in vitro* BBB model.
Kirkstall *Quasi-vivo*® systems (QV500 and QV600) and three primary cells from ScienCell [Human Brain Endothelial cells (HBECs, Human Brain Vascular Pericytes (HBVPs) and Human Astrocytes (HAs)] were used to from the barrier. Cell viability was assessed by MTT assay. Cells morphology was studied by immunohistochemistry using anti-GFAP for HAs, anti-α-actin for HBVPs and anti-*zonula occludens 1* for tight junctions formed by HBECs. The TEER values were measured using an EVOM2 Volt/Ohm Meter and the barrier permeability examined by measuring sodium fluorescein with a novel laser based method. Experiments were carried out first in static conditions in order to achieve the best TEER values (>100ohms/cm2) and cell combinations before the transwells were placed inside QV600 and compared the TEER values after 5 days with static model in the same conditions
Cells showed different flow tolerance amongst the cell types, with HAs withstanding a maximum flow rate of 75 µl/ml. Viability of HBECs was increased under flow conditions (n=3, p>0.01). TEER values tend to increase after the cells under flow and fluorescein passage through the barrier was significantly lower than that observed in the empty coated transwells.
The results confirmed the optimal conditions in terms of media, flow speed and the optimal number of cells to be seeded for this novel functional BBB system under dynamic flow. This model will provide a platform to understand BBB function in health and disease as well as to develop strategies for effective drug delivery, toxicity testing and biomarker analysis and also reduces animal uses in these areas.
(1) Brown et al. Biomicrofluidics 2015;9:054124. (2) Abbott NJ. J Anat 2002; 200:629-638. (3) Nakagawa et al. Cell Mol Neurobiol 2007; 27:687-694.

Disclosures: **P. Miranda-Azpiazu:** None. **G. Jose:** None. **S. Panagiotou:** None. **S. Saha:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.13/NNN29

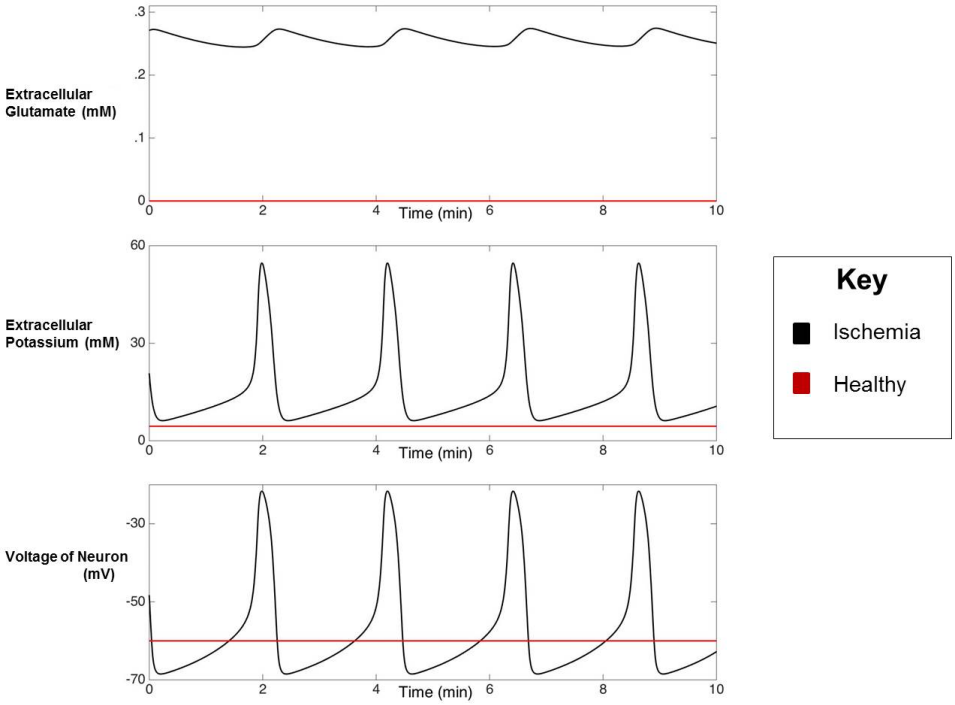
Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant 1410935

Title: A mathematical model of ischemic stroke

Authors: ***M. SARKAR**, C. CONTE, R. LEE, D. H. TERMAN;
Ohio State Univ., Columbus, OH

Abstract: Ischemic stroke, in which blood flow to the brain is interrupted, accounts for 87% of 795,000 strokes per year in the United States. When blood flow is disrupted, oxygen and glucose levels in the affected area decrease, preventing formation of adenosine triphosphate (ATP). Ion gradients are no longer maintained and neurons in ischemic regions depolarize. Intracellular calcium and extracellular glutamate accumulate, and neurons are overstimulated and die. Recurrent waves of depolarization spread from the core of damage to surrounding areas called the penumbra. Penumbra cells may or may not survive, depending on insult severity. However, a model that describes the major components of stroke pathology has not yet been developed. The purpose of this study is to create a mathematical model of a neuron and an astrocyte in the penumbra during ischemia and determine conditions that allow for cell survival. A model that incorporates dynamics of cell receptors, ion pumps and channels, glutamate transporters and exchangers, aerobic respiration and astrocyte-neuron interactions is developed. Simulations show that when the strength of the sodium-potassium pump is increased, depolarization of the neuron can be reversed. In addition, the equilibrium potential of the sodium-glutamate transporter increases and the current of the leak channel decreases. Intracellular glutamate rises and extracellular glutamate drops as well. However, when the pump strength is lowered, the opposite occurs, leading to pathologically high levels of extracellular glutamate. Oscillations in neuron and astrocyte voltages occur, as well as in intracellular and extracellular levels of ions like potassium and sodium. This suggests recurrent waves of spreading depolarization. Results are shown below. The model is being used to help identify key biological processes, which underlie the development of stroke-like conditions. This includes the role of the sodium-glutamate exchanger in both the neuron and astrocyte. Once the mechanics of ischemic stroke are understood more fully, better treatments can be developed.



Disclosures: **M. Sarkar:** None. **C. Conte:** None. **R. Lee:** None. **D.H. Terman:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.14/NNN30

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

Support: DFG SPP1665

Title: A bayesian hierarchical model for the biophysical properties of melanopsin

Authors: ***B. V. EHINGER**[1], D. EICKELBECK[2], K. SPOIDA[2], S. HERLITZE[2], P. KÖNIG[1,3]; [1]Inst. of Cognitive Sci., Osnabrück Univ., Osnabrueck, Germany; [2]Dept. of Gen. Zoology and Neurobio., Ruhr-University Bochum, Bochum, Germany; [3]Dept. of Neurophysiol. and Pathophysiology, Univ. Med. Ctr. Hamburg Eppendorf, Hamburg, Germany

Abstract: Melanopsin is a photoactive protein in retinal ganglion cells. It has recently become attractive as an optogenetic tool: Melanopsin can easily be activated using blue light and deactivated using green/yellow light. This allows for a precise control of the activation and

deactivation of the GPCR pathways and/or the neuronal firing while having limited phototoxicity at the same time.
For its effective application in optogenetics it is important to understand the underlying processes and develop mechanistic models which implies a quantitative description of the data. In recent years a new tool set, Bayesian hierarchical modeling, has seen rapid development. We use these methods to model the kinetics of melanopsin.
In this poster, we develop, implement, fit and discuss several Bayesian generative models of melanopsin signaling dynamics. We start with a sketch of a basic model and translate it into a formal probabilistic language (STAN). As melanopsin occurs in at least two states, a resting and an active state, the basic model is defined by a non-stationary two state hidden Markov process. Subsequently we add complexities in terms of (1) a hierarchical extension to fit multiple cells; (2) a wavelength dependency, to investigate the response to a stimulation with different colors of light; (3) differences between sub-types of melanopsin as found in different species. This application of modeling melanopsin signaling dynamics demonstrates several benefits of Bayesian methods. They directly model the uncertainty of parameters, are flexible in the distributions and relations of parameters in the modeling, and allow to include prior knowledge, for example parameter values based on biochemical data.

Disclosures: **B.V. Ehinger:** None. **D. Eickelbeck:** None. **K. Spoida:** None. **S. Herlitze:** None. **P. König:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.15/NNN31

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

Title: Determinants of spontaneous synchronized network activity in primary neuronal cultures: a computational approach

Authors: ***D. LONARDONI**, H. AMIN, A. MACCIONE, T. NIEUS, L. BERDONDINI; Neurosci. and Brain Technologies, Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Unravelling the electrophysiological principles of neuronal network resolved at cellular resolution is a challenging objective that can be addressed with emerging neurotechnologies, experimental studies and computational modelling. High density multielectrode arrays (Berdondini, 2009) offer nowadays the unique possibility of simultaneously recording neuronal spiking activity from up to several thousands of closely spaced recording sites, thus providing a high spatial resolution information of the ongoing

spiking network activity. Currently, neuronal networks grown in-vitro allow to achieve the lowest cellular under sampling and are therefore an interesting model to investigate. Based on detailed high-resolution recordings from primary neuronal cultures, we have developed a computational model that serves as a ground true for investigating determinants underlying the generation of spontaneous spiking activity in these networks. After a few weeks of development, networks display spontaneous and propagating network-wide bursting events (NBs) that can be classified into a few classes of spatiotemporal patterns. Previous observations highlighted that: (i) NBs correspond to propagating spiking activities that originate from a few and specific regions of the network (Gandolfo, 2010) and (ii) share similar spatio-temporal firing patterns (Raichman, 2008). By including only a few topological constraints on the topology of the network, we will show that the model generates realistic NBs that are comparable to the statistics of our experimental data. The responsiveness of the model to chemical perturbations is also remarkably robust and shows responses in line with known results obtained with the manipulation of synaptic receptors. Given the reliability of the synthetic model with respect to experimental data, we have exploited the complete information on its cellular and structural constituents to performed functional connectivity analysis aimed at characterizing the sub-circuits that determine these NBs. Interestingly, this analysis reveals that these functional sub-circuits are peculiar network regions that: (i) display similar spatio-temporal spiking pattern before triggering an NB and (ii) are more susceptible to amplify local perturbations or spontaneous spiking activities than other regions of the network. These local properties might be determining the initiation of NBs. In a next step, the model will be extended to include different plasticity rules as required to investigate how changes in the strength of the synaptic connections can impact the emerging firing properties.

Disclosures: D. Lonardoni: None. **H. Amin:** None. **A. Maccione:** None. **T. Nieus:** None. **L. Berdondini:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.16/NNN32

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

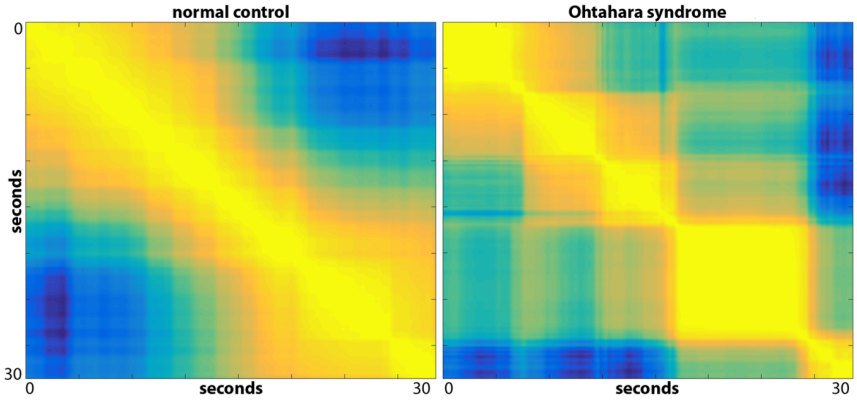
Support: Wellcome Trust: 106556/Z/14/Z

Title: Network dynamics in early infantile epileptic encephalopathies

Authors: *R. E. ROSCH[1,2], F. MOELLER[5], T. BALDEWEG[3], G. BAIER[4];
[2]Wellcome Trust Ctr. for Neuroimaging, [3]Ctr. for Developmental Cognitive Neuroscience, Inst.

of Child Hlth., [4]Cell and Developmental Biol., [1]Univ. Col. London, London, United Kingdom; [5]Clin. Neurophysiol., Great Ormond Street Hosp. for Children NHS Fndn. Trust, London, United Kingdom

Abstract: Epilepsy is one of the most common neurological conditions with particularly high incidence in childhood. Some of the infantile epilepsies - the epileptic encephalopathies - are associated with developmental delay, thought to be caused by the abnormal electrical brain activity. In addition to paroxysmal epileptic seizures, many of these epilepsy syndromes show marked disruptions in the background activity of the brain as measured by electroencephalography (EEG). This can range from burst suppression patterns as seen in Ohtahara syndrome to desynchronised, high amplitude abnormalities described as hypsarrhythmia, characteristically associated with infantile spasms.
These syndromes are typically age specific and individual patients can transition from burst suppression to hypsarrhythmia during development. The aim of this study is to evaluate the visually apparent EEG differences between different epileptic syndromes in terms of network-level dynamics. We report a network analysis of clinical EEG recordings of patients with either Ohtahara syndrome or infantile spasms, and age-matched normal controls. We estimate trajectories of band-specific network correlation patterns as well as band power distributions. We use these measures to track switching between different network states.



Correlation dynamics: delay-delay matrices showing temporal dynamics of correlation pattern changes in EEG channels across the scalp.

We find independent dynamics governing the network state switching in all groups: band power and network correlation transitions occur independently from each other. These network state transitions are different between patients and controls, as well as the patient groups. The biggest differences are apparent in the smoothness of transitions between correlation pattern states. These results reveal underlying dynamics not readily apparent in visual examination of the respective EEG patterns and will form the basis for further modelling of interacting pathological and developmental processes giving rise to these specific epilepsy phenotypes. The approach presented here may also enable automatic EEG classification in the future.

Disclosures: **R.E. Rosch:** None. **F. Moeller:** None. **T. Baldeweg:** None. **G. Baier:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.17/NNN33

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

Support: W81XWH-11-D-0011

W81XWH-16-C-0042

Title: From callosal axonal injury to neurobehavioral dysfunction: computational modeling of cortical network dynamics in mild traumatic brain injury

Authors: *J. CUI, L. NG, V. VOLMAN;
L-3 Communications/Applied Technologies, Inc, San Diego, CA

Abstract: Neurobehavioral sequelae of mild traumatic brain injury (mTBI) include impaired attention, increased reaction time, and impaired working memory. Quantitative electroencephalography (qEEG) analysis of mTBI subjects often reveals “slowing down” of resting-state brain rhythms. However, little is known about the mechanisms linking mTBI to its sequelae and injury doses for inducing these sequelae. To address these questions, we developed a large biologically plausible neuronal network model of cortical tissue, describing two sub-networks connected by myelinated corpus callosum axons. The network model featured realistic axonal conduction delays and short-term synaptic depression and facilitation. Based on diffusion imaging and histological data, we hypothesized that the corpus callosum, the largest white matter tract in the brain, is the primary locus of injury in mTBI. Injury-induced dysfunction of callosal axons was operationally defined as injury severity-dependent alteration in axonal spike amplitude and conduction speed. For both intact and injured model networks, the network dynamics were characterized for a task-free resting state and response to attention-like stimulation. A number of changes were observed in model networks following callosal injury, consistent with clinical findings: 1) slowing down of the network rhythms, from consciousness-dominated alpha band in the intact network, to lower-frequency bands in injured networks; 2) altered response to attention-like stimulation; 3) increased population response time (proxy of reaction time); and 4) reduced population persistence time (proxy of working memory). Importantly, these changes correlated with the severity of callosal injury. The model helps to bridge the gap between the biophysical mechanisms of mTBI and the emerging cognitive sequelae.

Disclosures: **J. Cui:** None. **L. Ng:** None. **V. Volman:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.18/NNN34

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

Support: PLSI supercomputing resources of Korea Institute of Science and Technology Information (KISTI)

GIST under the Practical Research and Development support program supervised by the GIST Technology Institute (GTI)

GIST Research Institute (GRI) in 2016

Title: Tms-induced neuronal activation - a computational study

Authors: *H. SEO[1], N. SCHAWORONKOW[2], J. TRIESCH[2], S. JUN[1];
[1]Sch. of Electrical Engin. and Computer Sci., Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [2]Frankfurt Inst. for Advanced Studies, Frankfurt am Main, Germany

Abstract: 1. Introduction. Transcranial magnetic stimulation (TMS) is a noninvasive method that modulates neural activity in the brain. Recently, researchers have attempted to precisely estimate the stimulus-induced electric field by using an anatomically realistic head model based on magnetic resonance imaging (MRI). However, it remains a challenging question how exactly individual neurons in different parts of the neural tissue become activated through such stimulation. In this work, we employed computational modeling to analyze the activation of layer 5 pyramidal neurons (L5 PNs) by TMS and to predict the spatial extent of activated neural tissue.
2. Methods. A three-dimensional volume conduction model of the head was constructed based on human magnetic resonance imaging. The magnetic vector potential was determined for a Magstim 70 mm figure-8 coil that was positioned to target the hand knob and the stimulus-induced electric fields were then calculated via a finite element method. The L5 PNs were virtually fitted into the head model and distributed from the precentral to the postcentral gyrus. For each neuronal compartment we estimated the electric field gradient at its center and used it to calculate the so-called activating function, i.e., the magnitude of the electric field gradient along the direction of the neuronal compartment, whose effect was modeled as an extra current injected into that compartment. These computations were performed in the NEURON environment.
3. Results & Discussion. We analyzed the activated neural tissue at certain excitation thresholds for two coil orientations (0° and 180°), and focused on the excitable cortical area for a stimulator output of 67, corresponding to the average value of the motor threshold. Interestingly, the wall of the gyrus is the most excitable area, while the top of the gyrus is not as excitable. When changing

the coil orientation from 0° to 180° (simulated by changing the direction (sign) of the current through the stimulation coil) the most strongly activated area jumped from the precentral to the postcentral gyrus. In conclusion, by incorporating L5 PNs with detailed morphology into the head model, we found that the gyrus walls were the most excitable areas for a standard TMS protocol. We analyzed targeted neural tissue for different coil orientations and found that the magnitude of electric fields, which is highest at the crown of the gyrus, is a poor predictor of tissue activation. We conclude that the combination of detailed neuronal models with brain-wide electric field calculations is critical for accurate estimation of brain areas targeted by TMS.

Disclosures: **H. Seo:** None. **N. Schaworonkow:** None. **J. Triesch:** None. **S. Jun:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.19/NNN35

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

Title: Learning with discrete representations using continuous chaotic. neural populations.

Authors: ***S. HAXBY**[1], E. PETERSON[2];
[1]Univ. California, San Diego, LA Jolla, CA; [2]Cognitive Sci., UCSD, La Jolla, CA

Abstract: Representations in the neural code, perception, decision making may rely on discrete representations. Yet organisms make smooth movements. And people have seemingly continuous lines of thought. How the human brain transforms these discrete representations into the continuous output required for such actions is not well understood. As a first step, we developed a chaotic recurrent neural network (RNN) whose activity is transformed into a discrete representation during learning, while still producing a smooth output.
We modified a prominent method for training RNNs, FORCE, introducing a discrete, threshold-based, decoding mechanism within the FORCE procedure. Preliminary experiments with our new "DFORCE" algorithm suggest discrete decoding offers surprisingly similar performance to the original algorithm. The discrete representations intrinsic to DFORCE however increases the robustness of learning in the presence of noise. Early experiments suggest it may also prove superior in learning certain difficult problems, such as a N-dimensional Lorentz attractor.

Disclosures: **S. Haxby:** None. **E. Peterson:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.20/NNN36

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

Support: NIH NIBIB grant R01EB018297

Title: Effects of synaptic transmission probability on functional network structure

Authors: ***M. BUDAK**, M. R. ZOCHOWSKI;
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Abstract: In the developing brain, wiring the complex network is energetically and metabolically expensive. To minimize wiring cost to create highly connected network, parsimonious principle dominates in the developing network. Thus, the topology of the network has both local and long-range connections - so called 'small-world' network - with a high level of modularity between neurons in these networks.
The functional connectivity of the network depends however on many parameters, not only actual anatomic connectivity. Here we investigate how synaptic transmission probability differentially affects networks of different structural connectivity. We model networks with integrate-and-fire excitatory neurons and define a probability of synaptic transmission, a parameter to randomly determine whether neurons send signals to the others they're connected to or not. We use small-world network to mimic the brain's parsimonious structure, as well as scale-free network to model its modularity. We show that changes in probability of synaptic transmission have differential effect on functional network structure depending on the structural connectivity. Namely the dynamics of networks with local connectivity are more robust to the decrease of synaptic transmission probability.

Disclosures: **M. Budak:** None. **M.R. Zochowski:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.21/NNN37

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

Title: Dynamics of rate-model networks with separate excitatory and inhibitory populations

Authors: *M. STERN[1], L. ABBOTT[2];
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Abstract: Randomly connected networks of rate-model neurons have a rich dynamics [1], a feature that has been exploited to model a variety of phenomena [2]. These model networks typically do not distinguish between excitatory and inhibitory neuron classes. Doing this requires constraining the network connectivity matrix to have columns with exclusively positive entries, representing input from excitatory neurons, and with negative entries, representing input from inhibitory neurons. The eigenvalue spectra of random matrices satisfying this constraint have a number of interesting properties [3,4]. Here we study the dynamics of rate-model networks that result from using such connectivity matrices. We find that neural activity is correlated across all neurons, including both excitatory and inhibitory subpopulations. This correlation depends on the difference between the mean strengths of the excitation and inhibition connections. and it increases as this difference is increased. For very large values of this difference, the network reaches a stable fixed point, otherwise it is chaotic. Chaos arises from the residual activity deviating from the correlated mean network activity, and it acts to reduce these correlations. The magnitude of the residual chaotic activity is determined by the variances of the synaptic strengths within the excitatory and inhibitory populations. In summary, unlike models with a single mixed excitatory/inhibitory population, in which the activity between pairs of neurons is uncorrelated for every value of synaptic gain, networks with distinct excitatory and inhibitory subpopulations exhibit strongly correlated activity across the entire network reminiscent of the up/down states seen in neural recordings [5]. [1] Sompolinsky, H., Crissanti, A., and Sommers, H.J. (1988) *Phys. Rev. Lett.*, 61:259-262. [2] Reviewed in Sussillo, D. *Curr. Opin. Neurobiol.* **25**, 156-163 (2014). [3] Rajan, K. and Abbott, L.F. *Phys. Rev. Lett.* 97, 188104 (2006). [4] Tao, T. *arXive*:1012.4818v6. [5] Steriade, M., Nunez, A., and Amzica, F. *J. Neurosci*. 13, 3252-3265 (1993).

Disclosures: M. Stern: None. **L. Abbott:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.22/NNN38

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

Support: KAKEN 16K00386

Title: Cortical circuit organized through the log-STDP leads to an internal representation of sensory experience during development: a model study

Authors: T. MATSUMURA[1], T. YUASA[1], *S. KANG[1,2];
[1]Yamagata Univ., Yonezawa-Shi, Yamagata, Japan; [2]Lab. for Neural Circuit Theory, Brain Sci. Institute, RIKEN, Wako-shi, Saitama, Japan

Abstract: Recent *in vivo* multi-unit recording study revealed that primary visual cortex showed prominent similarity between spontaneous activity (SA) and evoked activity (EA). Furthermore, such the similarity gradually increased depending on the sensory experience with progress of developmental stage. However, it is still unclear how the dynamics is organized through the learning of local circuit in cerebral cortex.
To explore underlying mechanism, we computationally examined recurrent network model whose synapses were modified through the logarithmic spike-timing-dependent plasticity (log-STDP) that could guarantee both of synaptic specialization and network stability. Model network was driven by repetitive application of sensory inputs modeled as Poissonian spike trains with non-stational rate or correlation. In our study, the similarity of network dynamics was quantified as repetitive spike sequence (RSS) that appeared both during SA and EA with millisecond accuracy. As a result, it was demonstrated that model network showed the significant increase of RSS when the log-STDP mediated excitatory synapses and synaptic weights in network leaded to a long-tail distribution during learning phase. Furthermore, the number of RSS corresponded to the statistics of sensory stimuli such as intensity or interval when network received external inputs with rate modulation. Under the correlation modulation, a network showed rich variety in phase space of instantaneous population activity obtained as rate heterogeneity over individual neurons. It was also found that only the stimulus previously applied during learning phase could evoke the sufficient RSS. On the other hands, un-experienced stimuli failed to generate such spike sequences. Our result suggests that an internal representations of cortical circuit during development employ spatiotemporal population activity in an experience-dependent manner.

Disclosures: **T. Matsumura:** None. **T. Yuasa:** None. **S. Kang:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.23/NNN39

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

Support: Cognitive Science and Technology Council

Title: Quasi cycle induced cross frequency

Authors: *R. FARHOUDI[1], A. ABBASIAN[2], M. FOTOUHI[3];
[1]Mathematics, Sharif Univ., Tehran, Iran, Islamic Republic of; [2]Inst. For Res. In Fundamental Sci., Tehran, Iran, Islamic Republic of; [3]Shrif Univ. of Technol., Tehran, Iran, Islamic Republic of

Abstract: It is widely known that cross-frequency couplings in brain activity may result from interacting physiological processes. Among these couplings the phase amplitude couplings is by far the most studied as for example, the theta-gamma rhythm in the hippocampus .Although a common starting point is the observed spectral correlations based on the recorded neural activity there is as yet no consensus on the exact mechanism of such dynamical interactions. Mathematically these oscillations could be derived from deterministic models such as the well known Wilson-Cowan equations where the existence of multiple limit cycles belonging to different neural populations plays a key role. Here, we approach the problem through fixed point dynamics in the presence of noise where instead of limit cycle dynamics we observe a quasi-cycle behavior due to the pulling action of noise away from the fixed point dynamics. In the case of a simple non-linear stochastic dynamics we show that to obtain meaningful results with respect to cross frequency modulation we need a second order approximation to the corresponding stochastic equation. Indeed, restriction to first order linear approximation may introduce artificial spectral correlations not related to real physiological interactions among neural populations. To our knowledge this is the first time to show how in the absence of intrinsic oscillations a phase-amplitude modulation may arise in the presence of noise for a given range of network connectivity among different population of neurons.

Disclosures: R. Farhoudi: A. Employment/Salary (full or part-time): Sharif unversity. **A. Abbasian:** None. **M. Fotouhi:** None.

Poster

368. Models of Excitability: Networks and Single Neurons I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 368.24/NNN40

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

Support: EPSRC CDT Studentship

GlaxoSmithKline

Title: PyPN - a tool for simulating peripheral nerves

Authors: ***C. H. LUBBA**[1,2], Y. LE GUEN[1], S. JARVIS[1], N. JONES[3,2], S. SCHULTZ[1,2];
[1]Dept. of Bioengineering, [2]Ctr. for Neurotechnology, [3]Dept. of Mathematics, Imperial Col. London, London, United Kingdom

Abstract: Manipulations of the peripheral nervous system (PNS) may have great potential for clinical applications, as suggested by the Bioelectronic Medicines initiative. In aid of this, a computational model of the peripheral system is an important long term goal, allowing normal, pathological and modulated function to be investigated virtually. We propose here a simulation tool, PyPN (Python Peripheral Nerve simulator), as a first step towards such a large scale model. In its current form PyPN allows the simulation of a single nerve bundle with axons carrying individual spike trains and being exposed to external stimuli.
PyPN is a modular Python wrapper for NEURON and LFPy (Lindén et al, Front. Neuroinf 7:1-15, 2014). It models axons as independent from each other and surrounded by a homogeneous medium. Myelinated axons follow the McIntyre model (J Neurophysiol, 87(2):995-1006, 2002), with parameter extrapolation for smaller diameters. The main building block is a peripheral nerve bundle containing a variable number of axons, and following a given path. In order to give these axons a realistic shape, they follow the nerve core with segment direction changes drawn from a random distribution, with parameters that can be extracted from confocal microscopy (e.g. Brainbow mouse data). Individual fibres or groups can be stimulated either via model electrodes with defined geometry or optogenetic stimulation using a kinetic opsin model (Grossmann et al J Computat Neurosci 34:477-77, 2012). Recording electrode configurations are also defined geometrically.
We found good agreement between our simulation and experimental recordings for conduction velocities, membrane voltages, single spike shapes and compound action potentials (CAPs). As peripheral nerves are thin and long, an accurate simulation needs to solve the cable equations with many individual segments causing relatively long runtimes (7.5 s to simulate 30 ms of activity per fibre on an i7-4600U processor); however, as axon simulations are independent in the model, substantial parallelization is possible.
PyPN is the first computational toolbox for peripheral nerve simulation that brings together models for stimulation, nerve morphology and recording electrodes. It can already be used in various ways, e.g. to investigate the effects of stimulation, build the relationship between CAP and fiber distribution or to generate surrogate data for decoding algorithms. In the future the combination of multiple nerve bundles, automated branching and merging functionality and models for varicosities and organs will pave the way for large scale PNS simulation.

Disclosures: **C.H. Lubba:** 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; GSK. **Y. Le Guen:** None. **S. Jarvis:** None. **N. Jones:** None. **S. Schultz:** None.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

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Program#/Poster#: 369.01/NNN41

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

Support: NIH Grant RO1GM098578

Title: The capacity of information integration of alpha band is associated with non-responsiveness

Authors: *H. KIM, U. LEE, V. PHILLIP, B.-B. TARIK, J. LEE, G. MASHOUR;
Univ. of Michigan, Ann Arbor, MI

Abstract: Background: The integrated information theory suggests that the level of consciousness can be measured with the amount of information integrated in the brain. Tononi suggested Phi to quantify the amount of integrated information a system generate as a whole above the amount of information its parts generates independently. However, because of heavy computation of Phi, the application to empirical data has been limited to small channels of the brain activities. In this study, we modulated the level of consciousness for human subjects with anesthesia, and quantified it with a statistical method to estimate Phi from a high-density EEG data. **Method:** Three different anesthetics, propofol, isoflurane, and ketamine, were applied to modulate the level of consciousness of human volunteers from light to deep anesthesia, recording 128 EEG channels. We examined the average Phi value of 100 sets of 8 EEG channels, which were selected randomly at resting state and compared across the other states. We focused on the relative change of average Phi rather than the exact Phi value of a 128 channel EEG. In addition, spectral power, connectivity (weighted Phase Lag Index), and network modularity of diverse frequency bands (delta, theta, alpha, beta and gamma) were investigated across states of consciousness (resting, responsive with slow waves, non-responsive with slow waves, non-responsive with burst suppression, and recovery). **Results:** Deep anesthesia with burst suppression induced zero Phi value for all frequency bands. However, the state with non-responsiveness and slow waves still showed large frequency specific Phi values. Across all anesthetics, the average Phi value was increased in delta, theta and gamma, whereas decreased in alpha band. In the brain network, the number of modules was reduced for the delta, theta and gamma bands, whereas increased for the alpha band during non-responsive state. It implies that each frequency wave has distinctive way of information integration in the network level. **Conclusion:** Despite of non-responsiveness, the brain has a large Phi value. Only Phi of the alpha waves correlates with non-responsiveness, irrespective to the type of anesthetics, while complimenting with large Phi of the other brain waves.

Disclosures: **H. Kim:** None. **U. Lee:** None. **V. Phillip:** None. **B. Tarik:** None. **J. Lee:** None. **G. Mashour:** None.

Poster

369. Computational Tools for Human Data I

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

Support: NIH R01 GM098578

James S. McDonnell Foundation Collaborative Grant #220020419

Title: Reintegration of regional brain functions during gradual and abrupt brain recoveries after a major perturbation

Authors: ***U. LEE**[1,2], M. KIM[1,3], G. MASHOUR[1,4,2];
[1]Anesthesiol., Univ. of Michigan Med. Sch., Ann Arbor, MI; [2]Ctr. for Consciousness Sci., Ann Arbor, MI; [3]Dept. of Physics, Pohang Univ. of Sci. and Technol., Pohang, Korea, Republic of; [4]Neurosci. Grad. Program, Ann Arbor, MI

Abstract: Background: How the brain reconstitutes consciousness and cognitive functions after a major perturbation is an important question with significant neuroscientific and clinical implications. Recent empirical studies with animal and human demonstrated that the emergence from unconsciousness is not random but ordered, demonstrating gradual and abrupt transition patterns. However, there has been no principled explanation about how the disintegrated brain functions are reintegrated during gradual and abrupt transitions. To study the underlying network mechanism, we investigated explosive synchronization, which suggests that if a network has a specific network configuration, it facilitates abrupt transition from incoherent to synchronized network. Otherwise, the network shows gradual transition.
Method: We simulated gradual and abrupt transitions with anatomical human brain network from diffusion tensor images, which include 82 cortical and subcortical regions. Kuramoto model was implemented to each node as neural population activity, and a positive correlation between node degrees (the number of connections) and natural frequencies of nodes was provided to induce explosive synchronization. To quantify the reintegration order for nodes, we defined the reintegration time for each node counting the steps of coupling strength taken to reach a high level of network synchronization (from 0.2 to 0.7), which corresponds to the brain activity at unconscious state and resting state, respectively. The relationship between the reintegration time and node degree in both gradual and abrupt transitions was studied.

Results: We found that for gradual transition the reintegration time of 82 brain regions has a significant correlation (Spearman coefficient of -0.45; $p<0.01$) with the node degrees, whereas for abrupt transition the integration order is predictable only among hub nodes (nodes with dense connections) before the transition. Furthermore, we found that the typical modular structure of human brain network plays a crucial role to determine the integration time and the overall emergence patterns.
Conclusion: The brain network structure determines the reintegration order of regional brain functions during gradual transition. The reintegration order during abrupt transition is partially predictable in early emergence progress. Regarding the generality, this novel network approach would provide a principled explanation about how the brain reconstitutes consciousness and cognitive functions after physiologic (sleep), pharmacologic (anesthesia), and pathologic (coma) perturbations.

Disclosures: **U. Lee:** None. **M. Kim:** None. **G. Mashour:** None.

Poster

369. Computational Tools for Human Data I

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

Support: NIH: ULTTR001108

NIH: 1U54MH091657

Indiana University College of Arts and Sciences

Title: Sparse-tensor framework for computational analysis of brain connectomes

Authors: ***C. F. CAIAFA**[1], F. PESTILLI[2];
[1]Indiana Univ. / CONICET, Bloomington, IN; [2]Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Diffusion weighted Magnetic Resonance Imaging (dMRI) combined with fiber tracking algorithms enables the measuring of anatomy and tissue properties of the human white-matter in living brains (Wandell 2016; Jbabdi et al. 2015). By measuring living brains, this technology makes it possible to correlate white matter properties with human behavior and cognition, as well as development and aging processes. The availability of these modern measurements has the potential to allow for the transition from simple qualitative descriptions of white matter to full quantitative models of tissue organization and to enhance the renewed

interest in mapping human connectomes - the full map of brain connections. We present a computational framework to represent brain connectomes efficiently using sparse-tensors (multidimensional arrays). The framework takes as input the anatomy of a full set of brain connections generated using any dMRI data and tractography method and returns as output a tensor representing fundamental fascicles properties, such as position, identity and angle of curvature. We describe the computational framework and show applicability to the analysis of in-vivo human brain measurements using several brains from multiple data sets. We report results on data with more than 1,000 connectomes and two dMRI datasets (Van Essen et al. 2013; Pestilli et al. 2014). Ten connectomes were generated for each brain using multiple tractography methods (constrained-spherical deconvolution (Tournier et al. 2012; Descoteaux et al. 2009) and the tensor (Basser et al. 2000) models). Results describe computational means of performing fundamental anatomical operations on white matter connectomes. More precisely, we describe mechanisms to perform the following operations on brain tissue using multidimensional arrays: (1) we show that the framework can efficiently implement forward models of diffusion signal (Pestilli et al. 2014), and (2) identify brain connections and establish their statistical evidence in individual brains. Our results show that mapping white matter fascicles to tensors allows for connectome anatomy to be studied more efficiently. Because of its computational efficiency, the framework opens new avenues of investigation to understand the white matter structure in individual brains and across large populations of brains.

Disclosures: **C.F. Caiafa:** None. **F. Pestilli:** None.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

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Program#/Poster#: 369.04/NNN44

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

Support: NIMH Grant MH071589

Title: Studying intersubject networks and standard graph measures during dynamic threat processing

Authors: ***M. NAJAFI**, L. PESSOA;
Univ. of Maryland, College Park, MD

Abstract: Different individual brain regions act in synchrony when perceiving natural stimuli, such as movies. At the same time, network properties within individuals are reorganized in important ways under different emotional and cognitive conditions. What is the relationship

between intersubject synchrony and networks defined via within-subject data (as in standard graph analysis)? To answer this question, we investigated functional MRI data (N=64) during a dynamic threat of shock paradigm used to create anxious states. In this experiment, the proximity of receiving shock was continuously manipulated by the distance of two colored circles moving around randomly on the screen; if the circles collided, a mild electric shock was delivered to the participant's hand. This paradigm allowed participants to continuously track the proximity of threat via the distance between the two circles, and whether threat was approaching or receding. In standard intersubject correlation analysis, each voxel (or ROI) time series is correlated with itself in other subjects. Here, we generalized this strategy to perform intersubject network analysis by computing the correlation of the each participant's ROIs time series with that of all of the other ROIs of other participants. The standard within-subject network that is used in graph theory applications to brain data was defined via correlating ROI time series with one another within each subject. We investigated several graph centrality measures including betweenness, which captures the participation of a region in the flow of information through the entire network. As in previous studies, we considered several large-scale brain networks (salience, task-positive, -negative), in addition to the amygdala and bed nucleus of the stria terminalis (BNST). Intersubject network analysis revealed increased connectivity in the salience network. Furthermore, we detected a positive linear relationship of betweenness scores (across ROIs) computed on within-subject and inter-subject networks. During threat approach (vs. withdrawal) betweenness increased for the salience network and the BNST. Together, these initial findings help uncover the relationship between intersubject synchrony and other frequently utilized graph measures used in the literature to study large-scale brain networks.

Disclosures: **M. Najafi:** None. **L. Pessoa:** None.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

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Program#/Poster#: 369.05/NNN45

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

Title: A quantitative systems pharmacology platform of brain and serum progranulin (PGRN) to investigate targets in frontotemporal dementia (FTD)

Authors: ***C. FRIEDRICH**, M. M. PRYOR;
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Abstract: Frontotemporal dementia (FTD), the second most common form of neurodegenerative dementia, is characterized by extensive neuronal loss, TDP-43 pathology, and gliosis. FTD can

be caused by loss of function mutations in the *GRN* gene that results in a haploinsufficiency of the progranulin (PGRN) protein. Therapies are being developed to restore the expression and distribution of PGRN. Here, we describe the development of a PGRN PhysioPD™ Research Platform, a graphical and mathematical model of PGRN production, uptake, clearance, and transport in brain and periphery. Quantitative integration of public and proprietary data sets during development led to interesting insights, including: 1. Microglial PGRN production far exceeds neuronal PGRN production on a per cell basis, yet neuronal PGRN production *in vivo* contributes significantly to overall brain PGRN concentration due to the higher numbers and longer PGRN intracellular half-life of neurons relative to microglial cells. 2. Modeling of two proprietary datasets revealed an apparent inconsistency in the intracellular half-life of PGRN in neurons. To reconcile the apparent conflict, the team formulated and tested hypotheses, revealing insights about neuronal PGRN production and uptake. 3. There are significant differences in PGRN dynamics in periphery vs. brain, suggesting that caution should be used in interpreting serum PGRN level as a biomarker for brain PGRN level. A recent study appears to support this modeling research insight (Wilke et al. Curr Alz Res 2016 Mar 14, Epub). The PGRN PhysioPD Research Platform has proven useful to simulate the effects of modulating different targets and model drug effects on increasing PGRN for the treatment of FTD.

Disclosures: C. Friedrich: None. **M.M. Pryor:** None.

Poster

369. Computational Tools for Human Data I

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

Support: DARPA HAPTIX Contract No. N66001-15-C-4014

VA Merit Review #I01 RX00133401

NIH Grant 2T32EB004314-16

Title: Developing patient-specific, in-situ computational models using intraoperative ultrasound.

Authors: *I. CUBEROVIC[1], M. A. SCHIEFER[2], J. ANDERSON[3], D. J. TYLER[1]; [1]Biomed. Engin., [2]Case Western Reserve Univ., Cleveland, OH; [3]Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: Electrical stimulation of the median and ulnar nerves through cuff electrodes evokes sensory percepts in multiple unique locations across the hand. Models derived from human

cadaver neuroanatomy suggest that increasing contact count and using field shaping with multiple simultaneously active electrodes can further refine stimulation selectivity. However, even when stimulation parameters are limited to: 1-4 electrodes, -3.0 - 3.0 mA, 0 - 255 μs, and 0 - 1000 Hz, the stimulus search space exceeds an intractable 180 million combinations. Modeling and large parameter search algorithms can reduce this search space. The morphology of the nerve affects the shape of the electric field produced by an extraneural electrode. Typically, models are developed with Monte Carlo techniques based on cadaver anatomy. We hypothesize that using patient-specific fascicular mapping will improve the accuracy of patient specific models and lead to more optimal clinical stimulation paradigms while minimizing the clinical time necessary for stimulation tuning. This study reports on the use of intraoperative high-resolution ultrasound (US) to develop patient-specific, *in-situ* nerve models.
One subject was implanted with Composite Flat Interface Nerve Electrodes (CFINE) in April 2016. While undergoing surgery to implant a bidirectional neuroprosthesis, the median and ulnar nerves were exposed via medial incision proximal to the subject's left elbow. The site was flooded with saline and an L8-18i hockey stick ultrasound probe (GE Healthcare, UK) encased in a sterile sleeve was placed directly on each nerve with a short-axis orientation. 18 MHz B-mode images of the nerve were obtained on a GE Logiq E9 US machine (GE Healthcare, UK). After surgery, the video was imported into MATLAB (The MathWorks, Inc., Natick, MA). Each frame was filtered with a debaucheries 2 wavelet filter to reduce speckle noise. The filtered frames were then spatially registered and temporally averaged to obtain a single cross-sectional image of the nerve and the fascicular arrangement at the CFINE location. We converted the image to a biophysical finite element method (FEM) model of the median and ulnar nerves in a 10 x 1.5 mm CFINE. We calculated voltages along axons positioned within the fascicles of the patient-specific FEM model. A linear approximation determined regions of axon activation within the nerve as a function of stimulus parameter. Using these modeling techniques we are able to "test" millions of stimulation paradigms *in-silica* to find the few most likely to produce desired sensory perceptions. The predicted stimulation codes can then be applied experimentally to validate and further refine the model.

Disclosures: I. Cuberovic: None. **M.A. Schiefer:** None. **J. Anderson:** None. **D.J. Tyler:** None.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

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Program#/Poster#: 369.07/NNN47

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

Support: Universidad Autonoma de Occidente

Title: Fear - driven changes of mind in a decision-making neural network model

Authors: *P. A. GONZALEZ-PARRA[1], J. HURTADO-LOPEZ[1], D. F. RAMIREZ-MORENO[2];
[1]Mathematics, [2]Physics, Univ. Autonoma de Occidente, Cali, Colombia

Abstract: A model for a decision making neural network that expresses different modes of decision and the possibility of transitions among them is developed. The DM network proposed follows a bottom-up guided process, exhibits the co-existence of different modes of decision depending on an external modulatory parameter, allows a way for approaching the problem of considering “changes of mind”, on a basic and effective synaptic circuitry.
There are natural scenarios where humans or animals subjects evaluate to stay or flee places in front of distant or close threats and modify the responses while considering and estimating complementary information such as economic valuations or emotional conditions.
Our DM network provides two behavioral choices (the "stay" and the "go" options) and deploys three modes of decision. The first one drives the DM network into the “stay” choice for any input, the second mode allows the subject to choose between the two alternatives, and the third mode is fixed into the “go” choice. The first and third modes drive the DM network into a single choice regardless of the input stimuli while the second mode falls into the common mode of choosing the best of two choices.
Our DM network has the capacity of making transitions among these modes due to a modulatory parameter provided by an external neural network. The external neural network considered as a modulatory source is a fear-processing neural network. The proposed DM neural network model has a first layer that operates in a WTA mode and successive feedforward inhibitory projections that allow the modes of decision described above. Under certain values of the modulatory variable a particular decision mode appears. The modulatory input acts as a control variable on inducing transitions among the decision modes.

Disclosures: P.A. Gonzalez-Parra: None. **J. Hurtado-Lopez:** None. **D.F. Ramirez-Moreno:** None.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 369.08/NNN48

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

Support: NIH Grant R25 GM61331

Title: A hybrid computational model for optimizing neuromuscular electrical stimulation of peripheral nerve fibers

Authors: *P. D. ARGUELLO, I. PEREZ, L. TONG, D. S. WON;
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Abstract: Neuromuscular electrical stimulation (NMES) has been proven to restore a degree of ambulatory functionality in patients who have sustained spinal cord injury (SCI). These therapeutic effects are enabled via stimulation of both the efferent and afferent fibers found in the peripheral nervous system. It has been shown that by changing stimulus parameters, the efferent or afferent pathways could be activated preferentially (Veale et al., 1973). However, a physiological model to explain how certain combinations of stimulation parameters lead to preferential activation of the peripheral nerve fibers has yet to be elucidated. By developing an electrophysiologically accurate model of the neuromuscular control of force generation in a rodent hindlimb model, we hope to identify A) the stimulation pattern and combination of stimulation parameters that most selectively activates the afferent fibers thus optimizing force output and B) a feasible physiological mechanism that helps explain the optimal stimulation parameters. Our system model includes a compartmental efferent fiber modeled in NEURON, the extracellular electrical field potentials generated by a finite element model of an intramuscular stimulating wire microelectrode, an object-oriented 2-level central pattern generator model, and a musculoskeletal model of a rodent hindlimb using MSMS (MDDF, University of Southern California) which produces measures of muscle force. We hypothesize that stimulation pulse widths of 1 ms and frequencies less than 100 Hz generate the most force, indicating achievement of maximal afferent fiber recruitment, in accordance with experimental data obtained by Collins, by activating afferent pathways which lead to recruitment of nerve fibers through activation of spinal central pattern generator circuitry (Collins et al., 2002). The results of this study could inform and improve future applications of NMES for spinal cord injury rehabilitation. The funding for this work was provided by NIH Grant R25 GM61331

Disclosures: **P.D. Arguello:** None. **I. Perez:** None. **L. Tong:** None. **D.S. Won:** None.

Poster

369. Computational Tools for Human Data I

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Program#/Poster#: 369.09/NNN49

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

Support: NIH R01 MH081023

NIH R01 MH101173

Title: Diagnostic prediction of autism in resting-state functional mri using conditional random forest

Authors: *B. T. FAIRES[1], C. A. NASAMRAN[1], A. JAHEDI[2], C. CHEN[2], J. FAN[3], R.-A. MÜLLER[4];
[1]Biomed. Informatics, [2]Computat. Sci., [3]Mathematics and Statistics, [4]Psychology, San Diego State Univ., San Diego, CA

Abstract: Background
Autism spectrum disorder (ASD) is characterized by social and behavioral impairments. Although it is a neurodevelopmental disorder, no unique brain biomarkers for ASD are known. Previous research has used machine learning and computational statistics to mine MRI data for ASD biomarkers. Chen et al. (*NICL* 2015) achieved 90.8% diagnostic accuracy using the ensemble learning technique, random forest (RF). However, RF is known to have an intrinsic variable selection bias (Strobl et al., *BMC Bioinformatics* 2007). In order to eliminate this bias and to increase the interpretability of the results, we developed a conditional random forest (CRF) ASD diagnostic prediction model for resting-state functional MRI data (rs-fMRI).
Methods
Rs-fMRI data from 252 patients (126 ASD and 126 TD) were selected from the Autism Brain Imaging Data Exchange (ABIDE). Preprocessing techniques and participant selection criteria were adopted from Chen at al. to allow for direct comparisons between the RF and CRF models. Connectivity matrices for each participant were created using 220 regions of interest (ROI) from Power et al. (*Neuron* 2011). The dimensionality of the dataset was reduced to eliminate noise and for computational feasibility.
Results
The CRF model achieved a diagnostic accuracy of 92.5-95% (in two runs with different seeds) from 180 most informative connections between ROIs. Most informative connections (normalized based on total number of possible connections per network) were heavily represented in somatosensory/motor (especially mouth region), ventral attention, salience, cingulo-opercular, memory retrieval, and cerebellar networks. Raw numbers for default mode network (before normalization) were also high.
Discussion/Conclusions
CRF reached very high, though not perfect, diagnostic prediction accuracy based on a complex set of 180 functional connectivities. Reduced variable selection bias compared to the earlier RF study (Chen et al., 2015) resulted not only in slightly improved accuracy, but also in some changes in the relative network composition of most informative connections. Whereas preponderance of somatosensory ROIs was slightly lower for CRF (compared to RF), cingulo-opercular, ventral attention, and cerebellar ROIs were found to be more informative. Overall, the findings suggest that rs-fMRI data may be a source for complex biomarkers of ASD.

Disclosures: **B.T. Faires:** None. **C.A. Nasamran:** None. **A. Jahedi:** None. **C. Chen:** None. **J. Fan:** None. **R. Müller:** None.

Poster

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

Support: K01 MH097972

R01 MH081023 (RAM)

Title: Resting state fMRI connectome differentiating autism from typical development

Authors: ***A. JAHEDI**[1], V. MEENI[2], A. LINKE[5], S. NAIR[3], C. P. CHEN[4], B. A. BAILEY[1], R.-A. MÜLLER[3];
[1]Dept. of Mathematics and Statistics, [2]Bioinofrmatics, [3]Dept. of Psychology, [4]Dept. of Computat. Sci., San Diego State Univ., San Diego, CA; [5]Dept. of Pshycology, SDSU Brain Imaging Develop. Lab., San Diego, CA

Abstract: Background: Autism spectrum disorder (ASD) denotes a set of neurodevelopmental conditions characterized by an impaired social interaction and repetitive behaviors. Although considered a neurological disorder, brain biomarkers remain elusive. We used multivariate distance matrix regression (MDMR), a connectome-wide association framework (Shehzad et.al., 2014), to detect voxels with brain connectivity patterns that differ between typically developing (TD) and ASD participants.**Methods:** We included 6-minute resting state fMRI scans from 45 ASD and 38 typically developing (TD) participants, ages 8-17 years. Groups were matched on age, non-verbal IQ, and head motion. Data were preprocessed using nuisance regressors from six motion parameters, white matter and ventricles, and derivatives. Time points with motion >0.5 mm and two subsequent time points were censored. All included subjects had >80% remaining time points. We used MDMR to detect voxels for which whole-brain connectivity differed in ASD vs TD groups. Whole brain results were corrected using cluster-extent thresholding ($p<0.05$) as implemented in FSL. We then conducted whole-brain functional connectivity analyses using significant clusters from MDMR as seeds. **Results:** MDMR analysis identified 10 clusters in occipito-temporal regions, posterior cingulate cortex (PCC), and cerebellum as maximally distinctive between ASD and TD groups. For left lingual gyrus, whole-brain functional connectivity results showed overconnectivity across extended visual regions, contrasted by frontal and temporo-parietal underconnectivity. A similar pattern of

overconnectivity with visual areas and fronto-temporal underconnectivity was seen for an MDMR hotspot in left cuneus. PCC seeds showed overconnectivity with prefrontal executive regions and ventral visual stream. MDMR clusters in left cerebellum were characterized by extensive bilateral overconnectivity with cerebral cortex. **Conclusions:** Our results suggest that whole brain connectomes of occipital and neighboring parietal and temporal regions as well as cerebellum differentiate children and adolescents with ASD from their TD peers. While these findings and those from post-hoc functional connectivity analyses for MDMR hotspot seeds are in line with some previous studies, they highlight that posterior (mostly visual and default mode) brain regions and their connectomes may critically underlie autistic symptomatology. MDMR findings for left cerebellum - consistent with a previous report (Khan et al. *Biol Psychiatry* 2015) - suggest cerebro-cerebellar overconnectivity as a candidate biomarker for ASD.

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Poster

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Title: Explicitly linking regional activation and functional connectivity: community structure of weighted networks with continuous annotation

Authors: ***A. MURPHY**[1], S. GU[2], N. F. WYMBS[3], S. T. GRAFTON[3], D. S. BASSETT[2]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Bioengineering, Univ. of Pennsylvania, philadelphia, PA; [3]Psychological and Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: A major challenge in neuroimaging lies in understanding how neurophysiological dynamics map on to cognitive functions. Traditionally, these maps have been constructed by linking a region to a cognitive function by assessing changes in activity magnitude related to task performance. More recent lines of work in the emerging field of network neuroscience have begun to construct maps linking the patterns of connectivity between many regions to specific cognitive functions by drawing on mathematical tools from network science and graph theory. However, the two views offered by activity and connectivity have rarely been addressed in concert, leading to a fundamental lack of understanding in the relationship between them. This gap can be largely explained by the fact that few tools have been developed that explicitly account for connectivity patterns **between** nodes at the same time as accounting for activation values **on** nodes. Here we address this gap by developing a new technique that can be used to uncover groups of brain regions (nodes) that are both functionally connected (edges) and share similar activation magnitudes (annotations). More specifically, we solve the problem of community detection on weighted networks with continuous annotations by deriving a generative probabilistic model. This model generates communities whose members connect more densely to nodes within their own community than to nodes in other communities, and whose members share similar annotation values. Therefore, communities are a function of both the network and the annotations. We demonstrate the utility of the model in the context of neuroimaging data and offer intuitions and quantitative results to show the influence of model parameters on estimated community structure. Then, we apply the method to data gathered during a motor learning paradigm, where edges are task-based functional connectivity and annotations to each node are beta weights from a GLM that encoded a linear decrease in BOLD activation with practice. Interestingly, we observe that individuals who learn at a higher rate exhibit the greatest dissimilarity between functional connectivity and activation magnitudes. This tool offers an explicit, mathematically principled link between functional activation and functional connectivity, and can be readily applied to a wide variety of neuroimaging datasets.

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Poster

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Program#/Poster#: 369.12/NNN52

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

Title: Interrogating temporal functional cortical connectivity patterns with source level narrowband induced activity and deep recursive neural networks

Authors: Z. HARPER[1], *C. M. WELZIG[2];
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Abstract: Magnetoencephalography (MEG) can capture functional connectivity with high spatiotemporal resolution; however, complex, subject-specific cortical interaction features and temporal patterns require novel techniques to define them (Larson-Prior, Oostenveld et al. 2013). We have developed a semi-supervised machine learning system to study such connectivity dynamics. First, feature extraction targets interactions between clusters of intrinsic functional connectivity that differentiate cognitive states across two working memory tasks. Input for training is based on a graph representation of narrowband network activity across these clusters. We emphasize preserving critical spatiotemporal information inherent to dynamically changing oscillatory and synchronistic patterns of functional activity across the cortex (Hutchison, Womelsdorf et al. 2013). The input for our machine learning system is a bipartite graph with nodes and directed edges derived from source-localized MEG signals. Nodes are produced by clustering resting state network (RSN) cortical parcellations (Yeo, Krienen et al. 2011) and RSN consensus communities (Power, Cohen et al. 2011). Edge weights represent source level average narrowband induced activity calculated between nodes. Across samples, these weights are time-locked with task-related stimulus events. This system uses a deep recursive neural network built on a Long-Short Term Memory architecture to learn temporal oscillatory dynamics that cannot be captured within a single sample. This method allows for training on modulating oscillatory patterns by preserving elements of a specified range of contiguous samples relative to each sample used for training. As this system allows for examination of trained network activation at each layer, we are able to identify dynamic patterns of network interactions that mediate differentiation. Thus, interpretation of weights at specific layers within the network can highlight cluster interactions that mediate functional connectivity within classified cognitive states over time.

Disclosures: Z. Harper: None. **C.M. Welzig:** None.

Poster

369. Computational Tools for Human Data I

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

Support: NSFC Grant 91432105

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Title: Video compression applied to 10-TB-sized volumetric brain images: a preliminary study

Authors: ***A. LI**, Y. CHEN, Y. LI, Q. LUO, H. GONG;
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Abstract: We can obtain 10-TB-sized volumetric images with the development of microscopic technique, but such large datasets raise many issues on data storage, transmission and sharing. There is much information redundancy in continuous images, in general case, we can not use the redundant information in axial direction when coding the images in compressed format. In recent years, there have many studies on 3-D compression methods for volumetric images. Here we applied H.265, a new digital video coding standard, to compress different types of continuous microscopic images of brain tissue. The results show that video compression can effectively reduce the amount of data, particularly for sparse labeled tissue, the compress ratio can less than 10%. In the future, we can compress the whole brain dataset by adopting H.265 standard, this significantly lowers the threshold for making use of the large data in brain studies.

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Poster

369. Computational Tools for Human Data I

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

Support: NSF Grant 1514622

Title: Prediction of EMG trajectory using stochastic dynamical operators and neural recordings

Authors: ***M. ABOLFATH-BEYGI**[1], T. D. SANGER[1,2], S. F. GISZTER[3];
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Abstract: Our goal is to provide a new framework with the theory of stochastic dynamical operators (SDOs) that allows quantitative prediction of the relationship between any two scales of the nervous system during movement. The premise of the SDO theory is that it tremendously facilitates analyzing the nonlinear dynamics of neural populations in motor control by providing a mathematical framework where the dynamics effect of neurons can be linearly superimposed. The theory is capable of describing the sensory-motor effects of neurons, which is inherently

difficult due to the nonlinearity of their dynamics. As an initial step, we aim to link the firing patterns of interneurons to overt motor behavior in a spinal rhythmic movement. We present preliminary results for validation of this theory with the simulations of a Hodgkin-Huxley spinal network. We use a simulated two-level CPG model of the neural circuit, with 27 populations, which generates locomotor rhythmic activity simulating control of four muscles (Shevtsova et al. 2015). We first generate simulated EMG signals by filtering the spike trains of motoneuron pools and estimate their individual SDOs using their spiking event times and the changes in the EMG. An SDO stochastically maps the current state of the system into a change in the state. When a neuron fires, its activated SDO updates the probability distribution of EMG, which represents an internal belief of the system about EMG. When multiple neurons fire together and they are only coupled via the system dynamics, we can linearly superimpose their SDOs. Initially, we estimate the SDOs of individual neurons ahead of time and hold them constant during the dynamics. We use the spike number of each neuron in a 40ms time interval before the current time point to scale the strength of its SDO in the prediction of EMG in the next time step. Using the SDO framework, we are able to predict the trajectory of the EMG signal extracted from the Extensor motoneuron pool. For prediction generation, we use the superposition of the SDOs of four populations of pattern formation and rhythm generators, primary afferents and Renshaw cells. Starting with perfect a priori knowledge about the zero value of EMG at the onset of the extensor burst, we are able to regenerate the extensor EMG trajectory for 1000ms, which is the period of the rhythmic movement. The correlation coefficient between the estimated EMG trajectory and the simulated EMG is obtained as 0.996±0.001 (95% CI). This result is a primary step that demonstrates SDO theory has the potential to describe nonlinear network dynamics that was generated by a simulated neural network with realistic Hodgkin-Huxley models for the neurons.

Disclosures: **M. Abolfath-Beygi:** A. Employment/Salary (full or part-time): University of Southern California. **T.D. Sanger:** A. Employment/Salary (full or part-time): University of Southern California. **S.F. Giszter:** A. Employment/Salary (full or part-time): Drexel University College of Medicine.

Poster

369. Computational Tools for Human Data I

Location: Halls B-H

Time: Monday, November 14, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 369.15/OOO3

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

Title: Hyperalignment improves between subject classification of fmri brain activity during motor imagery

Authors: *S. M. AL-WASITY[1], A. VUCKOVIC[1], S. VOGT[3], Y. KOIKE[4], F. POLLICK[2]; [1]Sch. of Engin., [2]Sch. of Psychology, Univ. of Glasgow, Glasgow, United Kingdom; [3]Dept. of Psychology, Lancaster Univ., Lancaster, United Kingdom; [4]Precision and Intelligence Lab., Tokyo Inst. of Technol., Tokyo, Japan

Abstract: Introduction: A major limitation of Multivariate Pattern Analysis (MVPA) is the difficulty of aligning features of fMRI data across brains due to high variability in individual responses. To address this issue Haxby et al. (2011), suggested a method called Hyperalignment to align participant's patterns of ventral temporal cortex during object recognition into a common high dimensional space to facilitate the Between Subject Classification (BSC). In this study, we studied the capability of Hyperalignment to align the representational space of left hemisphere motor cortex during the Motor Imagery (MI) of complex right arm movements. **Method**: Ten right-handed participants (2 female, age=32.5 ±7.6) participated in two fMRI experiments using a 3T Siemens MRI scanner. In Experiment 1 participants performed MI of knocking, lifting and throwing movements for 8 runs of 456s each. In Experiment 2 participants performed 7, 320s runs of combined MI and action observation of a specially constructed set of animations containing movement blends of knocking, lifting and throwing (Vangeneugden, et al., 2008). The data of the first experiment were used for classification purposes while the data of the second one were used for Hyperalignment. Data were analysed using a combination of Brainvoyager QX2.8.4 and PyMVPA. Functional scans were pre-processed and co-registered to a common anatomical space and a mask used to extract functional data from Brodmann areas 4&6 of left hemisphere. For information decoding we used a Support Vector Machine (SVM) classifier for each pair of movements (lift vs knock, lift vs throw and knock vs throw). A SVM-Searchlight approach with a sphere radius of 3 voxels was used for each class pair to define a Region of Interest of the most discriminative voxels. Within Subject Classification (WSC) was performed by training a pair-wise linear SVM classifier using a leave run out cross validation approach. For Between Subject Classification (BSC), the SVM classifier was trained using leave one subject out cross validation. BSC was performed on the data that were aligned anatomically using Talairach space and on the data that were aligned using the Hyperalignment based on data from the blended movements. **Results**: We compared the classification accuracy of the pair-wise SVM for averaged WSC to anatomically aligned BSC and Hyperaligned BSC. Results showed that performance of Hyperaligned BSC was greater than anatomically aligned BSC, though averaged WSC still generally performed best. **Conclusion**: For a novel task (MI) and brain region (motor cortex), hyperalignment improved the performance of a multi-subject classifier to decode brain state.

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Poster

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NFL-GE Head Health Challenge I

Title: Sensitivity of the human brain structural networks to brain atlases, weighting methods, and tractography parameters

Authors: *K. WEI[1], M. CIESLAK[2], C. GREENE[3], S. T. GRAFTON[2], J. M. CARLSON[1]; [1]Dept. of Physics, [2]Dept. of Psychological & Brain Sci., [3]Dept. of Electrical Engin., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Network neuroscience leverages diffusion magnetic resonance imaging and tractography to quantify structural connectivity of the human brain. However, scientists and practitioners lack both a standardized tractography pipeline and a clear understanding of the effects of varying tractography parameters on the structural networks constructed using these methods. Taking advantage of the high quality diffusion images and the large sample size of the Human Connectome Project (HCP), we quantify how connectivity networks are impacted by the spatial resolution of the chosen brain atlas, total number of tractography streamlines, grey matter dilation, and network weighting methods. We investigate networks generated by varying these tractography parameters using graph metrics, including path length, assortativity, modularity, and clustering coefficient. Using path length, we demonstrate how injudicious combinations of highly refined brain atlas parcellations and low numbers of streamlines may inadvertently lead to pathologies in the network model construction involving isolated grey matter network nodes. We provide solutions to significantly reduce the likelihood of generating representations with detached regions, and examine the characteristics of the resulting network models before and after the solutions are applied. In addition, for different tractography parameters, we investigate the distributions of values taken by various graph metrics across the population of HCP subjects. While specific values of some metrics are sensitive to parameter choices, the placement of individual subjects within the overall distribution describing the population is generally preserved. Our work serves as a guideline for researchers to select the optimal tractography parameters and lays the foundation to establish a standardized tractography pipeline.

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Poster

369. Computational Tools for Human Data I

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

Support: GE/NFL Head health challenge

Title: Effect of different spatial normalization approaches ontractography and structural brain networks

Authors: ***C. A. GREENE**[1,2], M. CIESLAK[3], S. GRAFTON[3];
[2]Electrical and Computer Engin., [3]Psychological and Brain Sci., [1]UC Santa Barbara, Santa Barbara, CA

Abstract: We describe a critical analysis of the effect of different spatial normalization approaches on fiber tractography and structural brain networks. Population based tractography analysis is typically performed after spatially normalizing the diffusion weighted images (DWIs) followed by streamline reconstruction to enable comparisons in a standardized neuroanatomical space. Prior work has shown that tracts generated after normalization from reoriented DWIs do not match the tracts generated in native space. We show that spatial warping applied directly to the streamlines avoids the problems that arise when streamlines are generated from reoriented diffusion tensors, fiber orientation distributions (FODs), or orientation distribution functions (ODFs). We compare direct streamline warping against two publicly available DWI spatial normalization techniques where tractography is performed after normalization by measuring the impact of the different normalization schemes on structural brain networks and topologic properties from a subject pool of 440 HCP subjects. We use pairs of identical twins, fraternal twins, non-twin siblings, and non-related subjects to characterize the inherent variability in structural brain networks and show that they are heavily influenced by the spatial normalization approach with some changing the same subject's connectivity and network metrics after normalization to something more comparable to a distant or non-twin family member than themselves. Direct streamline normalization readily outperforms the other methods at preserving key native tract structure and anatomic properties of structural brain networks after spatial normalization.

Disclosures: **C.A. Greene:** None. **M. Cieslak:** None. **S. Grafton:** None.

Poster

369. Computational Tools for Human Data I

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Program#/Poster#: 369.18/OOO6

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

Support: 2 R01 NS047293-09A1

Title: Non-invasive skull conductivity estimation and EEG source localization

Authors: *Z. AKALIN ACAR, S. MAKEIG;
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Abstract: We have previously described an iterative skull conductivity and source location estimation (SCALE) algorithm for simultaneously estimating skull conductivity and brain source locations [Akalin Acar et al., *Neuroimag*e, 2016]. SCALE uses a realistic FEM head model and scalp maps of near-dipolar sources identified using independent component analysis (ICA) decomposition of sufficient high-density EEG data. For 2 young adult subjects, we applied SCALE to MR head images and 13 near-dipolar independent components (ICs) obtained from decomposition of 45 minutes of 128-channel EEG data by multi-model adaptive mixture ICA (AMICA). SCALE estimated brain-to-skull conductivity ratio (BSCR) as 34 and 54 respectively We also applied SCALE to 20 minutes of 64-channel EEG data and magnetic resonance (MR) head images from 4 12-month infants. Applied to 15-16 near-dipolar independent components from the AMICA decomposition, SCALE returned BSCR estimates in the 10-12 range. Concurrently, SCALE found plausible, compact sulcal or gyral cortical source distributions for the IC sources. For one of the adults (BSCR=34), we tested the error in dipole source localization arising from using different BSCR values in source localization. Skull conductivity estimation by SCALE improved the equivalent dipole source estimation by 13 mm in average. Here, we used 13 independent components (ICs) for SCALE source localization. To test the best number of IC maps to use in SCALE, we performed SCALE simulation studies using 20, 15, 10, and 5 simulated IC scalp maps, again modeling the skull as a uniform conductivity layer. In all these (noise-free) simulations SCALE estimated skull conductivity correctly but converged sooner when we used fewer IC maps. In actual data, however, skull conductivity is not homogeneous; if the brain ICs are predominantly in one brain region, then the skull conductivity estimated using these components should reflect the skull conductivity near that region. We explored this by performing 4 SCALE fits, each using 8 IC maps randomly selected from the 13 available. Two of the fits again converged to BSCR = 34. However, others converged to BSCR values of 25, and 41. Using 18 and 22 ICs from this subject also gave BSCR = 34. We were able to obtain more dipolar ICs for this dataset using 2-4 model AMICA decomposition. Increasing the number of ICs to 22 or 28, SCALE again gave BSCR = 34. These results suggest that when the ICs used

in SCALE represent processes in brain areas more or less evenly distributed across the brain volume, the SCALE skull conductivity estimate does not vary.

Disclosures: Z. Akalin Acar: None. **S. Makeig:** None.

Poster

369. Computational Tools for Human Data I

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

Support: ULTTR001108

1U54MH091657

Title: The posterior associative white matter network between the human temporal and parietal brain lobes

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Abstract: Two major cortical pathways of visual information have been hypothesized in the primate brain; the ventral and dorsal streams (Goodale & Millner 1992). New evidence for direct white matter pathways within the posterior human cortex, communicating information between these streams, has renewed interest in this hypothesis. Recent findings suggest that there are at least three vertical fiber tracts connecting the dorsal and ventral streams. These tracts proceed all the way from the occipital lobe, in the case of the vertical occipital fasciculus (Yeatman et al 2014; Takemura et al 2015), to the parietal lobe, as is the case for the parietal arcuate (Catani et al 2005; Weiner et al 2016). In the present work, we compare the statistical strength of evidence (Pestilli et al 2014) for the major tracts connecting the dorsal and ventral stream in the posterior human cortex.
We used diffusion-weighted magnetic resonance imaging data from two publicly available data sets (Human Connectome Project; Rokem et al 2015). A total of 26 hemispheres were analyzed. We used ensemble tracking methods (Takemura et al 2016) to build whole-brain connectomes using multiple probabilistic tracking methods (600,000 fascicles) per brain. White matter tract

identification was performed using published methods (Yeatman et al 2016; Takemura et al 2015). In addition, we developed an automated tract segmentation method using a combination of cortical parcellation (Destrieux et al 2010), fascicle projection, and density measures.
We report a new tract-segmentation method and measure the statistical evidence for multiple white matter tracts connecting the two visual information processing streams, such as the arcuate fasciculus, the parietal arcuate, and vertical occipital fasciculus. Our results reproduce previous findings from both in-vivo and postmortem studies (Lawes et al 2008; de Champfleur 2013, Takemura et al 2015, Weiner et al 2016). Our findings extend beyond previous results, suggesting a more complex network of ventro-dorsal communication than previously established.
Historically, neuroanatomical observations have focused on a number of associative white matter tracts running predominantly rostro-caudally, for example, the superior and inferior longitudinal fasciculi (Catani et al 2012). The dorsal and ventral streams, running posterior to anterior, have typically been described as parallel and structurally segregated. Our results provide quantitative evidence that an extensive network of communication pathways exist between these two functional streams. We provide a detailed description of the major structural organization of such a network.

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