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**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 553.01/A1

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

**Support:** NTU

The Ministry of Science and Technology

**Title:** A single calcium-binding domain is sufficient to mediate the Syt III's regulation of retinal waves

**Authors: *H.-Y. CHEN**[1], C.-T. WANG[1,2,3,4]
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

**Abstract:** Patterned spontaneous activity in the developing vertebrate visual system, known as retinal waves, is important for the establishment of correct neural circuits. Particularly, stage II retinal waves are essential for refining retinal projection to central brain. The stage II waves during the first postnatal week is initiated by acetylcholine release from starburst amacrine cells (SACs) via calcium-regulated exocytosis, further propagating onto neighboring SACs and retinal ganglion cells (RGCs). We previously showed that a calcium sensor protein, Synaptotagmin I (Syt I), is consistently expressed in developing SACs and RGCs. Weakened calcium binding to either C2A or C2B domain of Syt I can abolish the Syt I's regulation of retinal waves from either SACs or RGCs, suggesting the importance of the C2AB domains of Syts in regulating the spatiotemporal properties of stage II retinal waves. In contrast to Syt I, Syt III is abruptly upregulated in developing RGCs during the stage II wave period, and has been shown important for regulating wave properties. However, it remains unknown which calcium-binding domain has a dominant effect on the Syt III-mediated regulation of stage II wave properties. To answer the question, RGCs were transfected with genes of interests, including Syt III and its two mutants (Syt III-C2A*, harboring D386N; Syt III-C2B*, harboring D520N), by using the RGC-specific promoter (pBrn3b). Live calcium imaging was further conducted to detect the changes in wave-associated calcium transients after transfection. We found that Syt III in neonatal RGCs enhanced the frequency but reduced the spatial correlation of retinal waves compared to control. In contrast, the spatiotemporal properties of wave-associated calcium transients remained unchanged by overexpressing Syt III-C2A* or Syt III-C2B* compared to Syt III. Thus, our results revealed that unlike Syt I, a single calcium-binding domain is sufficient to mediate the Syt III's regulation of retinal waves. Given that RGCs release glutamate, our results imply that Syt

III in RGCs may play an important role in mediating glutamate release from RGCs, through calcium binding to either C2A or C2B domain.

**Disclosures: H. Chen:** None. **C. Wang:** None.

**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 553.02/A2

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

**Title:** Role of RNA Binding Proteins in the stress response: RNA metabolism and consequences in motor behavior in mice

**Authors: *C. M. FREIRE-COBO**, B. JORDAN
Albert Einstein Col. of Med., New York, NY

**Abstract:** How chronic stress unbalances synaptic protein homeostasis to exacerbate synaptopathies is unknown. Regulation of translational and transcriptional pathways constitutes part of the cell response to environmental stress. Sam68 (Src associated protein in mitosis, 68kD) and FMRP (Fragile X Mental Retardation Protein) are RBP RNA-binding proteins (RBPs) that mediate downstream signaling in response to neuronal activity and other internal/external stimuli. Sam68 and FMRP regulate the splicing, translation and transport of important synaptic mRNA cargoes in neurons and immune system components. The main effect caused by the lack FMRP is elevated protein synthesis and mGluR-dependent long-term depression (mGluR-LTD), a form of synaptic plasticity that requires protein translation. mGluR-LTD is dysfunctional in a number of neurodevelopmental disorders including Autism spectrum disorders (ASD). Our group has shown that mGluR-LTD expression is impaired in Sam68 KO mice. We hypothesize that maladaptive response to stress stimuli alter proteostasis through Sam68 function, and that this process regulates how stress aggravates behavioral phenotypes in Sam68 KO mice. We addressed how pharmacologically induced stress altered Sam68 and FMRP-dependent RNA metabolism in primary cultures, and in Sam68 and FMRP KO mice. We compared how stress impacts the ataxic phenotype observed in Sam68 and Fmr1 mutant mice at different developmental stages. Moreover, we analyzed how external stressors influence the availability and function of core elements that link translation to synaptic function. Finally, we compare how stress differentially affects Sam68 and FMRP cargo expression. Overall, our work provides insights into how stress affects the homeostatic control of neural networks. We propose that stress alters the expression of factors that affect brain plasticity, which ultimately aggravates synaptopathy-based diseases. The significant impact of environmental stressors on quality of life of ASD patients highlights the importance of further studies on how stress regulates symptoms that might be considered as diagnostic of disease progression.

**Disclosures:** **C.M. Freire-Cobo:** None. **B. Jordan:** None.

**Poster**

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

**Support:** R01-NS070005

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R00-NS072192

Disorder Fund for Neuro- science Research at Harvard University

**Title:** FGF22-IGF2 signaling regulates hippocampal synaptic stabilization and affective behaviors

**Authors: *A. TERAUCHI**[1], E. JOHNSON-VENKATESH[1], B. BULLOCK[1], M. LEHTINEN[2], H. UMEMORI[1]
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**Abstract:** Neurons in the developing brain must build complex networks by forming appropriate synaptic connections with one another. Synaptic development can be separated into two critical stages. First, a new synapse is assembled, and then the synaptic contact is stabilized. Initial synapse assembly is a local event at the contact sites and requires the exchange of molecules between pre and postsynaptic sites, while subsequent synaptic stabilization requires transcriptional regulation and is regulated by neural activity. Previously, we found that fibroblast growth factor 22 (FGF22), released from postsynaptic hippocampal CA3 pyramidal neurons, is required for initial presynaptic assembly of glutamatergic synapses. However, much less is known about the signals that regulate the stabilization of synapses and the genes that are involved. Here, we asked whether retrograde FGF22 signaling regulates gene expression in presynaptic neurons to control the stabilization of presynaptic terminals. By using microarray analysis, we found that a gene encoding insulin-like growth factor 2 (IGF2) was less expressed in the dentate granule cells (DGCs), major presynaptic neurons of CA3 pyramidal neurons, in *Fgf22*KO mice compared to wild-type mice. Application of FGF22 on DGC axons induced IGF2 expression in the cell body of DGCs. IGF2 is then transported to DGC presynaptic terminals in an activity-dependent fashion to stabilize these structures. We further found that IGF2 is not required for initial assembly of DGC-CA3 synapses but is required for their stabilization both in vitro and in vivo. Interestingly, FGF22-IGF2 signaling is not involved in the stabilization of CA3-CA3 synapses. These results indicate that FGF22 controls both the assembly and

stabilization of synapses: FGF22 directly controls local synapse assembly, and then FGF22 via IGF2 expression regulates synapse stabilization in a pathway specific manner.
The hippocampus plays a key role in multiple behaviors such as mood, anxiety, and social cognition. We found that *Fgf22*KO (null) mice exhibit passive stress-coping behavior in the forced swim test and tail suspension test as well as anhedonia in the sucrose preference test without changes in other behavior tasks relevant to anxiety, social cognition, and motor phenotypes. *Igf2*KO mice also exhibit passive stress-coping behavior. These results suggest unique roles of FGF22-IGF2 signaling in affective behaviors. We are currently identifying specific neurons and developmental periods that are critical for FGF22-IGF2 signaling in regulating affective behaviors.

**Disclosures: A. Terauchi:** None. **E. Johnson-Venkatesh:** None. **B. Bullock:** None. **M. Lehtinen:** None. **H. Umemori:** None.

**Poster**

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

**Support:** NWO, TOP ZonMw, no. 912.10.009

ALW Open Program, no. 819.02.017

Vici, no. 865.12.001

**Title:** Functional mapping of synaptic inputs *In vivo* followed by molecular identification of synaptic origin

**Authors: *A. H. LEIGHTON**[1], N. ZABOURI[1], J. E. CHEYNE[2], C. LOHMANN[1]
[1]Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [2]Univ. of Auckland, Auckland, New Zealand

**Abstract:** During development, spontaneous waves of depolarizations sweep across the visual system, shaping connections into a refined network by strengthening useful synapses and weakening others. This activity also allows inputs to be organized along the dendritic branch, resulting in a system where co-active synapses are located close together. Modelling studies had predicted that such sub-cellular organization would greatly increase the computational ability of the cell. However, we do not yet know the exact function of these clusters in the visual system. They could separate inputs, segregating synapses that arise from different sources. Alternatively, they could act as integration centers that mix inputs from different sources, allowing the cluster to act as a coincidence detector. To test these options, the origins of synaptic inputs onto a single

cell must be identified. Here, we describe a method to image synaptic inputs *in vivo* and subsequently characterize the source of those same synapses using immunolabelling. First, we recorded individual synaptic inputs along the dendrite *in vivo* in mouse pups (P8-P13), by performing simultaneous whole-cell recordings and 2-photon calcium imaging of GCaMP6 and dsRed expressing pyramidal cells in primary visual cortex (V1) layer 2/3. Recording pipettes were coated in Alexa-594 for visualization in the brain and contained biocytin for post-hoc identification of imaged cells. Brains were then perfused and whole cortices were cleared using the SeeDB2 method. Synaptic inputs from the thalamus and cortex into V1 are mutually exclusive populations, and can be distinguished as such by immunohistochemistry for vesicular glutamate transporters (VGluT1), namely VGluT1 (cortical) and VGluT2 (thalamic) expressing inputs. Confocal z-stacks of the target (biocytin+) cells were acquired at high resolution, up to a depth of 240 µm. The confocal images were then deconvolved and rendered in 3D with Huygens software, allowing cortical and thalamic contacts to be identified. We have obtained reliable labeling, clearing and confocal imaging and we have been able to locate target cells and target dendrites.

**Disclosures:** **A.H. Leighton:** None. **N. Zabouri:** None. **J.E. Cheyne:** None. **C. Lohmann:** None.

**Poster**

**553. Synapse Maturation**

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

**Support:** NIH 5R01NS031651

NIH 1R21NS053807

Grass Fellowship

**Title:** Non-hebbian activity-dependent synaptic refinement at the *Drosophila* nmj

**Authors: *F. J. VONHOFF**, H. S. KESHISHIAN
Molecular, Cellular, and Developmental Biol., Yale Univ., New Haven, CT

**Abstract:** The removal of off-target contacts is essential for establishing precise synaptic connectivity. In many systems Hebbian spike timing is involved, often associated with low frequency calcium and cyclic nucleotide oscillations. Here we describe a novel form of activity-dependent refinement at Drosophila neuromuscular junctions (NMJs) that may be non-Hebbian: silencing the presynaptic but not the postsynaptic side leads to extensive synaptic miswiring. Low frequency oscillatory presynaptic activity and calcium signaling regulate the motoneuron's

response to a muscle-derived chemorepellant, Sema2a, acting via the PlexinB receptor. Live imaging of intact embryos expressing the Ca indicator GCaMP reveals episodic calcium signals in motoneuron growth cones and filopodia. Ca transients are evident in both native and ectopic contacts, oscillating at 0.03Hz towards the end of embryogenesis. Genetic and RNAi knockdown tests show that the refinement depends on both the Ca(v)2.1 and Ca(v)3 channels. We are testing the effects of Ca dynamics on filopodial exploration and withdrawal at both correct and off-target muscles by manipulating Ca transients using the temperature-gated channel TrpA1 and the red-shifted channelrhodopsin Chrimson. Downstream of calcium, three Ca-dependent effectors act within a common signaling pathway to regulate refinement: the Ca-dependent adenylyl cyclase Rutabaga, the kinase CaMKII, and the phosphatase Calcineurin. Loss of function mutations in the corresponding genes disrupts normal refinement, leading to ectopic neuromuscular synapses on up to 30-40% of the examined muscle fibers. We have optogenetically activated the bPAC adenylyl cyclase and demonstrated that intracellular cAMP levels are required to be dynamically maintained within an optimal range for proper refinement. Downstream of cAMP, both PKA and protein phosphatase 1 (PP1) interact with CaMKII and Calcineurin to regulate the neuron's responsiveness to Sema2a-dependent chemorepulsion. Live imaging reveals that partial loss of Sema2a increases ectopic filopodial contacts onto off-target muscles. An off-target contact frequency of 0.85 filopodia/min was observed in Sema2a/+ heterozygotes compared to 0.35 filopodia/min observed in controls. We are currently testing the effects of manipulating the different molecular components of the pathway on filopodia dynamics on correct and incorrect muscle targets. These results reveal a novel mechanism for synaptic refinement, where the response of the innervating cell to retrograde chemorepulsion is regulated by oscillatory presynaptic Ca activity.

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

**Poster**

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**Title:** Dendritic spine remodeling promoted by NrCAM in cortical pyramidal neurons during adolescence through the Semaphorin3F holoreceptor

**Authors: *P. F. MANESS**[1], V. MOHAN[1], C. SULLIVAN[1], J. GUO[2], S. WADE[1], S. MAJUMDER[1], A. AGARWAL[3], E. S. ANTON[2], B. TEMPLE[1]
[1]Dept Biochem., [2]Neurosci. Res. Ctr., UNC Sch. of Med., Chapel Hill, NC; [3]Johns Hopkins Med. Inst., Baltimore, MD

**Abstract:** Dendritic spines of excitatory cortical pyramidal neurons are dynamic actin-rich protrusions that are overproduced postnatally, eliminated in substantial numbers during adolescence, then stabilized during synaptic maturation. Although much is known about spine morphogenesis, less is understood about mechanisms of adolescent spine pruning and maturation. Neuron-glial related cell adhesion molecule (NrCAM) is an Ig- class cell adhesion molecule that is required for limiting the density of populations of dendritic spines and excitatory synapses in mouse cerebral cortex. NrCAM is an obligate component of the receptor complex for Semaphorin 3F (Sema3F), a secreted ligand that induces spine elimination in cultured neurons through NrCAM, Neuropilin-2 (Npn2) and PlexinA3 (PlexA3). To determine if NrCAM functions in pyramidal neurons and to define its time course of action *in vivo*, we generated a novel NrCAM floxed mouse line and crossed it to Nex1Cre-ERT2 mice to inducibly delete NrCAM specifically from pyramidal neurons. By deleting NrCAM at different time windows, we determined that NrCAM constrains spine density of cortical pyramidal neurons in early adolescence, but deletion in adulthood had no effect. NrCAM regulated spine density on apical but not basal dendrites of pyramidal neurons in both visual (layer 4) and medial prefrontal cortex (layers 2,3). Re-expression of NrCAM by *in utero* electroporation of NrCAM-null embryos demonstrated that NrCAM regulates spine density *in vivo* in a cell autonomous manner, and requires the NrCAM PDZ binding motif. This motif (SFV) bound the synapse-associated scaffold protein SAP102, which localizes to nascent synapses.
Molecular modeling and mutagenesis identified an interface between the NrCAM Ig1 domain (TARNER) and Npn2 a1 domain (E56) that promoted clustering of Npn2 and PlexA3 in the dendritic membrane. Receptor oligomerization, in turn, activated PlexA3 Rap-GAP signaling essential for Sema3F-induced spine pruning in cortical cultures. Further structure-function analyses supported the conclusion that binding of NrCAM Ig1 to the Npn2 a1 interface, together with cytoplasmic PDZ scaffold protein interaction, stabilizes weaker Npn2-PlexA3 binding, so that Sema3F dimers can more efficiently form a signaling holoreceptor.
Temporal restriction of NrCAM function to adolescent rather than adult stages adds to evidence that neocortical mechanisms of juvenile and adult plasticity differ. Genetic linkage of NrCAM, Npn2, PlexA3, and Sema3F to autism spectrum disorders, in which increased spine density has been observed, may implicate the mechanisms identified here in neurodevelopmental disease pathology.

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**Poster**

**553. Synapse Maturation**

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

**Support:** NIMH F32MH100745

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**Title:** Conditional deletion of all neurexins defines diversity of essential synaptic organizer functions for neurexins

**Authors: *L. Y. CHEN**, M. JIANG, B. ZHANG, O. GOCKE, T. C. SUDHOF
Stanford Inst. for Neuro-innovation & Translational Neurosciences, Stanford Univ., Stanford, CA

**Abstract:** Neurexins has been thought as the key organizers of synapses that are essential for normal brain function. To date, it remains unclear whether neurexins are fundamental building blocks of all synapses with similar overall functions, or context-dependent specifiers of synapse properties. To address this question, we produced triple conditional knockout mice that allow ablating all neurexin transcripts in mice. Using neuron-specific manipulations combined with immunocytochemistry, paired-recordings, and two-photon $Ca^{2+}$-imaging, we analyzed excitatory synapses formed by climbing fibers on Purkinje cells in cerebellum, and inhibitory synapses formed by parvalbumin- or by somatostatin-positive interneurons on pyramidal layer 5 neurons in the medial prefrontal cortex. After pan-neurexin deletions, we observed in these synapses severe but dramatically different phenotypes that ranged from major impairments in their distribution and function (climbing-fiber synapses) to large decreases in synapse numbers (parvalbumin-positive synapses) and severe alterations in action-potential-induced presynaptic $Ca^{2+}$-transients (somatostatin-positive synapses). Our results suggest that neurexins primarily function as context-dependent specifiers of synapses.

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**Poster**

**553. Synapse Maturation**

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

**Support:** NTU

The Ministry of Science and Technology

**Title:** The role of ipRGCs in mediating Synaptotagmin I's regulation of retinal waves during development

**Authors: Y.-W. LIN**[1], *C.-T. WANG[1,2,3,4]
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

**Abstract:** The refinement of visual circuits requires the patterned spontaneous activity in developing retinas, named retinal waves. Particularly, stage II retinal waves are essential for retinal projection to central brain. During the first postnatal week in rodent, stage II retinal waves are initiated by neurotransmitter release from starburst amacrine cells (SACs), propagating through the neighboring SACs and retinal ganglion cells (RGCs). However, we previously found that manipulating a calcium sensor during exocytosis (synaptotagmin I, Syt I) in RGCs by using the Brn3b promoter can regulate the spatiotemporal properties of stage II retinal waves. The effects can be abolished by ionotropic glutamate receptor antagonists (RGCs release glutamate), suggesting that a retrograde circuit may exist between RGCs and SACs, further regulating wave properties. Previous studies showed that melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) project axon collaterals to SACs, forming a retrograde circuit. However, it remains unknown whether ipRGCs may involve in Syt I's regulation of retinal waves during development. To answer this question, we determined whether Syt I's regulation of retinal waves can be attributed to ipRGCs. First, developing rat retinas were transfected with pBrn3b-HA-Syt I. Further, immunofluorescence staining was performed in whole-mount retinas to analyze the localization of ectopic Syt I expression. By immunostaining transfected RGCs with HA antibodies, ipRGCs with melanopsin antibodies, and SACs with ChAT antibodies, we found that 25% of HA-labeled cells demonstrated immunoreactivity against melanopsin and 85% of HA-labeled cells showed immunoreactivity against Brn3. By constrast, only ~5% of colocalization was found between HA-labeled cells and ChAT-labeled cells. Thus, our analysis indicated that the Brn3b promoter may drive ectopic Syt I expression in a population of ipRGCs, suggesting that ipRGCs may play a role in mediating Syt I's regulation of retinal waves during development.

**Disclosures:** **Y. Lin:** None. **C. Wang:** None.

**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

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

**Support:** NIH/NINDS Grant F31NS089223

DoD NDSEG Program

**Title:** Climbing fiber synapse proliferation precedes pruning at Purkinje cells in the developing mouse cerebellum

**Authors:** ***A. M. WILSON**[1], R. SCHALEK[1], A. SUISSA-PELEG[1], T. JONES[2], S. KNOWLES-BARLEY[3], J. W. LICHTMAN[1]
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**Abstract:** For many vertebrate animals, the nervous system undergoes a drastic rewiring between birth and adulthood. This process, known as synapse elimination, is essential to normal development, but little is understood about the mechanisms that make it work, especially in the central nervous system. To study the developmental changes that occur during the earliest phase of synaptic rewiring in the cerebellum, we have used high-throughput electron microscopy to trace out the synaptic connections between climbing fibers and Purkinje cells in mice at postnatal days 3 and 7. Our analysis shows that many more climbing fibers innervate developing Purkinje cells than previously estimated and that each climbing fiber innervates a group of nearby Purkinje cells. Throughout the first postnatal week these climbing fibers functionally differentiate by adding synapses, as opposed to changing the sizes of existing synapses or removing them. Specifically, climbing fibers preferentially add synapses onto Purkinje cells they already strongly innervate, so that by the end of the first postnatal week typically one or at most a few climbing fibers dominate each cell. As they add synapses onto some target cells, climbing fibers remain weakly connected with others. These results argue that the eventual dramatic phase of climbing fiber synapse elimination in the cerebellum does not occur until a dominant climbing fiber is designated for each Purkinje cell.

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**Poster**

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

**Support:** NIH Grant R35NS097224

CIHR fellowship 358761

**Title:** Stromalin is a master regulator of synaptic vesicle biogenesis

**Authors: *A. PHAN**[1], C. I. THOMAS[3], M. CHAKRABORTY[2], J. A. BERRY[2], N. KAMASAWA[3], R. L. DAVIS, Prof[2]
[2]Neurosci., [1]The Scripps Res. Inst., Jupiter, FL; [3]Max Planck Florida Inst., Jupiter, FL

**Abstract:** The proper wiring of neural circuits during critical periods is necessary for proper adult cognitive function. However, the mechanisms required to orchestrate the precise wiring of memory systems remain unclear. Here we show that *stromalin*, a subunit of the cohesin complex known to be important for cell division, plays a role in *Drosophila* memory formation. We were surprised to find Stromalin knockdown (KD) in dopaminergic neurons (DAn) increased aversive olfactory memory acquisition. Strikingly, using a temperature-inducible RNAi expression system, we found that Stromalin KD in DAn during the 3$^{rd}$ instar larval stage was both necessary and sufficient to increase memory scores in adult flies. To elucidate how Stromalin affects DAn to enhance memory, we performed functional imaging experiments to examine DAn to mushroom body neuron (MBn) communication, which is important for the formation of olfactory aversive memory. Interestingly, we found Stromalin KD in DAn resulted in an increased cAMP signal generated in the MBn upon DAn stimulation, but that the DAn themselves did not have increased $Ca^{2+}$ responses to the stimulation. Thus, Stromalin KD in DAn strengthened the functional connection between DAn and MBn, which appears to underlie the enhanced memory acquisition seen in these flies. To probe the mechanisms behind this strengthened connection, we next examined whether Stromalin KD produced neuroanatomical effects in DAn. While the cohesin complex is known for its role in cell division, KD of Stromalin did not change DAn cell numbers, nor did it obviously affect their morphology. Stromalin KD did however increase levels of the presynaptic marker synaptotagmin:GFP (syt:GFP) in the DAn innervating the MB lobe. We showed, using super resolution structured illumination microscopy (SIM) of syt:GFP, that Stromalin KD did not affect the number or size of DAn synapses. We then turned to electron microscopy to image synaptic vesicles in DAn of the fly brain. Surprisingly, Stromalin KD DAn had 2-fold greater numbers of synaptic vesicles and dense core vesicles than control neurons. This appears to underlie the strengthened DAn to MBn connection. Remarkably, we report here that Stromalin KD does not affect the

neuroanatomy or network connectivity of DAn, but specifically increases the strength of otherwise normal connections by increasing synaptic vesicle numbers at the synapse. Our data demonstrate that the role of Stromalin is to developmentally suppress memory acquisition and synaptic strength by negatively regulating synaptic vesicle biogenesis. Funded by NIH R35NS097224 and CIHR fellowship 358761.

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**Poster**

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

**Support:** Vici, no. 865.12.001

**Title:** Spontaneous activity and mitochondrial dynamics during synapse development

**Authors: *C. SILVA**[1], M. V. F. BUSCH[3], M. V. ZWIETEN[4], C. LOHMANN[2]
[1]Netherlands Inst. For Neurosci., Amsterdam Zuidoost, Netherlands; [2]Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [3]Netherlands Inst. for Neurosci., Amsterdam Zuidoost, Netherlands; [4]Univ. of Amsterdam, Amsterdam, Netherlands

**Abstract:** Developing neurons constantly undergo functional and structural plasticity as they establish and eliminate synaptic contacts. Synapses are extremely energy demanding and mitochondria, the major ATP providers, are transported throughout axons and dendrites presumably to meet these high-energy demands at developing synapses. Furthermore, defects in mitochondrial motility can lead to impaired synaptic function and plasticity.
During development, spontaneously generated activity fine-tunes synaptic connectivity. Previous studies have shown that artificially enhancing activity arrests mitochondria at synapses. It is unknown, however, whether spontaneously occurring activity affects mitochondrial dynamics in the developing cortex, and, in turn, the formation of cortical networks. To address this question, we performed calcium imaging in organotypic slices and *in vivo* and investigated the relationship between spontaneously occurring calcium transients and mitochondrial motility during synapse development.
In organotypic slices obtained from P5 mice and kept in culture for 3 to 6 days (P5+DIV3-6), the average percentage of moving mitochondria, at a given moment, was 5.3% ± 3.6%. There were no differences in mitochondrial motility at different days *in vitro*. However, in slices obtained from older mice (P8+DIV3), mitochondrial motility was lower (1.5% ± 1.0%). *In vivo* (P8-11), the percentage of moving mitochondria was very low (0.1% ± 0.1%). Preliminary data showed

that at P5/6 *in vivo*, mitochondrial motility was 3.8% ± 0.9%. Together, this suggests a developmental decrease in mitochondrial motility *in vivo* that is not replicated in slice cultures. When we investigated the relationship between mitochondrial motility and spontaneously occurring calcium transients; neither *in vitro* nor *in vivo*, we found evidence that individual spontaneously occurring calcium transients could directly affect mitochondrial motility.
We observed, however, that stationary mitochondria seem to accumulate in stretches of dendrite with higher synaptic density.
We are currently further investigating the details of mitochondrial motility throughout synapse development, as well as the relationship between stationary mitochondria and synapses.

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**Poster**

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**Title:** Synergistic action of mAChR receptors,adenosine receptors and TrkB receptors in synapse elimination during neuromuscular junction development

**Authors: *N. GARCIA**, L. NADAL, E. HURTADO, A. SIMÓ, M. TOMÀS, V. CILLEROS, M. A. LANUZA, J. M. TOMÀS
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**Abstract:** The development of the nervous system involves an initially exuberant production of neurons, which establish an excessive number of synaptic contacts, and the subsequent reduction in both neurons and synapses as maturation proceeds. Hebbian competition between axons with different activities leads to the loss of roughly half of the neurons initially produced so connectivity is refined and specificity gained. The skeletal muscle fibers in the newborn neuromuscular junction (NMJ) are polyinnervated by several motor axons but by the end of the axonal competition, two weeks later, the endplates are innervated by a single axon. This peripheral synapse has long been used as a convenient model for synapse development.We used quantitative confocal imaging of the autofluorescent axons from transgenic B6.Cg-Tg (Thy1-YFP)16 Jrs/J and C57BL/6J mices to investigate the possible cooperation of the muscarinic autoreceptors (mAChR, $M_1$-, $M_2$- and $M_4$-subtypes), adenosine receptors (AR, $A_1$- and $A_{2A}$-

subtypes) and the TrkB receptor in the control of axonal elimination after the mice *Levator auris longus* muscle (at P9) had been exposed to several selective antagonist of the corresponding receptor pathways *in vivo*. We investigated the effect of simultaneous incubation with two inhibitors (two antagonists of two different receptor subtypes) as a pharmacological tool for revealing the possible occlusive or additive crosstalk effects between the corresponding receptors.We found that a potent effect of the $M_2$ receptor is largely independent of the other receptors in favouring axonal competition and loss. However, the other receptors ($M_1$, $M_4$, $A_1$, $A_{2A}$ and TrkB)cooperate and add their respective individual effects to increase axonal elimination rate. Thus both, cooperative and non-cooperative signaling mechanisms contribute to developmental synapse elimination in the neuromuscular system.This work was supported by a grant from the Catalan Government (2014SGR344) and a grant from MINECO (SAF2015-67143-P).

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**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 553.13/B3

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

**Support:** NRF Grant 2016M3C7A1905481

NRF Grant 2016R1A2B4006811

**Title:** BMP signaling modulates presynaptic neurotransmitter release in *Drosophila* neuromuscular junction synapses

**Authors: S.-H. LEE**[1], Y.-J. KIM[2], *S.-Y. CHOI[2]
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**Abstract:** Regulation of synaptic structure and function via interaction between presynaptic and postsynaptic neurons is critical to neural functions. In particular, Bone morphogenic protein (BMP) is a secreted molecule mediating retrograde signaling that is involved in the formation and maintenance of synaptic structure throughout many animal species. However, how BMP signaling modulates presynaptic neurotransmitter release is not yet clear. We studied the function of BMP signaling factors in neurotransmitter release in Drosophila neuromuscular synapses using loss-of-function mutants in genes for BMP modulators, Wit, Mad, and Dad. Larvae with mutations in wit and mad commonly showed a decreased synaptic bouton number in neuromuscular synapses. Larvae with dad mutations showed an increased bouton number. The

amplitudes of miniature EJC (mEJC) were normal for these mutants. Wit and mad mutants showed decreased evoked EJC (eEJC) amplitude and increased paired pulse facilitation, implying impaired presynaptic neurotransmitter release. We found a reduction in readily releasable neurotransmitters pool sizes in wit and mad mutants. However, dad mutants showed a normal probability of neurotransmitter release and readily releasable pool sizes and normal eEJC amplitude even with clear abnormalities in synaptic structure. These results suggested that BMP signaling was critical for each step of presynaptic neurotransmission. The results also suggested that BMP signaling modulated both synaptic structure and function independently and specifically.

**Disclosures: S. Lee:** None. **Y. Kim:** None. **S. Choi:** None.

**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 553.14/B4

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

**Title:** ArhGAP22 is a new regulator of spines development and function

**Authors: *A. LONGATTI**[1,2], L. MURRU[1], L. PONZONI[3,4], E. MORETTO[1], M. SALA[1], M. PASSAFARO[1]
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**Abstract:** ArhGAP22 is a member of the RhoGTPase-activating protein (GAP) family. It is involved in many processes in eukaryotic cells including organization of cytoskeleton, regulation of cell polarity, gene transcription and vesicular trafficking. In particular, one of the main function of ArhGAP22 is the inhibition of Rac1, a protein involved in cytoskeleton remodeling and in dendritic spines morphology.
The main aim of this project is to define molecular and functional mechanisms underlying biological properties of ArhGAP22 in nervous system taking advantage of a mouse model knockout (KO) for ArhGAP22. In fact, although previous works demonstrated that ArhGAP22 is expressed in brain (cortex, cerebellum, hippocampus) and preferentially in the post-synaptic compartment of excitatory synapses, specific mechanism and functional consequences of its activity in neurons have not been demonstrated yet.
Since ArhGAP22 is a selective inhibitor of Rac1, we analyzed the levels of active Rac1 in adult mouse brain. Rac1-GTP levels are significantly increased in KO mice. Moreover, since Rac1 is a positive regulator of dendritic spine formation, we performed morphological analyses of excitatory synapses in hippocampus. KO mice showed an increase in dendritic spines density and an altered morphology.

In parallel, we analyzed the effect of ArhGAP22 knock down on the expression profile of several pre and post -synaptic markers in hippocampal synaptosomes deriving from WT and KO mice. Surprisingly, western blot experiments showed that KO mice had a significative reduction of AMPAR GluA1 and GluA2/3 subunits expression.
To explore the functional role of ArhGAP22, we studied electrophysiological properties of hippocampal neurons of WT and KO mice. Although there were no differences in CA3-CA1 synapses baseline function and release probability between the two genotypes, LTP induction at the CA3-CA1 synapse was strongly impaired in KO mice compared to WT. Furthermore, we tested ArhGAP22 KO mice in several behavioral tests. Significant alterations in anxiety and learning/memory profiles have been observed.
Overall, these data suggest that ArhGAP22 silencing in mice leads to marked deficits in hippocampal-dependent cognitive functions. These alterations could be explained by abnormal dendritic spines number and maturation together with altered AMPA receptors composition.

**Disclosures: A. Longatti:** None. **L. Murru:** None. **L. Ponzoni:** None. **E. Moretto:** None. **M. Sala:** None. **M. Passafaro:** None.

**Poster**

**553. Synapse Maturation**

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**Program#/Poster#:** 553.15/B5

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

**Support:** DoD Grant W81XWH-09-1-0088

**Title:** Role of serotonin signaling on synaptic plasticity in tuberous sclerosis complex

**Authors: *W. FRANCESCONI**[1], R. KIRCHNER[2], F. BERTON[3], A. YOSHII[4]
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**Abstract:** Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutations in *TSC1* or *TSC2*. While it is a multi-organ disease, neurological symptoms are prominent and include epilepsy, intellectual disabilities and autistic behaviors. Dysregulated synapse formation and plasticity is likely to play a major role in pathophysiology of TSC. The TSC-1 and -2 proteins form a complex that downregulates the mammalian target of rapamycin (mTOR) pathway, and a gene mutation results in mTOR overactivation. mTOR is a critical regulator of protein synthesis as well as synaptic plasticity. Consequently, suppression of mTOR by rapamycin or its derivatives is one way to correct the pathophysiology of TSC. However, mTOR pathway has multiple roles and rapamycin neither specifically nor completely cures all

neurological symptoms. A greater understanding of dysregulated synaptic function will enable identification of specific therapeutic targets for epilepsy and cognitive disabilities in TSC. To identify the cause of these abnormal synaptic activities, we conducted an RNA-seq analysis and found an excess of transcripts associated with the serotonin signaling pathway. The amount of protein was also higher and its intracellular distibution was altered in pyramidal cells of the *TSC1*$^{-/-}$ cortex. We used calcium imaging and found that *TSC1*$^{-/-}$ cultured cortical neurons had frequent $Ca^{2+}$ bursts throughout the soma and dendrites that depended on a specific serotonin pathway while wildtype neurons showed random and scattered transients mostly within dendritic spines. Furthermore, daily injection of the sertonin pathway antagonist rescued the premature death of the mutant pups. Our results indicate synaptic dysregulation in TSC can be corrected by modulating serotonergic pathway and set the stage for developing a new therapeutic approach.

**Disclosures: W. Francesconi:** None. **R. Kirchner:** None. **F. Berton:** None. **A. Yoshii:** None.

**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

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**Program#/Poster#:** 553.16/B6

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

**Support:** NIH/NINDS NS069660

**Title:** Altered perineuronal net structure in Ptprz1 knockout mice

**Authors: *G. J. EILL**, *G. J. EILL, A. SINHA, R. T. MATTHEWS
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**Abstract:** A unique lattice-like substructure of neural extracellular matrix (ECM), perineuronal nets (PNNs), are key regulators of plasticity in the mature central nervous system (CNS). PNNs are found on a subset of neurons in the CNS, for example, they are prominently surrounding parvalbumin interneurons in the cerebral cortex. Despite their limited distribution, they profoundly impact developmental plasticity in areas such as the visual cortex as well as plasticity in numerous brain regions in the adult. The prominent role in these processes has led to their implication in multiple neuropsychiatric disorders, neurodegenerative disease and recovery after injury. However, despite clear evidence for a role of PNNs in regulating neural function, their exact molecular composition and structure is not well understood, which, in turn, has made it difficult to determine precise molecular function of PNNs. From previous work, PNNs are composed of a hyaluronan backbone saturated with an array of hyaluronan-binding chondroitin sulfate proteoglycans (CSPGs), link proteins, and glycoproteins. Previous work has suggested that in addition to the hyaluronan-binding CSPGs (primarily members of the lectican family), the CSPG RPTPζ/phosphacan is localized in PNNs. Receptor-type protein tyrosine phosphatase zeta

(RPTPζ), and a secreted splice variant (phosphacan), are derived from the *Ptprz1* gene and have been implicated in a variety of developmental processes. RPTPζ/phosphacan is interesting among the CSPGs because not only can it serve typical ECM functions, but direct signaling functions as well. However, the contribution of RPTPζ/phosphacan to PNN structure and function is not well understood. Using *Ptprz1* KO mice we investigated PNN formation via immunohistochemistry and biochemical techniques. We found that in the cortex and hippocampus, PNNs were severely disrupted in the absence of RPTPζ/phosphacan. Further analysis using cell culture and biochemical techniques revealed that RPTPζ/phosphacan mediates key interactions between known components of the PNN and the cell surface of net-bearing neurons. Therefore, our work demonstrates that RPTPζ/phosphacan is a central player in the formation of PNNs. We further disected how membrane bound versus secreted splice-variants of *Ptprz1* contribute to the formation and structure of PNNs. Overall, this work sheds new light on the structure of PNNs and is an important step towards understanding PNN function.

**Disclosures: G.J. Eill:** None. **A. Sinha:** None. **R.T. Matthews:** None.

**Poster**

**553. Synapse Maturation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 553.17/B7

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

**Title:** Synaptic pruning and phenylketonuria

**Authors: *G. M. RUNE**, G. SCHLEGEL
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**Abstract:** High levels of phenylalanine in serum due to mutations of the gene coding for the enzyme phenylalanine hydroxylase are considered to account for mental retardation in Phenylketonuria. In the hippocampus, which is related to learning and memory, of corresponding mutants ($Pah^{enu2}$) with high levels of phenylalanine, we found delayed synaptic pruning thus higher synaptic density. Physiologically, synaptic pruning is aimed at elimination of weak synapses by microglia and required for rearrangement of synaptic connectivity after birth. The delay in synaptic pruning in the $Pah^{enu2}$ mouse was consistently associated with reduced neuronal activity and reduced C3 complement expression, which is essential for activation of microglia. In order to control whether these effects are actually caused by high concentrations of phenylalanine in serum of these mutants, we studied the effects of the amino acid on synaptic connectivity in hippocampal cultures, where microglia was either absent or unaffected by elevated levels of phenylalanine. Unlike in the $Pah^{enu2}$ mouse, synapse density and dendritic length were dramatically reduced in hippocampal slice cultures and dispersion cultures respectively, indicating strong neurotoxic capacity of high levels of phenylalanine. Changes in

the p-/n-cofilin ratio, which were rescued by increasing neuronal activity, and reduced activation of the small GTPase Rac1 likely underlie structural alterations. Since C3 complement expression, which is required for microglia recruitment, was reduced in the $Pah^{enu2}$ mouse but not in hippocampal slice cultures, our data strongly suggest a two-fold effect of mutations in the gene coding for phenylalanine hydroxylase: reduced C3 complement accounts for increased synapse density due to delayed synaptic pruning, while the supraphysiological levels of phenylalanine impair neuronal activity followed by synapse loss and reduced dendritic length.

**Disclosures: G.M. Rune:** None. **G. Schlegel:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.01/B8

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

**Support:** MRC DTG

**Title:** Mesoscale calcium imaging of neonatal cortical connectivity

**Authors: *C. CROSS**, J. AGULLO CAMPELLO, M. C. ASHBY
Univ. of Bristol, Bristol, United Kingdom

**Abstract:** Early postnatal life is a period of rapid neuron growth, migration and synaptogenesis. With these anatomical developments, come changes to the functional activity of neural networks, but it is not yet fully understood how and when these changes occur, or when the network reaches a mature state. Activity in the primary sensory cortex in response to peripheral stimuli is not always present at birth, with different sensory modalities emerging at different stages. Spontaneous neural activity occurs before sensory inputs shape the network, also contributing to network maturation, including modulating of axon guidance and synaptic plasticity. The developing patterns of activity in the neuronal network of the cortex in early postnatal life are the focus of this study, using recordings of both spontaneous activity, and responses to sensory stimuli. It is hypothesised that both types of network activity will change with age, converging towards a mature pattern of activity. To assess cortical activity with high temporal and spatial resolution in the neonatal mouse brain, we used imaging of the genetically-encoded calcium indicator, GCaMP6. Emx1-IRES-cre (Jax # 005628) and Ai95d (Jax # 024105) mice were crossed to obtain offspring expressing the calcium indicator protein GCaMP6f in excitatory cortical neurons. Following removal of the scalp and implantation of a miniature head-fixation post, the cortex of awake neonatal pups was imaged using a tandem lens fluorescence macroscope. Spontaneous and sensory stimulus-evoked cortical activity was imaged at 50Hz, alongside recording of body movements by mechanical and optical sensors. This approach

allows the measurement of activity across almost the entire cortical surface at a resolution of ~50μm$^2$. Using this, we assessed the development of cortical activity patterns across neonatal maturation. Cortical activity, both spontaneous and triggered by somatosensory stimulation, is present from postnatal day 1. During postnatal maturation patterns of spontaneous and evoked activity undergo significant changes, including an increase in the frequency of spontaneous events and alterations in the spatial organisation of activity that suggest maturation of inter-regional connectivity. As such, we have assessed the developmental profile of early cortical network activity. This approach is not only useful for informing of the mechanisms of ontogenetic development, it can also offer a template for comparison with animal models of disease, providing a new mode of investigating aberrant neural network development.

**Disclosures: C. Cross:** None. **J. Agullo Campello:** None. **M.C. Ashby:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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**Program#/Poster#:** 554.02/B9

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

**Support:** Priority Program 1665 of the German Research Foundation

Collaborative Research Centre/Transregio 166 of the German Research Foundation

**Title:** Contribution of NKCC1-mediated GABAergic depolarization to neuronal network activity in the neonatal mouse hippocampus

**Authors: *J. GRAF**[1], C. ZHANG[1], O. W. WITTE[1], C. A. HÜBNER[2], K. HOLTHOFF[1], K. KIRMSE[1]
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**Abstract:** The developmental shift from depolarizing to hyperpolarizing GABAergic responses is a widely accepted developmental concept. The shift reflects a change in the intracellular chloride concentration in postsynaptic neurons which, in most cell types, is due to a differential expression of the chloride importer NKCC1 and the chloride exporter KCC2. Whereas the mechanisms of change in GABA signaling are well understood, the developmental impact on network maturation is still under debate. To address this issue, we here generated a mouse line with a conditional deletion of NKCC1 in cortical glutamatergic neurons. Electrophysiological recordings from CA3 pyramidal cells *in vitro* confirmed that NKCC1 deletion attenuated the depolarizing-excitatory effects of $GABA_A$ receptor-activation, whereas miniature IPSCs and EPSCs were found to be unaffected. Confocal $Ca^{2+}$ imaging revealed a strong reduction of highly synchronized network activity in hippocampal slices of conditional knock-out mice,

similarly to acute pharmacological inhibition of NKCC1. In addition, *in vivo* wide-field $Ca^{2+}$ imaging in the hippocampal CA1 region unveiled a minor reduction in the frequency of network events without significant effects on spatial extent, amplitude or kinetics. Conversely, local pharmacological block of $GABA_A$ receptors profoundly increased both frequency and amplitude of network events *in vivo*. In summary, our preliminary data support the view that in neonatal mice depolarizing GABAergic transmission can both facilitate and profoundly inhibit the generation of hippocampal network activity in a context dependent manner.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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**Program#/Poster#:** 554.03/B10

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

**Support:** NSF IOS 1353044

**Title:** Functional network connectivity during looming stimulus detection in midbrain tectal networks

**Authors: *A. S. KHAKHALIN**[1], C. D. AIZENMAN[2]
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**Abstract:** One of the important open questions in connectomics and neuroscience is whether feature detection in small networks relies more on specialized cells with highly selective input connections, or on distributed activation of sparse neural networks. In this study, we use in-vivo high acquisition rate calcium imaging to record network activation in the optic tectum of Xenopus tadpoles, while these networks respond to visual stimuli delivered to the tadpole eye. We first describe overall patterns of activity in tectal networks, and provide a classification of visually evoked responses in individual tectal cells. We then use several alternative computational methods (based on either coupled linear models or machine learning, and with different underlying assumptions) to reconstruct elements of tectal network topology from its activation data. While it is impossible to fully reconstruct network topology from a recording of a subset of its neurons in a situation of shared driving inputs, we attempted to correct for some of these complications using data-based modeling and hidden parameters sensitivity analysis, and report reliable differences between connectivity estimates for cells selective to either looming stimuli or full-field visual flashes. We then compare estimations of network topology and selectivity of individual tectal cells in older tadpoles to that in animals at earlier stages of development, to better describe the trajectory of functional network maturation and connectomic

reorganization. Finally, we propose a computational model of tectal network development that relies on spike-time-dependent plasticity, homeostatic plasticity, and reinforcement learning, to provide a general framework for the emergence of looming selectivity in visual sensory cells.

**Disclosures: A.S. Khakhalin:** None. **C.D. Aizenman:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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**Program#/Poster#:** 554.04/B11

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

**Support:** DFG (Priority Program PP1608:Fr1784/7-1)

NIH Grant 1002383

**Title:** Short-term plasticity and vesicle pool replenishment at inhibitory synapses of the auditory brainstem: Adaptations and refinements after hearing onset

**Authors: *D. J. WEINGARTEN**[1,2], N. MÜLLER[1], E. FRIAUF[1], H. VON GERSDORFF[2]
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**Abstract:** Mammals have evolved the ability to accurately pinpoint the location of high frequency sounds in the horizontal plane. This requires an auditory system that can distinguish sound signals that differ by minute amounts of intensity. Indeed, reliable synaptic transmission is a hallmark of the ascending auditory pathway where highly specialized excitatory synapses allow the processing of auditory information with exquisite temporal precision. However, less is know about how inhibitory synapses shape the processing of sound signals after hearing onset. Here we investigated the inhibitory synapses of the lateral superior olive (LSO) in the mouse auditory brainstem. A speeding of evoked excitatory and inhibitory postsynaptic currents (EPSCs & IPSCs) has been observed in the LSO during postnatal development. Yet, if changes occur in the presynaptic parameters remains unclear. Using whole-cell patch clamp recordings in acute brainstem slices we characterized inputs from the medial nucleus of the trapezoid body (MNTB) and the cochlear nucleus (CN) to principle neurons of the LSO (MNTB-LSO and CN-LSO synapses) via electrical stimulation of the respective axons. Recordings were done at 36±1°C from pre-hearing mice at postnatal day P10-12 and young adults at P28-34. Evoked IPSCs of MNTB-LSO synapses showed a 2-fold speeding of their kinetics from P10-12 to P28-34. In addition, IPSC onset latency was reduced from ~1 ms to below 500 µs (P10-12: n=9, P28-34: n=14). Miniature IPSCs (mIPSCs) showed a 2-fold speeding of their kinetics between both age groups. Furthermore, whereas mIPSC amplitudes remained unchanged around 25 pA, the

spontaneous mIPSC frequency doubled from 3 to 6 Hz. Via application of high-frequency stimulation trains (50 pulses of 50, 100, and 200 Hz) the readily releasable pool (RRP) and the delay and extent of vesicular replenishment in both ages were estimated. Surprisingly, a strong reduction of RRP vesicles from 600 to 200 vesicles was observed (P10-12: n=13, P28-34: n=7), whereas, the vesicular release probability tripled after hearing onset from 5 to 15%. To cope with this accelerated emptying of the RRP, MNTB-LSO synapses stimulated at 50 Hz showed a shorter onset of replenishment, which shifted from 150 ms to 100 ms (P10-12: n=13, P28-34: n=7). In both age groups this onset became shorter with higher stimulation frequencies, hinting at a $Ca^{2+}$-dependent mechanism. Introducing gaps of different lengths (10-5000 ms) in between stimulation trains revealed an increased $Ca^{2+}$-dependent RRP replenishment in P28-34. In summary, the mature MNTB-LSO synapse has remarkably fast and resilient presynaptic machinery for replenishing synaptic vesicles.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

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

**Support:** DFG(Priority Program PP 1608:Fr1784/19-1)

**Title:** Impaired topographic map refinement and synaptic strengthening of an inhibitory auditory microcircuit in Otoferlin knock-out mice

**Authors: *N. MÜLLER**[1], M. SONNTAG[2], E. FRIAUF[1]
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**Abstract:** During development, sensory neural circuits are established broadly and become subsequently refined prior to sensory input in an activity-dependent manner involving spontaneous activity. In the auditory system, the impact of spontaneous activity on circuit refinement is much less described than in other sensory systems, especially for inhibitory projections. Spontaneous prehearing activity is generated in the cochlea, and the activity pattern is conserved in downstream nuclei. To analyze the isolated role of spontaneous activity on circuit refinement of the inhibitory projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), we studied P11 Otoferlin$^{deaf5/deaf5}$ mice (Otof). Otoferlin is highly expressed in the inner ear, but not in MNTB and LSO. Otof mice show a strongly reduced vesicle exocytosis from inner hair cells. In line with this, *in vivo* recordings in the MNTB suggest

a lower number of active units. Furthermore, the frequency of residual MNTB activity was reduced and showed atypical patterns. Focal flash photolysis of caged glutamate in the MNTB while recording from LSO neurons in acute slices of Otof mice revealed a 2-fold broader mediolateral and dorsoventral input width. This strongly implies impaired synapse elimination, resulting in a distorted and imprecise topographic map. Besides synapse elimination, synaptic strengthening is a hallmark of circuit refinement. To assess this issue, we recorded from LSO neurons in acute slices while stimulating MNTB fibers with stepwise increasing stimulus intensities resulting in gradual recruitment of converging MNTB fibers. The number of MNTB fibers in Otof mice was increased by 50%, confirming the impaired elimination seen in the glutamate uncaging experiments. Furthermore, the single fiber strength was reduced by 40%, demonstrating impaired synaptic strengthening. To reveal its origin, we determined quantal parameters and found a 40% lower quantal content per MNTB fiber. We obtained further insight into the mechanism of strengthening via fluctuation analysis. The reduced quantal content was due to 40% fewer release sites per fiber, while the release probability and the number of vesicles released from a release site were normal. In conclusion, we demonstrate the requirement of spontaneous prehearing activity in topographic map refinement and strengthening of the inhibitory MNTB-LSO circuit. Our findings are novel in that they address effects of missing spontaneous activity restricted to the cochlea. This contrasts to other deafness models (VGlut3 and Cav1.3), in which relevant proteins are abolished both in the cochlea and the central auditory system.

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**Poster**

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

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**Title:** Mechanisms of a plasticity window closure in the CA1 region of the hippocampus

**Authors: *A. RODRIGUEZ-MORENO**[1], L. E. ARROYO-GARCÍA[1], M. PÉREZ-RODRÍGUEZ[1], G. FLORES[2]
[1]Univ. Pablo de Olavide, Sevilla, Spain; [2]Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico

**Abstract:** Spike Timing-Dependent Plasticity (STDP) is a strong candidate for a synaptic mechanism involved in development and in learning and memory. We have previously shown in the hippocampus that a post-before-pre pairing protocol (pairing postsynaptic action potentials with EPSCs at 0.2 Hz) produced robust input-specific t-LTD and that the induction of this form of LTD was completely blocked by D-AP5, by the broad spectrum mGluR antagonist MCPG, and that this form of t-LTD is present only until the third week of development. The goal of this work was to determine the mechanisms responsible for the closure of this plasticity window. We performed experiments in the CA1 region of hippocampal slices prepared from P13-P28 mice using the whole-cell configuration of the patch-clamp technique. To induce t-LTD, a post-pre pairing protocol (with the presynaptic activity occurring 18 ms after a postsynaptic action potential) was applied. We found that a post-before-pre pairing protocol was unable to induce a significant depression at P22-P28 (103 ± 6%, n = 7) in contrast to the t-LTD observed at P13-P18 (74 ± 6%, n = 8). The lack of t-LTD at P22-P28 was not due to an increase in GABAergic inhibition during development as in the presence of bicuculline or gabazine no t-LTD is observed at P22-P28 (98 ± 6%, n = 7). By contrast, in the presence of A1 adenosine receptor antagonists (as 8CPT), t-LTD is present at P22-P28 (76 ± 9%, n = 8), thus suggesting the involvement of A1R in the closure of the plasticity windows. These results suggest that spike timing-dependent depression disappears during the fourth week of development due, at least in part, to an increase of inhibition mediated by the activation of adenosine A1 receptors.

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**Poster**

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

**Support:** James Madison University College of Science and Mathematics Faculty Assistance Grant

James Madison University Light Microscopy and Imaging Facility

**Title:** A role for integrin beta 3 in dendritic spinogenesis and pruning in cerebral cortex

**Authors: K. M. BLAND**, Z. O. CASEY, C. J. HANDWERK, Z. L. HOLLEY, *G. S. VIDAL Biol., James Madison Univ., Harrisonburg, VA

**Abstract:** Dendritic spinogenesis and pruning in the cerebral cortex is a normal developmental process. Early in postnatal development, the cerebral cortex normally overproduces dendritic

spines, and then undergoes a period in which spines are pruned away, leaving behind spines that are postsynaptic sites of excitatory synapses important for neural function. Dysregulation of spine pruning in excitatory neurons of the cerebral cortex may lead to neurological disorders such as schizophrenia and autism spectrum disorder. Itgb3 is an autism susceptibility gene and integrin beta 3 KO in mice leads to decreases in callosal, striatal, and hippocampal volume. While it is known that integrin beta 3 forms heterodimers with integrin alpha V in neurons, it is unknown what functional role integrin beta 3 has in shaping cortical circuits early in life. To answer this, here we labeled layer II/III pyramidal neurons of mouse cerebral cortex via in utero electroporation and examined their dendritic spine density immediately before eye opening and 1-2 weeks later. Loss of function of integrin beta 3 was achieved by in utero electroporation of Cre recombinase to a mouse line with loxP sites flanking exon 1 of Itgb3. Results show that control but not Itgb3 KO neurons followed a timeline of increased dendritic spine density from P14 and P23 followed by decreased dendritic spine density between P23 and P30. Taken together, results from histological preparations suggest periods of dendritic spinogenesis and spine pruning in layer II/III of cortex that may be regulated by integrin beta 3.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.08/B15

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

**Support:** NINDS RO1 NS012601

T32 NS007220

TRDRP 25IP-0019

**Title:** MicroRNA-218 regulates early postnatal synchronized activity to promote proper hippocampal network function

**Authors: *S. R. TAYLOR**[1], G. LIPPI[1], M. KOBAYASHI[2], J. J. FAK[2], C. GIRGISS[1], J. LIU[1], R. B. DARNELL[2], D. K. BERG[1]
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**Abstract:** The development of functional mature neural networks relies on coordinated genetic programs and neural activity that together establish and refine patterns of connectivity. In the early postnatal rodent hippocampus, synchronized activity is thought to be crucial for network

formation. During the second postnatal week, network activity transitions from early, immature patterns to more complex, mature patterns. MicroRNAs (miRs) are promising candidates for regulating such transitions, as each miR is capable of modulating up to hundreds of genes. We hypothesized that miR-218 regulates hippocampal development because it increases in expression in the first two postnatal weeks, is highly abundant in the adult, is down-regulated in some forms of epilepsy, and putatively targets many genes involved in neural development and activity. We tested the roles of miR-218 *in vivo* using a locked nucleic acid (LNA) antagonist followed by *ex vivo* slice calcium imaging and dendritic spine analysis. We find that transient inhibition of miR-218 *in vivo* alters synchronized activity in an age-dependent manner, causing long-lasting changes in hippocampal activity. Transient inhibition of miR-218 in the first three postnatal weeks increased both the number of spontaneously active cells and the spontaneous activity per cell in hippocampal area CA3 at postnatal day 40-45 (P40-45). Testing the effects of miR-218 inhibition on early patterns of activity revealed that blockade of miR-218 starting at P2 decreased the frequency of synchronized activity at P5, but increased synchronized event frequency at P8 in CA3. Acute blockade of $GABA_A$ receptors with picrotoxin similarly altered synchronized activity at P5 and P8. Additionally, early blockade of miR-218 resulted in a decrease in spine density in stratum radiatum of CA1 at P11. Hi-throughput RNA profiling using RNAseq revealed multiple activated genes upon miR-218 inhibition, including factors involved in synapse formation, neurite outgrowth, and neurotransmission. These results indicate that miR-218 is essential for fine-tuning early patterns of spontaneous activity that promote proper mature network function.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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**Program#/Poster#:** 554.09/B16

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

**Support:** DC012592

MH102698

RB3-02186

**Title:** A non-sensory activity based checkpoint for interneuron wiring in the olfactory system

**Authors: *B. THROESCH**, K. N. JAMES, K. K. BALDWIN
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**Abstract:** The olfactory bulb (OB) is a dynamic system where newly born interneurons are integrated throughout the lifetime of an organism. The largest population of these interneurons are granule cells (GCs). While GCs are present at birth, additional precursors migrate into the OB where they mature and form reciprocal synapses with second-order projection neurons, mitral and tufted cells (MTs), continuously reshaping the OB circuit. Therefore, the olfactory system is an attractive model to identify signals that regulate the integration of neurons into a pre-existing neuronal circuit. To examine the impact of sensory vs non-sensory neuronal activity on OB circuit architecture, we selectively blocked neurotransmission from either olfactory sensory neurons or MTs using genetically encoded tetanus toxin. While the loss of sensory input has a modest effect, blocking MT signaling dramatically alters the structure of the OB. The increased disorganization is due to delayed maturation and increased cell death of most populations of inhibitory interneurons, including GCs. Single cell tracing analyses of GCs showed these neurons exhibit decreased dendritic branching as well as spine density, resembling immature GCs. Furthermore, this developmental blockade cannot be rescued by blocking apoptosis, uncovering a direct role for activity in regulating GC dendritic maturation. Transcriptome analyses of mature GCs and those stalled at activity-dependent developmental milestones identify gene regulatory networks that correlate with activity dependent maturation and functional integration. These studies establish a new role for non-sensory neuronal activity in regulating the development and integration of interneurons into adult circuits, which has implications for understanding olfactory coding and neurological disease.

**Disclosures: B. Throesch:** None. **K.N. James:** None. **K.K. Baldwin:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.10/DP01/B17 (Dynamic Poster)

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

**Support:** VR (Swedish Research Council)

**Title:** Cortical parvalbumin interneurons require postnatal expression of Sox6 for synaptic maturation and function

**Authors: *H. MUNGUBA**[1], J. N. CORRICO[2], S. NILSSON[1], P. OBERST[3], A. MUNOZ-MANCHADO[1], R. BATISTA-BRITO[4], G. J. FISHELL[5], B. CHATTOPADHYAYA[6], G. DI CRISTO[7], J. HJERLING LEFFLER[1]
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Montreal, Montreal, QC, Canada; [7]Res. Ctr., CHU Ste.justine-University of Montreal, Montreal, QC, Canada

**Abstract:** Cortical interneurons undergo extensive synaptogenesis and maturation of intrinsic properties during the first weeks after birth. In particular, parvalbumin (PV)-expressing interneurons go through a shift in their transcriptional profile during the second postnatal week, believed to be indispensable for their maturation. Because Sox6 is expressed in these cells throughout postnatal maturation, we investigated its role on late maturation and synaptic function and maintenance. For this, we utilized a conditional knockout strategy to specifically remove Sox6 in interneurons at different postnatal stages. Our results show loss of Sox6 in individual PV-cells (in otherwise wild type tissue) led to a robust decrease of size of PV interneuron axonal boutons contacting pyramidal neuron cell bodies, suggesting it to be a cell-autonomous effect. Furthermore, Sox6 was necessary for discrete aspects of cortical PV interneuron maturation independently of when it was removed (P7 or P21). By removing Sox6, although PV-expression was normal and their electrical properties mature, we observed a 30% decrease of PV-cells enwrapped by perineuronal nets (a hallmark for functional maturation). PV-cells lacking Sox6 also displayed reduced levels of TrkB-FL, which was sufficient to rescue the axonal phenotype when overexpressed in PV-cells lacking Sox6. Preliminary paired recordings of PV-cells and pyramidal neurons suggest that the decrease in bouton size leads to higher eIPSC failure rate. We are currently investigating if this role of Sox6 in PV-cells persists after adolescence.

**Disclosures: H. Munguba:** None. **J.N. Corrico:** None. **S. Nilsson:** None. **P. Oberst:** None. **A. Munoz-Manchado:** None. **R. Batista-Brito:** None. **G.J. Fishell:** None. **B. Chattopadhyaya:** None. **G. Di Cristo:** None. **J. Hjerling Leffler:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.11/B18

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

**Support:** NIH Grant R01

**Title:** Developmentally specific expression and network function of cb1 receptors in the newborn rat cerebellum

**Authors: *J. L. BARNES**, D. J. ROSSI
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**Abstract:** The type 1 cannabinoid receptor (CB1R) is widely expressed throughout the adult brain, especially the cerebellum, but while CB1Rs are also developmental neuromodulators, the

underlying mechanisms are not well understood. The cerebellum undergoes its most significant development during the third trimester of pregnancy, so *in utero* exposure to exogenous cannabinoids has the potential to alter cerebellar development. Thus, understanding how exogenous cannabinoids affect cerebellar developmental processes is important for providing usage guidelines for pregnant women, 3-6% of which consume cannabis during pregnancy, and for ascertaining potential therapeutic applications for neurodevelopmental disorders. Using immunohistochemistry, we find distinct CB1R expression in the third trimester-equivalent (PND 2-10) compared to adult (PND 30-35) cerebellar rat tissue: there is ubiquitous CB1R expression in all layers of the cerebellar cortex in developing tissue, whereas CB1R expression in adult tissue is more localized to the Purkinje and Molecular layers. CB1R staining was absent from all layers in CB1R knockout mice. In agreement with our immunohistochemistry studies, using whole-cell voltage-clamp recording in granule cells (GCs) of PND 2-14 rats, we found the CB1R agonist, WIN 55,212-2 (WIN; 5µM), reduces the frequency of both mossy fiber (MF) glutamatergic spontaneous EPSCs (sEPSCs) and Golgi cell GABAergic spontaneous IPSCs (sIPSCs). WIN also reduced the amplitude of the MF-evoked EPSC which was associated with an increase in the ratio of facilitation in paired pulse recordings. Lastly, WIN reduced the frequency of miniature EPSCs (recorded in TTX), which combined with the data above suggests that functional CB1Rs are expressed on MF axonal terminals. All actions of WIN were blocked by the CB1R antagonist SR141716A (2µM) and by germline genetic deletion of CB1R. In striking contrast to numerous studies in adult cerebellum, in voltage-clamped Purkinje cells (PCs), WIN did not affect sIPSC or sEPSC frequency. However, WIN did reduce MF-evoked disynaptic PC EPSC amplitude, suggesting cannabinoid effects on PCs stem from upstream activation of CB1Rs on granule cell synapses, despite apparent ubiquitous expression. In whole-cell voltage-clamp recordings from PND 30-35 GCs, WIN (5µM) did not affect sEPSCs, which combined with reduced immunostaining for CB1R in the adult GC layer, suggests that functional CB1Rs are only transiently expressed on incoming MF afferents during cerebellar development, but that their activation will also modify downstream output of developmental signals from GCs to developing PCs and possibly other developing neurons.

**Disclosures:** **J.L. Barnes:** None. **D.J. Rossi:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

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**Program#/Poster#:** 554.12/B19

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

**Support:** NIH Grant R01

**Title:** Nicotine enhances depolarizing $GABA_AR$ synaptic currents and signal transmission through the developing cerebellar cortex

**Authors: *H. SHIINA**, D. J. ROSSI
Washington State Univ., Pullman, WA

**Abstract:** Cerebellar injury or exposure to exogenous toxicants during development causes neurodevelopmental disorders in the offspring, leading to long-term decrements in cognition, affect and motor coordination. The cholinergic system plays a critical role in cerebellar development, and perturbation of that system by perinatal exposure to nicotine, an exogenous agonist of the nicotinic acetylcholine receptor (nAChR), retards cerebellar development, and increases risk for several cerebellar associated neuropathologies. Unfortunately, 10% of pregnant women still smoke during pregnancy due to nicotine's addictive nature, and the recent advent and rapidly growing use of e-Cigarettes is causing a resurgence of nicotine use and addiction. Despite well-established deleterious impacts of nicotine, relatively little is known about the mechanisms by which nicotine affects cerebellar development. Here we determined how activation of nAChRs by clinically relevant concentrations of nicotine (100-700nM) affects synaptic physiology and signal transmission through the cerebellar cortex during a critical developmental period. We used patch-clamp recording of GABAergic and glutamatergic synaptic currents in migrating and post-migratory granule cells, as well as Purkinje cells (mig-, GCs and PCs, respectively) in cerebellar slices from newborn rats (PND 3-9, equivalent to the human third trimester of gestation), and bath-applied nicotine to simulate *in utero* exposure. In voltage-clamped GCs, nicotine significantly increased GABAergic transmission from Golgi cells to GCs. Using perforated patch to record from GCs with unperturbed $[Cl^-]_{in}$, we determined that the reversal potential of $GABA_ARs$ relative to resting membrane potential is positive, making GABA depolarizing at this age. Thus, the nicotine-induced increase in GABA release excited GCs, which in turn increased glutamatergic transmission to developing PCs and mig-GCs. Mig-GCs express NMDARs, which drives their migration, and the nicotine-induced increased glutamate release enhanced NMDAR-mediated single channel activity. In addition to nicotine tonically activating these nAChR driven cascades, concomitant desensitization of nAChRs also reduced responses to transient pulses of pressure ejected exogenous ACh, as presumably occurs *in vivo* from cholinergic afferents. Given that nicotinic, $GABA_A$ and NMDA receptors are heavily involved in neurodevelopmental processes, these data suggest that nicotine usurps several endogenous developmental signals, which likely alter cerebellar development and contributes to the known smoking-induced impacts on the cerebellum and related behaviors.

**Disclosures: H. Shiina:** None. **D.J. Rossi:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

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

**Support:** International Anesthesia Research Society Mentored Research Award (2015-2017)

Boston Children's Hospital Anesthesia Research Trailblazer Award (2015, 2016)

**Title:** Postnatal development of GABAergic-dependent brain oscillations during sevoflurane anesthesia in children 0 to 3 years old

**Authors: *L. CORNELISSEN**[1,2], S.-E. KIM[3], J. M. LEE[2], E. N. BROWN[3], P. L. PURDON[4], C. B. BERDE[2]
[1]Boston Children's Hosp. (EN311.1), Boston, MA; [2]Anesthesiology, Perioperative & Pain Med., Boston Children's Hosp. & Harvard Med. Sch., Boston, MA; [3]Brain & Cognitive Sci., MIT, Cambridge, MA; [4]Anesthesia, Critical Care, and Pain Mgmt., Massachusetts Gen. Hosp., Charlestown, MA

**Abstract: BACKGROUND**: GABAergic interneurons are thought to drive circuit development throughout childhood; disruptions of these processes underlie many developmental disorders. Trajectories for development of GABAergic brain circuits have been characterized in animal models, but are difficult to measure in humans. Translation of insights from animal models could be better informed with a more detailed understanding of how these circuits develop in humans. Anesthetic drugs act through a GABAergic mechanism, and induce GABA-dependent stereotyped oscillations that relate fundamentally to circuit architecture and function (mPFC, Thal). Preliminary studies suggest that the neurophysiologic response to anesthetic drugs changes as a function of age, particularly in the first year of life [1,2]. Each year millions of children are administered general anesthesia for surgery, providing an experiment of nature that makes it possible to characterize how the brain responds to a strong GABA stimulus as a function of age. We therefore analyzed in detail how sevoflurane-induced brain oscillations change across the first three years of life to inform the developmental trajectory of GABAergic inhibitory circuits.
**METHODS**: We used multichannel scalp electroencephalograph (EEG) recordings in 120 children undergoing sevoflurane general anesthesia for elective surgery, to determine transitions in EEG power spectra and coherence across early infancy (birth - 3 years) as a reflection of naturally shifting excitatory-inhibitory circuit balance. We mapped spatial power and coherence over frontal, central, parietal, temporal and occipital cortices. Coherence was estimated between frontal channels FP1-F7 and FP2-F8.
**RESULTS**: During a surgical state of anesthesia, EEG power spectral properties: (1) all children

had slow (0.1-1Hz) and delta (1 to 4Hz) oscillations across the entire scalp; (2) theta (4 to 7Hz) and alpha (8-12Hz) oscillations emerged around 3-4 months; (3) alpha oscillations increased in power with postnatal age; (4) frontal alpha power was significantly greater than occipital power from ~7 months onwards; (5) frontal slow and delta coherence was observed from birth until 3-4 months and (6) frontal alpha coherence was observed from 10 months onwards.
**CONCLUSIONS**: Our results reveal key developmental milestones in the assembly and maintenance of the GABAergic-thalamocortical circuits. These data help explain why EEG-based indices provide inaccurate measures of anesthetic states in children and potentially identify critical periods of plasticity.
[1] Cornelissen et al (2015) *Elife*.4:e06513.
[2] Akeju et al (2015) *BJA*.115 Suppl 1:i66-i76

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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**Program#/Poster#:** 554.14/B21

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

**Support:** NIH R01 MH102365

**Title:** VIP interneurons mediate cortical phenotypes in a genetic model of schizophrenia

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**Abstract:** Schizophrenia is associated with altered cognitive and perceptual processing, as well as disruption of normal brain rhythms such as gamma oscillations (30-80Hz). Current evidence suggests that dysregulation of GABAergic interneurons contributes to neural and behavioral deficits in this disease. However, there are several major populations of interneurons and their respective roles in psychiatric disease remain poorly explored. Neuregulin 1 (*NRG1*) and its interneuron-specific tyrosine kinase receptor *ERBB4* are risk genes for schizophrenia. Using a conditional ErbB4 deletion model, we directly tested the role of vasoactive intestinal peptide (VIP)-expressing interneurons in schizophrenia-related deficits *in vivo*. ErbB4 removal from VIP interneurons during development leads to changes in their activity, along with severe dysregulation of the temporal organization and state-dependence of cortical activity. As a result of these neural circuit alterations, animals in which VIP interneurons lack ErbB4 exhibit reduced cortical responses to sensory stimuli and impaired sensory learning. Our data support roles for VIP interneurons in both normal cortical circuit development and the pathophysiology of

schizophrenia. These findings provide a new perspective on the role of GABAergic interneuron diversity in the disruption of cortical function in this complex psychiatric disease.

**Disclosures:** **R. Batista-Brito:** A. Employment/Salary (full or part-time):; 53000.00, Yale University. **J.A. Cardin:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

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

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**Title:** Development of neuronal microcircuits in neocortex layer1

**Authors: *S. JIANG**[1], Y.-C. YU[2], X. YAO[2]
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**Abstract:** Layer 1 is the outermost layer of the cerebral cortex, which originated from the neocortical marginal zone (MZ). Many studies strongly implied that layer 1 neurons (L1-INs) play crucial roles in neocortical development, synaptic integration and information processing. However, the functional microcircuits of L1-INs remain incompletely quantified. Previously studies have shown that on the basis of their electrophysiological properties, L1-INs were classified into two subtypes, the burst spiking (BS) interneurons and the late spiking (LS) interneurons. In this study, combining whole-cell patch clamp recordings with immunohistochemistry and microelectrode dye filling technologies, we investigated the electrophysiological properties of different neuronal types and the development synaptic microcircuits in layer 1 of somatosensory cortex. We found that 79.7% of L1-INs are late spiking (LS), and 20.3% of them are burst spiking (BS). Moreover, we found that the electrical connections exhibit cell-type selectivity among neocortical L1-INs, the LS pairs have more proportions of electrically coupled than LS to BS and BS pairs, but the coupling coefficients between coupled pairs revealed no significant difference between interneuron subtypes. Furthermore, the unidirectional chemical synapses between LS-LS pairs was significantly higher than between LS-BS pairs and BS-BS pairs. The peak amplitude of unitary inhibitory postsynaptic current between LS pairs was significantly larger than that between LS-BS pairs and BS-BS pairs. The unidirectional chemical synapses between LS and BS pairs showed directional selectivity. The majority of unidirectional pairs formed connections from LS to BS,

whereas only small number of them formed connections from BS to LS. Overall, our results can provide new insights into the mechanism of fundamental cortical computation involved by the superficial layer of the neocortex.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.16/B23

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

**Title:** Investigating synaptic plasticity and the balance of protein palmitoylation-depalmitoylation in the pathogenesis of a pediatric neurodegenerative disorder

**Authors: *K. P. KOSTER**[1], W. FRANCESCONI[2], F. BERTON[2], A. YOSHII[2]
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**Abstract:** The neuronal ceroid lipofuscinoses (NCLs) are a class of progressive pediatric neurodegenerative diseases. The infantile form, CLN1, causes visual failure, seizures, developmental regression and death by age 10. The cardinal neuropathological finding of all NCLs is the accumulation of proteolipid material, lipofuscin, which is readily visualized as autofluorescent lipopigment (AL) using fluorescence microscopy. Interestingly, AL is also identified in prominent neurodegenerative disorders, including Alzheimer's disease, suggesting conserved degenerative mechanisms. CLN1 is an autosomal recessive disorder caused by mutations in palmitoyl protein thioesterase 1 (PPT1), a depalmitoylating enzyme. Consequently, protein metabolism is dysregulated, leading to AL deposition. However, it remains unclear whether AL accumulation correlates with impaired synaptic functions in CLN1. Further, while the balance of palmitoylation-depalmitoylation is critical for synaptic protein function, the mechanisms by which mutations in PPT1 disrupt this balance and, thus, drive synaptic dysfunction in CLN1, are unknown. We first investigated the relationship between synaptic plasticity, protein palmitoylation and AL deposition in PPT1-knockout (KO) primary neuronal cultures and found: 1) ALs accumulate in PPT1-KO cells immediately following mature synapse formation, and 2) modulating neuronal activity or protein palmitoylation-depalmitoylation directly impacts this AL deposition. Next, we examined AL accumulation in the visual cortex (VC) of PPT1-KO mice as progressive visual failure is among the first clinical symptoms of CLN1. While AL deposition had previously been described only in adult PPT1-KO mice, we found the accumulation of AL immediately after eye opening at post-natal day (P) 14 in the PPT1-KO VC. Remarkably, AL accumulation appeared first in layer IV of the VC, the termination site of thalamocortical projections. Together, these results suggest that neuronal activity regulates the balance of protein palmitoylation-depalmitoylation and affects AL

accumulation. In the future, we aim to biochemically and electrophysiologically define key synaptic mechanisms underlying the relationship between neuronal activity, protein palmitoylation-depalmitoylation and the accumulation of AL, particularly as it pertains to the progressive synaptic dysfunction and cell death in CLN1. Importantly, novel therapeutic strategies for this monogenic disorder will have broader implications for adult-onset neurodegenerative diseases.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.17/B24

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

**Title:** Maternal regulation of pups' cortical activity: Role of serotonergic signaling

**Authors: E. COURTIOL**[1], D. A. WILSON[3], R. SHAH[1], R. M. SULLIVAN[3], *C. M. TEIXEIRA[2]
[2]EBI, [1]Nathan Kline Inst., Orangeburg, NY; [3]Child and Adolescent Psychiatry, New York Univ. Sch. of Med., New York, NY

**Abstract:** Neuronal activity during development shapes the new wiring and connectivity in the emergent brain. In this period of heightened plasticity the brain is shaped to adapt to the environment it will encounter. It is also a period of heightened vulnerability to factors that can derail adaptive brain development. Recently, pup cortical activity has been found to be influenced by maternal interactions, the major environmental input at this stage. It has been shown, using local-field-potential (LFP) recordings in behaving rat pups (P12-P19), that nipple attachment was related to an increase in low frequency neural activity while the absence of the mother from the nest was associated with decreased low frequency neural activity. The maternal interactions during early-life are thus a critical factor modulating pups neuronal activity. However, the mechanisms underlying these changes of neural activity are still not understood. Serotonin is a key neuromodulator involved in brain development. Postnatal life is a critical period when serotonergic activity can regulate the development of neuronal circuitry and specifically emotional neurocircuitry. For instance, increasing serotonergic signaling during postnatal days 2 to 11 (P2-P11) with a selective-serotonin-reuptake inhibitor (SSRI) has been shown to lead to emotional deficits in the adult. Furthermore, maternal tactile stimulation regulates glucocorticoid receptor expression via activation of the serotonergic system. Serotonin in the maternal brain is known to regulate maternal care. However, little is known on how maternal care regulates pups' brain activity. In our study, we found evidence suggesting that: (a) maternal presence increases LFP power in low-frequency bands in P11 rat pups; (b)

serotonergic signaling through 5-HT2 receptors is necessary for this modulation; and (c) enhancing serotonergic signaling, using a SSRI, in isolated P11 rat pups produces an enhancement in LFP power in low-frequency bands similar to the effect produced by maternal presence. Our results suggest a contribution of the serotonergic system in mediating changes of cortical activity in pups related to maternal care.

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**Poster**

**554. Neural Circuit Maturation and Remodeling II**

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

**Support:** NIH 1R15HD077624-01

NSF Grant 1257895 to MSS

**Title:** Role of calcium activity during neural development

**Authors: *S. PAUDEL**[1], M. SEHDEV[2], W. HERBST[3], M. SAHA[2]
[1]Applied Sci., [2]Biol., Col. of William and Mary, Williamsburg, VA; [3]UCLA, Los Angeles, CA

**Abstract:** The development of a functional nervous system is characterized by the generation and specification of a diverse array of neuronal subtypes that subsequently form an integrated system. This process is orchestrated by both intrinsic and extrinsic molecular signals. One of the key signals is calcium, a ubiquitous and essential messenger that plays an important role during various developmental processes from neural induction through synapse establishment. While a great deal is known regarding the role of calcium in the adult nervous system, far less is known regarding the widespread calcium activity prior to synapse establishment. Using *Xenopus laevis* primary neuronal cell culture, we show that at neurula stages neuronal progenitor cells exhibit frequent small amplitude spikes that span shorter duration and presumptive neurons fated to differentiate exhibit higher amplitude spikes spanning longer duration. However, the pattern, regularity, cellular molecular phenotype of calcium active cells and the underlying molecular mechanism(s) of this activity during early embryonic neural development remains unknown. Therefore, in this project we extend our investigation to an *in vivo* system and monitor calcium activity in *X. laevis* embryos during neural development using genetically encoded calcium markers and identify phenotype of the cells employing *in situ* hybridization and immunocytochemistry. We have developed a system to correlate molecular cellular phenotype of individual cells and their calcium dynamics that entails calcium imaging using GCaMP and

memRFP followed by in situ hybridization with genetic markers and immunocytochemistry against RFP. Preliminary closeness centrality analysis of *in vivo* calcium recordings revealed correlated clusters of cells as well as highly connected cells that may define important domains for early neurogenesis.

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**Poster**

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

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Grant-in Scientific Research on Innovation Areas ”Dynamic regulation of Brain Function by Scrap & Build System" (JP16H06459) from MEXT

**Title:** Patchwork-type spontaneous activity in layer 4 of neonatal barrel cortex transferred via thalamocortical projections

**Authors: *H. MIZUNO**[1,2], K. IKEZOE[3], T. SATO[1], K. KITAMURA[3], T. IWASATO[1,2]
[1]Natl. Inst. of Genet., Mishima / Shizuoka, Japan; [2]Dept. of Genet., SOKENDAI, Mishima / Shizuoka, Japan; [3]Univ. of Yamanashi, Yamanashi, Japan

**Abstract:** Establishment of precise neuronal connectivity in the neocortex relies on activity-dependent circuit reorganization in postnatal development; however, the nature of cortical activity during these periods remains largely unknown. Using two-photon calcium imaging of the barrel cortex during the first postnatal week, here we found a “patchwork-type” spontaneous activity, which was confined to the barrel map. In the absence of whisker-stimulation, layer 4 (L4) neurons belonging to the same barrel fired together. By generating transgenic mice expressing GCaMP6s in the thalamocortical (TC) axons, we showed that TC axon termini also exhibited the patchwork activity. This activity was diminished by peripheral anesthesia but

mostly uncorrelated with whisker movements. In the second postnatal week, the patchwork pattern disappeared and L4 neurons even within the same barrel fired in a desynchronized manner. The patchwork-type spontaneous L4 activity has features that are suitable as the template for TC circuit refinement in the neonatal barrel cortex.

**Disclosures:** **H. Mizuno:** None. **K. Ikezoe:** None. **T. Sato:** None. **K. Kitamura:** None. **T. Iwasato:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.20/B27

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

**Support:** Department of Biotechnology, India

**Title:** Elucidating the organization and regulation of lipid nanodomains on neuronal plasma membrane

**Authors:** ***M. J. DEEPAK**, D. KUMARAN NAIR, V. CHAUHAN
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**Abstract:** Lipid rafts (LR) are sphingolipid-enriched nanodomains on the plasma membrane which act as organizing centres for the assembly of signaling molecules influencing membrane fluidity. Several studies indicate the crucial role of lipid rafts in diverse biological processes such as cell division, development, migration, and organogenesis. Unfortunately, the precise physiological role as well as nanoscale organization remains elusive due to their small size and dynamic character with lifetimes varying from milliseconds to seconds. Previous studies on lipid rafts have shown that they are highly mobile structures on the plasma membrane with their size ranging from 10-200 nm. Conventional microscopy techniques for imaging protein-lipid interactions at the cell membrane are limited by optical diffraction, which does not allow characterization of structures below 200 nm. This calls for recently developed single molecule based superresolution techniques such as PALM (Photoactivation Localization Microscopy) and SPT-PALM (single particle tracking-PALM), which allow resolving structures few tens of nanometres apart by controlling the fluorophore emission and activation. SPT-PALM (single particle tracking-PALM) allows to address in living cells in real-time the nanoscale regulation of the transient lipid nanodomains at a resolution multifold better than the conventional microscopy techniques. Here, we try to elucidate the organization and regulation of lipid nanodomains on neuronal plasma membrane using single molecule based superresolution imaging to understand its relevance in diverse biological processes.

**Disclosures:** **M.J. Deepak:** None. **D. Kumaran Nair:** None. **V. Chauhan:** None.

**Poster**

**554. Neural Circuit Maturation and Remodeling II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 554.21/B28

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

**Support:** VICI grant 848203

**Title:** Mother-pup interactions and oxytocin during early brain development

**Authors:** ***P. P. MALDONADO**[1], A. NUNO-PEREZ[1], Y. HAN[2], M. CARRILLO[2], P. DE GOEDE[3], A. KALSBEEK[3], C. KEYSERS[2], C. LOHMANN[1]
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**Abstract:** Spontaneous network activity occurs before birth and during early postnatal brain development. This type of activity regulates the establishment of synaptic connections and subcellular compartments in order to prepare the brain before it can fully experience the environment. Though the senses of vision and hearing and most motor abilities become functional around the second postnatal week in rodents, tactile and odor cues are already perceived at birth, playing a significant role during mother-pup interactions in the nest. Moreover, natural occurring differences in maternal care behaviors such as licking and grooming affect adult brain functions. How brain development process is affected by this type of early social experience has not yet been investigated. Associated with adult social behaviours and already expressed during prenatal development, the neuropeptide oxytocin (OT) and OT receptors (OTR), are good candidates to mediate the effects of mother-pup interactions on brain development. Thus, here we aim to address the role of OT on spontaneous neuronal activity of primary visual cortex (V1). As shown by *in vivo* 2-photon calcium imaging recordings in layer II/III of V1, OT decreases the frequency of spontaneous calcium network events. *In vitro*, OT increases the frequency of inhibitory postsynaptic currents (IPSCs) without affecting excitatory postsynaptic currents (EPSCs). This OT-mediated increase in inhibition is concomitant with an increase in the excitability of somatostatin interneurons upon OTR activation. Moreover, by performing large-field calcium imaging recordings we observed that OT-mediated inhibition is selective to V1, without affect somatosensory cortex (S1). Consistently with this observation, OT has a balanced effect in S1, increasing the frequency of both IPSCs and EPSCs, therefore leading to a zero net effect of OT on synaptic activity. Finally, we used primi- and multiparous mouse mothers as a model to study the effect of differential maternal interactions on OT and OTR levels in the neonatal mouse brain. Our preliminary data suggest that OT and OTR mRNA levels, as

obtained from the cortex and the hypothalamus of mouse pups, are differentially modulated depending on the degree of maternal experience. Together, our results reveal that OT exerts specific cellular and network effects in the developing V1, changing the inhibitory-excitatory balance towards inhibition. Moreover, these results give evidence for a role of maternal interactions on postnatal brain development.

**Disclosures: P.P. Maldonado:** None. **A. Nuno-Perez:** None. **Y. Han:** None. **M. Carrillo:** None. **P. de Goede:** None. **A. Kalsbeek:** None. **C. Keysers:** None. **C. Lohmann:** None.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.01/B29

**Topic:** A.07. Developmental Disorders

**Support:** NHRI-EX103-10316NC

NTU102C101-42

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NTUH-UN104-018

NTUH-UN105-014

MoST105-2628-B-002-033-MY3

**Title:** Transcriptomic and allelic-specific expression analysis in a family quartet with autism spectrum disorder

**Authors: *C.-Y. LIN**[1,3], J.-Y. WU[1], C.-Y. LIN[1], H. COON[4], P.-H. HUANG[5], H.-N. HO[2,6], S. AKBARIAN[8], S.-F. S. GAU[1,7], H.-S. HUANG[1,9]
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**Abstract:** Autism spectrum disorder (ASD) is a highly prevalent neurodevelopmental disorder and the exact causal mechanism is unknown. Dysregulated allele-specific expression (ASE) has been identified in persons with ASD; however, a comprehensive analysis of ASE has not been conducted in a family quartet with ASD. To fill this knowledge gap, we analyzed ASE in a family quartet with ASD (one of the two offspring had been diagnosed with ASD) by using genomic DNA from both parents and offspring's and RNA from offspring's' postmortem prefrontal cortex (PFC). DNA- and RNA-sequencing revealed distinct ASE patterns from PFCs of both offspring. Noteworthy, only the PFC of the offspring with ASD exhibited a mono-to-biallelic switch for two autism susceptibility genes. We have also identified novel RNA-editing and monoallelically-expressed genes and miRNAs. These results demonstrate the prevalence of ASE in human PFC and ASE abnormalities in the PFC of a person with ASD and may provide mechanistic insights for the pathogenesis of ASD.

**Disclosures: C. Lin:** None. **J. Wu:** None. **C. Lin:** None. **H. Coon:** None. **P. Huang:** None. **H. Ho:** None. **S. Akbarian:** None. **S.S. Gau:** None. **H. Huang:** None.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.02/B30

**Topic:** A.07. Developmental Disorders

**Support:** SFARI

**Title:** Determining the role of gene-environment interactions in mouse models of neurodevelopmental disorders

**Authors: *M. KIELHOLD**, A. NARAYAN, B. BABINEAU, H. M. MOON, J. SU, T. PALMER
Neurosurg., Stanford Univ., Palo Alto, CA

**Abstract:** Neurodevelopmental disorders (NDD), such as autism and schizophrenia have a diverse and multi-faceted etiology that is poorly understood, though epidemiological studies suggest that environmental risks such as prenatal infections or other gestational immune events correlate with increased NDD risk. It is also known that there is strong heritability of NDDs, providing evidence that genetic predisposition for such disorders exist. Innate immune responses are evoked by toll-like receptor (TLR)-dependent signaling pathways, and TLR4 selective (bacterial) mimetic-mediated maternal immune challenges have been shown to result in brain and behavioral changes (as reviewed by Meyer 2014). Previously, we demonstrated that a TLR4-selective insult at E12.5 has adverse effects on fetal and placental health, proliferation of radial glial cells, altered cortical laminar patterning in the adult and behavioral deficits (Carpentier et.

al 2013). Here, we aim to determine if heterozygous expression of autism susceptibility genes act synergistically with TLR4-selective insult during mid-gestation to impact the developing fetus. Placental pathology and pregnancy outcomes were evaluated by quantifying tissue necrosis and fetal survival, respectively. Neocortical alterations in the developing fetuses were examined via immunohistochemistry for markers of cell proliferation and neural progenitor cell populations. Finally, behavioral outcomes were measured using tasks that evaluate behaviors analogous to the symptoms of NDDs, including pup vocalizations, social approach and marble burying. Thus far, we have observed increased placental hemorrhage and decreased fetal viability when mice heterozygous for an autism risk gene were exposed to bacterial infection. Behavioral analysis is ongoing. Our results have implications for understanding how environmental insults may act synergistically with genetic susceptibilities to induce greater levels of affection in individuals.

**Disclosures:** **M. Kielhold:** None. **A. Narayan:** None. **B. Babineau:** None. **H.M. Moon:** None. **J. Su:** None. **T. Palmer:** None.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.03/B31

**Topic:** A.07. Developmental Disorders

**Support:** NIMH MH096093

Harvey Family Endowment

**Title:** Differential methylation across multiple tissues as a means of determining causality in Autism Spectrum Disorder

**Authors: *E. L. BEARER**[1], B. S. MULLIGAN[2], J. M. STEPHEN[3]
[1]Dept. of Pathology, UNM Sch. of Med., Albuquerque, NM; [2]Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [3]Mind Res. Network, Albuquerque, NM

**Abstract:** In 2016 the CDC announced that 1 in 68 Americans are diagnosed with Autism Spectrum Disorder (ASD), with an estimated $265 billion a year (2015) attributed to care. ASD often cannot be reliably diagnosed until age 2 because children present neurotypically until that point in development, but current practice standards are inexact: 57% of children identified with autism do not receive their diagnosis until after age 3, and white children are up to 1.6 times more likely to be diagnosed than minority children. In order to diagnose ASD earlier, improvements in objective testing are needed. Genetic differences have been identified for various subtypes of this disorder, such as Rhett syndrome, but no single unifying DNA alteration has yet been found. Hence we propose that a global epigenetic change, possibly affecting one

gene or across the genome, that impacts the development of the nervous system in a child who will develop ASD that would not happen in a neurotypical (NT) child, and will be identifiable across a comparison of multiple tissues. We predict that this change in epigenetic status will be detected earlier than the behavioral manifestations, and further that these changes are environmentally regulated and thus would be targets for therapy. To test this, we recruited children with ASD and not (NT) for interviews and sample collection. From these children, 11 individuals' saliva was sequenced with an Illumina HumanMethylation450 BeadChip, 6 NT and 5 ASD. NT and ASD brain samples from a separate study analyzed on the same platform were used for cross comparison between tissues. After initial processing yielded little variation between ASD and NT whole genome DNA methylation (DNAme) patterns, saliva cell composition was found to be somewhat different, 62.419%( +/- 6.585%) keratinocyte (K) in ASD saliva samples and 57.639% (+/- 9.135%) K in NT samples, with a total average composition of 59.478% (+/- 7.850%) K, with the remaining being whole blood. This difference was enough to believe that reference-based cell type deconvolution is needed. After correction, ASD-driven whole-genome variation is more detectable between ASD and NT in saliva samples. Similar corrections made in the brain samples, and then the two compared again. This yielded a single gene with evidence to suggest differential methylation had taken place in the gene's promoter region. This gene's role in neural development and maintenance proves interesting for future exploration into the mechanism by which developing brains become autistic.

**Disclosures: E.L. Bearer:** None. **B.S. Mulligan:** None. **J.M. Stephen:** None.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

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**Program#/Poster#:** 555.04/B32

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant R01 NS089896

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NIH Grant T32 GM007863

University of Michigan Rackham Graduate School Research Grant

**Title:** Histone binding protein, PHF21A, is required for rapid induction of cAMP-responsive immediate early genes

**Authors: *R. S. PORTER**[1], Y. MURATA-NAKAMURA[1], H. NAGASU[1], H.-G. KIM[2], S. IWASE[1]

[1]Human Genet., Univ. of Michigan, Ann Arbor, MI; [2]Obstetrics and Gynecology, Augusta Univ., Augusta, GA

**Abstract:** Given the prevalence of neurodevelopmental disorders (NDD) such as syndromes of intellectual disability (ID) and autism spectrum disorders (ASD), there has been a great effort to identify genetic causes of these early cognitive disorders. Recent studies have identified a number of transcriptional, synaptic, and interestingly many histone methylation regulating genes. Histone H3 lysine 4 methylation (H3K4me) is the most extensively regulated histone modification in eukaryotes. Out of the 13 known enzymes that place and remove H3K4me marks, eight have been implicated in NDDs, as well as multiple H3K4me reader proteins that recognize the mark and recruit effectors. One such is example is Potocki Shaffer Syndrome, a rare neurodevelopmental syndrome associated with microdeletion of a region of Chromosome 11p11.2. Genetic evidence has implicated haploinsufficiency of *PHF21A*, which encodes a histone-binding protein, as the likely cause of intellectual disability and craniofacial abnormalities in Potocki Shaffer Syndrome. Previous work, however, has not investigated the molecular consequences of reduced *PHF21A* expression and how it leads to a cognitive phenotype. In this study, we analyzed the transcriptomes of two patient-derived cell lines with heterozygous loss of *PHF21A* compared to unaffected individuals by RNA-Sequencing and identified 1,885 genes that were commonly misregulated. Despite using lymphoblastoid cells, pathway analyses of the misregulated genes showed significant enrichment of genes involved in neuronal development and synaptic plasticity. Specifically, cAMP-mediated signaling was among the top downregulated pathways. Through luciferase reporter assays, we found that *PHF21A* is required for full induction of a CRE-Luciferase reporter following stimulation by the cAMP analog, forskolin. Furthermore, patient cells stimulated with forskolin showed a delayed induction of immediate early genes (IEGs) compared to control cells. IEG induction, by pathways including cAMP-signaling, is a key initial step in learning and memory. Our results suggest that an inefficient transcriptional response to cAMP-signaling might be involved in the pathology of *PHF21A* deficiency. This study emphasizes the importance of dynamic chromatin regulation in the temporal response to external stimuli. Furthermore, this work sheds light on the molecular mechanism that underlies intellectual disability in Potocki Shaffer Syndrome and may extend to other syndromes of intellectual disability associated with disruption of chromatin regulating genes.

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**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.05/B33

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

**Title:** Maturation of corticospinal tracts in children with congenital hemiplegia using diffusion tensor imaging

**Authors: *C. PAPADELIS**[1], M. RUBENSTEIN[1], H. L. KAYE[2], K. KAPUR[3], B. SNYDER[4], E. GRANT[6], A. ROTENBERG[5]
[1]Newborn Med., [2]Dept. of Neurology, Div. of Epilepsy and Clin. Neurophysiol., [3]Neurol., [4]Orthopedic Ctr., [5]Boston Children's Hosp., Boston, MA; [6]Div. of Newborn Med., Boston Childrens Hosp., Boston, MA

**Abstract: Objectives:** The corticospinal tracts (CSTs) are among the first white matter tracts to mature. During brain maturation, CSTs are susceptible to injury leading to neurological deficits, often resulting in the clinical presentation of cerebral palsy. Our objective is to describe changes in the developmental trajectory of CSTs maturation as a function of age in children with congenital hemiplegia and typically developing children. **Methods:** Diffusion tensor imaging (DTI) was performed in 13 children with congenital hemiplegia (7 females; 14.02 years ± 5.14; range: 6 to 17 years) and 16 age-matched typically developing children (9 females; 11.9 years ± 3.56; range: 7 to 18 years) with a 3-T MR scanner. The CSTs were reconstructed passing through brainstem and the pre-central gyrus using TrackVis software. The mean number of fibers, fractional anisotropy (FA), apparent diffusion coefficient (ADC), axial diffusivity (AD), and radial diffusivity (RD) of the CSTs were estimated for each participant and hemisphere. For each child with congenital hemiplegia, a pediatric radiologist identified a more affected (MA) and a less affected (LA) hemisphere. Each child in the typically developing group was assigned to have a MA hemisphere according to the matched congenital hemiplegia participant's side of damage. Analysis was performed using linear mixed-effects regression model with random intercept to account for within subject correlations for each diffusion measure. The fixed-effects in the model included age, injury side, group and all possible higher order interaction terms. The final model selection was performed using Akaike Information Criterion. **Results:** In the congenital hemiplegia group, AD, ADC, and RD increased linearly with age ($p<0.001$). This relationship was not present in the typically developing children in either hemisphere. For the congenital hemiplegia group, the linear relationship between the ADC and RD with respect to age was more pronounced for the MA compared to the LA hemisphere ($p<0.01$). In both groups, and in both hemispheres, the number of fibers and the FA did not change as a function of age. **Conclusions:** We identify for the first time that white matter injury in the brain of children with congenital hemiplegia slows the normal maturation of CSTs. These radiographic findings provide insight into both normal motor system development, and into the development of the motor system in the setting of early life brain injury.

**Disclosures: C. Papadelis:** A. Employment/Salary (full or part-time):; Boston Children's Hospital. **M. Rubenstein:** A. Employment/Salary (full or part-time):; Boston Children's Hospital. **H.L. Kaye:** A. Employment/Salary (full or part-time):; Boston Children's Hospital. **K. Kapur:** A. Employment/Salary (full or part-time):; Boston Children's Hospital. **B. Snyder:** A. Employment/Salary (full or part-time):; Boston Children's Hospital. **E. Grant:** A.

Employment/Salary (full or part-time):; Boston Children's Hospital. **A. Rotenberg:** A. Employment/Salary (full or part-time):; Boston Children's Hospital.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.06/B34

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant 5T32GM007198-42

**Title:** Genome clustering for clinical subtype detection in autism

**Authors: *D. N. AMATYA**[1,2], D. NGUYEN[3], S. NAVLAKHA[4], F. H. GAGE[5]
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**Abstract:** Autism spectrum disorder (ASD) is a developmental neuropsychiatric disorder characterized by a wide range of clinical manifestations and genetic heterogeneity. This complex genetic landscape poses a serious challenge for efforts to find a relationship between genotype and clinical phenotype. Therefore, the disorder remains poorly understand, and diagnostic and treatment tools are lacking. Though genetic overlap between patients is low, many mutations in autism likely affect stereotyped biological pathways, resulting in subgroups of autistic patients with similar functional mutation burden. The central hypothesis of this study is that patients with functionally similar mutations can be clustered together in order to reveal previously unidentified groups that share both molecular mechanisms and disease related cognitive traits.
This study describes the construction and application of a novel algorithm for genomic sub- type detection in ASD using graph clustering and a subject distance function that is based on gene ontology, a database that describes the functional relationship of genes. Using the Autism Speaks MSSNG Database, a large set of paired genetic sequencing and clinical data, it is shown that resulting subject clusters are defined by both unique and convergent involvement of genetic pathways, which provides insight into the variety of disease mechanisms that may be present in ASD. These potential mechanisms are validated at the cellular level by connecting the clustering algorithm with data from ASD patient derived neurons. Finally, cluster memberships are associated with differences in clinical and cognitive variables, validating the medical utility of the genomic subtypes. Genome clustering in autism is an important step towards defining both mechanistic and clinical subtypes in this complex disorder, and this study addresses a growing need for algorithmic tools that connect genotype to phenotype in the context of heritable mental illnesses.

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**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.07/B35

**Topic:** A.07. Developmental Disorders

**Support:** NIH NIGMS GM119831

NIG NIMH U54 HD079125

**Title:** Transcriptional regulation mediated via dosage-sensitive Chd8 genomic interaction in mouse forebrain

**Authors: *A. S. NORD**, A. A. WADE, L. SU-FEHER, I. ZDILAR, R. CATTA-PRETA, K. J. LIM, T. W. STRADLEIGH, A. L. GOMPERS
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**Abstract:** The general chromatin remodeling gene *CHD8* has received high levels of interest since emerging as a top candidate causal gene in Autism Spectrum Disorder (ASD) via patient sequencing studies. Studies of mouse and human iPSC models have validated the relevance of *CHD8* mutations to neurodevelopmental disorders (NDDs), yet specific mechanisms by which haploinsufficiency impacts gene regulation during brain development remain unknown. We generated a mouse line harboring a loss-of-function frameshift allele of *Chd8* and show that heterozygous mice exhibit macrocephaly and cognitive deficits but do not exhibit gross laminar or structural cortical defects. Differential gene expression and co-expression network analysis of $Chd8^{+/-}$ forebrain revealed perturbation to biological processes and pathways across developmental stages, from proliferation through neuronal migration and maturation to synaptogenesis. ChIP-seq performed on mouse forebrain showed that Chd8 interacts with many regulatory elements genome-wide, but is strongly enriched for promoter interaction of genes associated with RNA processing and chromatin organization. Many RNA processing and chromatin-associated genes directly targeted by Chd8 exhibit decreased expression in $Chd8^{+/-}$ brain, indicating regulatory activity of Chd8 is dosage-sensitive. Our findings suggest a model where Chd8 acts to set the epigenomic state of cells in the developing brain, with haploinsufficiency resulting in attenuation of cellular differentiation and identity. We verified increased neuronal proliferation and global perturbation of RNA splicing during brain development in $Chd8^{+/-}$ embryos. Alterations to chromatin structure and transcriptional regulation identified in $Chd8^{+/-}$ mice may represent generalizable mechanisms underlying NDD neuropathology produced by loss-of-function mutations to chromatin remodeling factors.

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**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.08/B36

**Topic:** A.07. Developmental Disorders

**Support:** Simons Foundation SFARI no. 219193

NIH/NINDS 5R25NS065743

**Title:** Topographical shifts in functional connectivity reveal correlation differences between large-scale networks in 16p11.2 deletion carriers

**Authors: *A. Y. QURESHI**[1,2], J. A. NIELSEN[1], W. CHUNG[3], T. P. ROBERTS[5], E. H. SHERR[6], J. SEPULCRE[1,7], R. L. BUCKNER[1,7], .. AND THE SIMONS VIP CONSORTIUM[4]
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**Abstract:** The Simons VIP consortium spearheaded a genetics-first approach towards autism (Neuron, 2012). Here we explored the influence of copy number variation (CNV) of a recurrent ~600 kb (BP4-BP5) microdeletion of 16p11.2 using resting-state functional connectivity. Twenty-six 16p11.2 deletion carriers and 42 age-matched controls were included. A significant portion of carriers harbored language impairment (Phonological Processing Disorder 15/25; Language disorders 10/25), and social impairment (ADI 10/18; ADOS 7/25; Clinical ASD 5/25). The intrahemispheric correlation matrix of deletion carriers showed preservation of large-scale network architecture. Local and distant connectivity was estimated using prior methodology (Sepulcre et al., 2010). The degree of local and distant connectivity was computed for the number of links $r>0.25$ using a 16mm radius. The mean global sum of degree connections did not significantly differ for local ($t=-1.39$, $p=0.18$) or distant ($t=1.21$, $p=0.23$) connectivity between groups. The data did not support a uniform, global process consistent with the *theory of underconnectivity* in autism (Just et al., 2004). Nor did it support the more recent *dysconnectivity model* of decreased distant & increased local connectivity (Uddin et al., 2013; DeMartino et al., 2013). Regional differences in degree were observed at $p<0.05$, but no regions survived FDR correction. Next, we found differential links - or distinct connectivity between groups - to be non-uniformly distributed across the cortex. Local correlations significantly differed at the auditory cortex, insula, middle-posterior cingulate and the paracentral gyrus at $p<0.001$, FDR

corrected. In distant connectivity, the auditory cortex and insula were again present, but additionally the inferior parietal lobule, left middle frontal gyrus, right inferior frontal gyrus, posterior mid-cingulate & posterior cingulate sulcus, and dorsal & anterior medial prefrontal cortex were regions with the greatest differential links at $p<0.001$, FDR corrected. To visually demonstrate topographical displacement of connectivity a seed-based analysis was run as proof-of-principle. This analysis suggested functional connectivity differences between separate large-scale resting-state networks. These functional correlation differences are discussed in the context of other analyses of resting-state data.

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**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.09/B37

**Topic:** A.01. Neurogenesis and Gliogenesis

**Support:** NIH Grant U01 MH104393

**Title:** Neuronal brain region-specific DNA methylation and chromatin accessibility are associated with neuropsychiatric disease heritability

**Authors: *L. RIZZARDI**[1], P. F. HICKEY[4], V. RODRIGUEZ DIBLASI[2], R. TRYGGVADÓTTIR[1], C. M. CALLAHAN[1], A. IDRIZI[1], K. D. HANSEN[4], A. P. FEINBERG[3]
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**Abstract:** Epigenetic modifications confer stable transcriptional patterns in the brain, and both normal and abnormal brain function involve specialized brain regions, yet little is known about brain region-specific epigenetic differences. Here, we compared prefrontal cortex, anterior cingulate gyrus, hippocampus and nucleus accumbens from 6 individuals, performing whole genome bisulfite sequencing for DNA methylation. In addition, we have performed ATAC-seq for chromatin accessibility, and RNA-seq for gene expression in the nucleus accumbens and prefrontal cortex from 6 additional individuals. We found substantial neuron- and brain region-specific differences in both DNA methylation and chromatin accessibility which were largely non-overlapping, and were greatest between nucleus accumbens and the other regions. In contrast, glial methylation and chromatin were relatively homogeneous across brain regions, although neuron/glia ratios varied greatly, demonstrating the necessity for cellular fractionation.

Gene expression was also largely the same across glia from different brain regions and substantially different for neurons. Expression was correlated with methylation and accessibility across promoters and known enhancers. Several classes of transcription factor binding sites were enriched at regions of differential methylation and accessibility, including many that respond to synaptic activity. Finally, both regions of differential methylation and those of differential accessibility showed a surprising >10-fold enrichment of explained heritability associated with addictive behavior, as well as schizophrenia- and neuroticism-associated regions, suggesting that common psychiatric illness is mediated through brain region-specific epigenetic marks.

**Disclosures: L. Rizzardi:** None. **P.F. Hickey:** None. **V. Rodriguez DiBlasi:** None. **R. Tryggvadóttir:** None. **C.M. Callahan:** None. **A. Idrizi:** None. **K.D. Hansen:** None. **A.P. Feinberg:** None.

**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.10/B38

**Topic:** A.07. Developmental Disorders

**Support:** MR/K022377/1 (AB and CF)

SFARI 344763 (AB)

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SFARI 400101 (AG)

**Title:** Investigating autism-associated behaviours and neural circuits in mice deficient for the chromatin-remodelling factor Chd8

**Authors: *S. HURLEY**[1], P. SUETTERLIN[1], K. L. H. RIEGMAN[1], M. PAGANI[3], A. GALBUSERA[3], A. CARUSO[4], J. ELLEGOOD[5], J. P. LERCH[5], M. L. SCATTONI[4], A. GOZZI[3], C. FERNANDES[2], M. BASSON[1]
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**Abstract:** Mutations of the gene encoding the chromatin remodelling factor CHD8 have been identified as one of the strongest autism risk factors to date. Autism is characterized by behavioural deficits, including impairments in social interaction and communication, as well as alterations in brain structure and connectivity. In addition to autism, *CHD8* haploinsufficiency is associated with macrocephaly and developmental delay. We developed several mouse lines to investigate the effects of *Chd8* deficiency on brain development and behaviour. *Chd8* heterozygous mice exhibited complex behavioural abnormalities including increased interest in social cues and hypo-activity. These anomalous behaviours were accompanied by a 2.7% increase in brain volume, with volumetric increases in autism-associated areas, including frontal cortex and hippocampus. Due to the unusual complement of abnormal behaviours identified in *Chd8* heterozygous mice, we explored whether these structural brain and behavioural phenotypes could be exacerbated by further decreasing *Chd8* expression to around 40% of wild-type levels. Indeed, $Chd8^{neo/neo}$ mice, homozygous for a hypomorphic $Chd8^{neo}$ allele, displayed more pronounced structural brain changes than *Chd8* heterozygous mice, with a 4.5% increase in total brain volume. Behavioural anomalies were largely similar to heterozygous mice, and included hypo-activity and an increased predisposition to anxiety but no social deficits or other autism-associated behaviours. To gain insight into altered neural circuitry that could underlie the anomalous behaviours we observed, we performed resting-state functional MRI (rs-fMRI) in *Chd8* heterozygous mice. The results from these studies will be presented. Overall, our work suggests that threshold differences may exist between mice and humans, where *Chd8* levels even below 50% are not sufficient for mice to develop autism-associated behaviours. Future work will aim to investigate atypical neural circuitry underlying observed behavioural phenotypes and to establish a causal association of such abnormalities with dysregulation of molecular developmental pathways arising from *Chd8* deficiency.

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**Poster**

**555. Genetics of Neurodevelopmental Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 555.11/B39

**Topic:** A.07. Developmental Disorders

**Support:** UCLA IDDRC U54HD087101

**Title:** Evaluating the effect of high-frequency beta oscillations on sleep and cognition in Dup15q syndrome

**Authors: *V. SARAVANAPANDIAN**[1], R. BHATT[2], J. T. LERNER[2], S. S. JESTE[3]
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**Abstract:** Duplications of 15q11.2-13.1 (Dup15q Syndrome) is one of the most common copy number variations associated with autism spectrum disorders (ASD) and is characterized by global developmental delay, hypotonia, intellectual disability (Battaglia et al., 2010) and an increased risk for epilepsy (Conant et al., 2014). This 15q region harbors genes critical for brain development, including three $GABA_A$ receptor genes. Recent studies in our lab have identified that children with Dup15q syndrome show an electrophysiological biomarker characterized by increased resting state beta power, compared with non-syndromic ASD cohort and typically developing children, and this EEG signature strongly resembles that induced by allosteric modulation of $GABA_A$Rs suggesting the biomarker may reflect disruptions in GABAergic tone (Frohlich et al, 2016). Brain rhythms vary across different states and this neuromodulation supports learning and cognition. Our study aims to evaluate the stability of high beta oscillations across brain states and investigate its the functional consequences. Specifically, we hypothesize that: a) High beta oscillations seen during resting will persist during sleep in Dup15q; b) Persistent beta oscillations in the brain may inhibit brain-state-dependent modulation of neural activity, therefore disrupt sleep architecture, which can affect cognition. We have a cohort of 12 Dup15q children (average age: 6.8yrs) with overnight sleep recordings from UCLA hospitals. Preliminary qualitative assessments of 10mins of continuous sleep EEG from all participants revealed sustained beta oscillations in sleep. Using EEGLAB, sleep and wake (resting state before entering into sleep) data from one participant was processed and beta power computed across 9 scalp regions of interest to confirm the presence of beta oscillations in both brain states. Emerging evidence suggests that sleep plays a critical role in healthy cognitive function. Over the next few months we will quantify EEG beta power during sleep and characterize sleep in Dup15q behaviorally. We will determine if there is a relationship between beta power in sleep and standardized questionnaires of sleep quality as well as cognitive and developmental questionnaires. We hypothesize that increased beta power in sleep will correlate with poor sleep quality and potentially more cognitive impairment. Stability of beta power across different brain states would underscore the hypothesis that high beta EEG signature seen in Dup15q is related to the underlying genetic mechanisms and may be a true biomarker for the syndrome which may serve as an important target for clinical and pharmacologic intervention.

**Disclosures: V. Saravanapandian:** None. **R. Bhatt:** None. **J.T. Lerner:** None. **S.S. Jeste:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.01/B40

**Topic:** A.07. Developmental Disorders

**Support:** Stanley Medical Research Grant

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Simons Foundation SFARI Grant 240559

**Title:** Rational design of exquisitely selective inhibitors of the GSK3 kinase isoforms for the treatment of Fragile X Syndrome

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**Abstract:** The serine/threonine kinase Glycogen Synthase Kinase-3 (GSK3), part of the canonical WNT signaling pathway, has been implicated in multiple human diseases, including neurological and psychiatric disorders. Several converging lines of evidence make GSK3 an attractive target for the treatment of psychiatric disease. First, a growing number of direct genetic associations for WNT signaling have been established in bipolar disorder, schizophrenia, and autism. Secondly, the rapid anti-depressant effects of ketamine may depend on GSK3 signaling. There are two paralogs of GSK3, α and β, and while numerous dual GSK3α/β inhibitors have been reported, none possess paralog selectivity. This lack of selectivity has plagued their clinical development due to elevation of β-catenin levels, a known toxic consequences of dual inhibition. To circumvent this toxicity, we identified small molecule isoform selective inhibitors of GSK3α and GSK3β and used them to delineate the biological function of each paralog. We probed the contribution of the GSK3 paralogs to the pathophysiology of Fragile X syndrome (FXS) in *Fmr1*$^{-/y}$ mice and found that pharmacological inhibition of GSK3α, but not GSK3β, corrects excessive basal protein synthesis and susceptibility to audiogenic seizures. Inhibition of GSK3α additionally reversed other FXS-relevant phenotypes, including sensory cortical hyperexcitability and deficits in learning and memory, but does not induce the known liabilities of $mGlu_5$ inhibitors. We are currently pursuing phosphoproteomic analysis to understand the mechanisitic underpinning of the selective effect of GSK3α inhibition and its impact on FXS biochemical signaling. Our results indicates that GSK3α selective inhibitors may offer a new treatment of FXS and related disorders.

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**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.02/B41

**Topic:** A.07. Developmental Disorders

**Support:** NIH/NINDS grant NS079775 to RFB

MIND Institute Intellectual and Developmental Disabilities Research Center (U54 HD079125) to RFB

**Title:** Investigating disease progression of Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) using an inducible mouse model

**Authors: *M. M. FOOTE**[1], E. NEVEROVA[1], C. VIEIRA[1], K. VALENTINE[1], A. SAHA[1], C. HSIEH[1], J. KOTNIK[2], E. DOISY[1], R. HUKEMA[3], R. WILLEMSEN[3], R. F. BERMAN[4]
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**Abstract:** Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late onset neurodegenerative disorder caused by a premutation (PM) of the Fragile X Mental Retardation (*FMR1*) gene, which is a CGG trinucleotide expansion of 55-200 repeats in the 5'UTR. The clinical symptoms of FXTAS include intention tremor, gait ataxia, cognitive decline. This PM can alter *FMR1* transcription and translation, more importantly, it is susceptible to repeat-associated non-AUG (RAN) translation that produces a toxic peptide, FMRpolyG. In the brain, the cellular pathology of FXTAS is marked by cell loss, or brain atrophy, as well as the presence of ubiquitin-positive intranuclear inclusions. While most male PM carriers will not show the signs of FXTAS until after the age of 50, research in mouse models indicates that the disease process may actually begin much earlier in life. In fact, in a dox-inducible mouse model of the PM, only 8 wks of expressing an ectopic CGG repeat alone is sufficient to produce the FXTAS hallmark cellular pathology, ubiquitin-positive intranuclear inclusions. Here, we investigated cell type-specific contributions in FXTAS pathogenesis using a doxycycline (dox)-inducible transgenic mouse model that ectopically expresses a CGG(90) repeat selectively in neurons. By giving inducible mice different dox treatments, we are able to assess FXTAS disease progression (comparing 8 vs 20wk on) and the potential for disease reversibility (8wk on, followed by a 12wk off). To assess disease progression, inducible mice were screened using a battery of behavioral assays followed by a variety of histological studies to characterize cellular pathology.

The mice show mild behavioral changes associated with the symptoms of FXTAS patients. Histological analysis revealed that these inducible mice produce FMRpolyG and ubiquitin-positive intranuclear inclusions. By comparing different time points, we determined that the number and size of inclusions increases over time in the inducible PM mice. These mice also show significant cell loss in key brain regions. Unexpectedly, we also identified the presence of inclusions that did not appear to reside within neurons. We hypothesize that these inclusions could be remnants of dead neurons, inclusions in non-neuronal cells, or extracellular inclusions. Further analysis may identify a novel mechanism of disease progression and transmission which, in turn, would aid in the development of treatments. Collectively, these results indicate that ectopic CGG(90) expression in neurons is sufficient to produce FXTAS-associated pathology in mice and supports the hypothesis of a RAN-associated mechanism underlying FXTAS pathogenesis.

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**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.03/B42

**Topic:** A.07. Developmental Disorders

**Title:** Development of a sensitive and quantitative assay to detect FMRP in cell lines and human tissues

**Authors: M. ROTH**[1], S. WEBB[1], H. WU[1], A. M. CACACE[1], *L. V. RONCO[2]
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**Abstract:** Fragile X Syndrome (FXS) is a monogenic neurodevelopmental disorder attributed to the loss of the protein Fragile X Mental Retardation Protein 1 (FMRP). Promoter methylation triggered by the expansion of greater than 200 CGG trinucleotide repeats at the 5' UTR of the X-linked gene results in the epi-genetic silencing of *FMR1*. FMRP is a widely-expressed RNA-binding protein with particularly high expression in neurons and gonads. FMRP has been shown to be essential for regulated protein synthesis at developing synapses and for maintenance of normal synaptic plasticity. There is a lack of quantitative and sensitive assays for measurement of FMRP. Furthermore, the relationship between FMR1 mRNA and FMRP levels and neuronal function has not yet been established. While assays to detect and measure FMRP have previously been described, none is sufficiently sensitive, precise and accurate to properly characterize such relationships. We evaluated, optimized and compared various immunoassay technologies,

including Luminex, Quanterix Simoa and Meso Scale Detection. We have successfully developed a novel electro-chemiluminescence immunoassay capable of detecting attomolar ($10^{-18}$) levels of FMRP with a lower limit of detection of 142 aM. With this assay, FMRP levels were quantified in normal cultured human iPSC, neuronal precursor cells and NGN2-induced neurons. Furthermore, we have successfully determined FMRP levels in primary tissues and fluids from healthy individuals and compared them to FXS individuals. The assay is suitable for quantitative assessment of FMRP levels in disease states and for evaluation of novel therapies involving *FMR1* gene activation.

**Disclosures:** **M. Roth:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. **S. Webb:** A. Employment/Salary (full or part-time):; Fuclrum Therapeutics. **H. Wu:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. **A.M. Cacace:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. **L.V. Ronco:** A. Employment/Salary (full or part-time):; FULCRUM Therapeutics.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.04/B43

**Topic:** A.07. Developmental Disorders

**Title:** Functional assessment of spontaneous and evoked activity in iPSC-derived Fragile X neurons using multielectrode array (MEA) and fluorometric imaging plate reader (FLIPR) platforms

**Authors: *J. D. GRAEF**[1], C. SUN[2], L. LIN[2], S. WEBB[2], V. VILLEGAS[2], S. T. WARREN[3], A. M. CACACE[2]
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**Abstract:** Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by a CGG repeat expansion in the 5'-UTR of the Fragile X mental retardation gene, *FMR1*. Expansions of >200 repeats can lead to hypermethylation of the CGG repeats and CpG islands in the upstream promoter region, resulting in heterochromatin formation and silencing of the *FMR1* transcript thereby preventing FMRP protein production. FMRP is a RNA-binding protein highly expressed in neurons where it plays a key role in regulating local activity-dependent synaptic translation. Therefore, the absence of FMRP has been shown to affect both synaptic formation and maturation, as well as different forms of synaptic and homeostatic plasticity. In this study, we have taken advantage of patient-specific iPSC-derived neurons retaining the pathological repeat expansion, as well as CRISPR/Cas9 technology to create isogenic clones to better understand the relationship between *FMR1* and FMRP expression levels with a specific FXS functional phenotype. Using both MEA technology to record electrical activity, and FLIPR technology to

characterize evoked calcium responses, we have elucidated disease-relevant functional impairments that are dependent upon the level of FMRP expression. Therapies aimed at reactivating *FMR1* transcription in hopes of restoring FMRP levels are therefore an attractive approach for disease-modification in FXS.

**Disclosures:** **J.D. Graef:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics. **C. Sun:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics. **L. Lin:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics. **S. Webb:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics. **V. Villegas:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics. **S.T. Warren:** F. Consulting Fees (e.g., advisory boards); Fulcrum Therapeutics. **A.M. Cacace:** A. Employment/Salary (full or part-time):; Fulcrum Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Fulcrum Therapeutics.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.05/B44

**Topic:** A.07. Developmental Disorders

**Title:** Quantitative assessment of the contribution of FMR1 to function in iPSC-derived Fragile X neurons

**Authors: *H. WU**[1], J. D. GRAEF[2], C. NG[1], C. SUN[2], M. ROTH[3], *L. WITT[1], S. WEBB[4], V. VILLEGAS[5], L. V. RONCO[3], S. T. WARREN[6], A. M. CACACE[7]
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**Abstract:** Fragile X syndrome (FXS) is a monogenetic neurodevelopmental disorder with an incidence of ~ 1/4,000 live male births. Patients with FXS display a broad spectrum of intellectual, cognitive, and social deficits. These deficits are attributed to the loss of the protein

FMRP as a consequence of heterochromatin formation, methylation, and silencing caused by CGG trinucleotide repeat expansions at the 5' UTR of the X-linked gene, *FMR1*. FMRP has been shown to be essential for regulated protein synthesis at the developing synapses and maintenance of normal synaptic plasticity. However, *in vivo* or *in vitro* quantitative correlations of the length and methylation status of the CGG repeat expansion and the levels of FMRP with the functional deficits have not been established. In this study, we used two distinct but complementary approaches to establish the genotype, epigenotype and phenotype relationship in iPSC-derived Fragile X neurons. First, we developed an mRNA titration approach in iPSC-derived NGN2 neurons from patients with FXS (FXS iPSC neurons). mRNAs for WT *FMR1* or *FMR1* with 450 CGG repeats in the 5' UTR were expressed in FXS iPSC neurons. The minimal level of FMRP, as measured by Meso Scale Discovery immunoassay, that was required to normalize both expression of FMRP responsive genes and neuronal spontaneous network activity was determined by RT-qPCR and multi-electrode array (MEA) analysis, respectively. In the second approach, CRISPR/Cas9 genome editing was applied to generate a panel of isogenic lines with diverse shortening of the CGG expansion from an FXS iPSC line. Systematic analyses of the repeat length, methylation status of the CGG expansion, *FMR1* mRNA and FMRP protein expression, along with the MEA functional analysis of the NGN2-induced neurons from these isogenic lines allowed us to establish a precise relationship among the genotype, epigenotype and phenotype of the FXS neurons *in vitro*. This study quantitatively assessed the minimal level of gene expression necessary to rescue the physiologic deficit of FXS neurons. It therefore provides quantitative molecular guidance for novel disease modifying therapeutic approaches that involves *FMR1* reactivation. Establishment of this correlation may also provide a useful predictor of FXS phenotypic severity.

**Disclosures:** **H. Wu:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **J.D. Graef:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **C. Ng:** None. **C. Sun:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **M. Roth:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **L. Witt:** None. **S. Webb:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **V. Villegas:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **L.V. Ronco:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics. **S.T. Warren:** F. Consulting Fees (e.g., advisory boards); advisory board member, Fulcrum Therapeutics. **A.M. Cacace:** A. Employment/Salary (full or part-time):; full time, Fulcrum Therapeutics.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** A.07. Developmental Disorders

**Support:** SFARI Explorer Award

FRAXA Fellowship

IDDRC Pilot Grant

**Title:** Rescue cognition defects in Fragile X mice by activating autophagy

**Authors: *J. YAN**[1], M. W. PORCH[1], M. V. BENNETT[1], R. ZUKIN[2]
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**Abstract:** Fragile X Syndrome (FXS) is the most common form of heritable intellectual disabilities and a leading genetic cause of autism. An effective treatment for the cognitive deficits associated with Fragile X Syndrome remains an unmet medical need. Recent findings have revealed that mammalian target of rapamycin (mTOR) is overactivated in the hippocampus and is causally related to an overabundance of immature spines in hippocampal neurons of Fragile X mice, linking cognition defects in Fragile X to down-stream processes of mTORC1. Autophagy is a process of programmed degradation and recycling of cellular components *via* the lysosomal pathway. In neurons, mTORC1 is strategically positioned at pre- and postsynaptic sites where it serves as a brake on autophagy. In the present study, we show that autophagic flux, a functional readout of autophagy, and degradation of synaptic proteins *via* the lysosomal/autophagy pathway are impaired in hippocampal neurons of Fragile X mice. We further show that activation of autophagy rescues aberrant spine morphology, synaptic function, and cognition behaviors in Fragile X mice. These findings implicate a causal relation between impaired autophagy and cognitive defects in Fragile X, and identify autophagy as a novel therapeutic target for Fragile X syndrome.
**Key Words:** *Fragile X syndrome, Autophagy, mTOR, Cognition defect, Spine morphology*

**Disclosures: J. Yan:** None. **M.W. Porch:** None. **M.V. Bennett:** None. **R. Zukin:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

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**Topic:** A.07. Developmental Disorders

**Support:** FRAXA Research Foundation

NIH NS064967

**Title:** Fmrp and mitochondria: Enhancement of mitochondrial efficiency in the treatment of fragile x syndrome

**Authors: *P. LICZNERSKI**[1], H.-A. PARK[1], P. MIRANDA[1], V. K. GRIBKOFF[1], L. EL-HASSAR[2], L. K. KACZMAREK[2], R. J. LEVY[3], E. A. JONAS[1]
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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. Loss of FMRP 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 neuronal 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 either Bcl-xL or FMRP disrupts mitochondrial membrane potential and decreases ATP content of neurons. We now show that FMRP associates with mitochondria, that absence of FMRP alters mitochondrial structure and function and that an FMRP-dependent relationship exists between ribosome-containing complexes and mitochondria. We also find that the elevated levels of protein translation in FMRP KO mouse neurons can be reduced by treatment with specific modulators of the ATP synthase, the function of which is to increase the efficiency of mitochondrial metabolism. These modulators also increase LTP in FMRP KO mouse hippocampal slice recordings. We suggest that FMRP is not only an mRNA binding protein but also enhances the normal relationship between the ribosome and the mitochondria. This relationship is crucial for normal synapse formation and function.

**Disclosures: P. Licznerski:** None. **H. Park:** None. **P. Miranda:** None. **V.K. Gribkoff:** None. **L. El-Hassar:** None. **L.K. Kaczmarek:** None. **R.J. Levy:** None. **E.A. Jonas:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.08/B47

**Topic:** A.07. Developmental Disorders

**Support:** FRAXA Fellowship Award

McKnight Foundation

**Title:** Restoration of FMRP in the prefrontal cortex of adult Fragile X mice post-development rescues prefrontal-associated deficits

**Authors: *J. J. SIEGEL**, R. A. CHITWOOD, J. M. DING, R. GRAY, B. V. ZEMELMAN, D. JOHNSTON
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**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. In neurons, FMRP regulates the transcription of hundreds of proteins related to development, synaptic plasticity, and numerous other cellular functions. The dysregulation of protein synthesis, particularly during development, is considered the primary cause of ongoing neural dysfunction in patients. A number of impairments in FX patients are associated specifically with prefrontal cortex (PFC) dysfunction, including attention deficits, hyper-responsiveness, impaired social inhibition, and working memory deficits. We have identified a behavioral task, trace eyeblink conditioning (TEC), which is particularly sensitive to PFC dysfunction in mice. Recently, we demonstrated that FX mice show an increased proportion of nonlearners and delayed acquisition in learners in TEC, which appears due to an excitation/inhibition (E/I) imbalance based on single-unit recordings. Interestingly, the PFC-associated deficits in TEC were fully recapitulated when FMRP was blocked in the PFC alone of adult (post-development) mice. The latter result suggested that 1) deficits are due to the absence of FMRP in the PFC alone, and 2) deficits cannot be the sole result of developmental dysregulation in the PFC. Here, we test whether restoration of the production of FMRP to the PFC post-development (at 10-15 weeks of age) may partially or fully rescue deficits in TEC. For these experiments, we injected an rAAV for the production of Cre into the PFC of adult conditional restoration (cON) mice. Control groups consisted of wild-type littermates receiving PFC Cre injections, and non-injected sham cON littermates. Although FMRP was absent in the rest of the brain, restoration of FMRP in the PFC of adult cON mice rescued both the proportion of observed nonlearners and the onset of learning to wild-type levels, and was significantly different than cON sham controls. The results suggest a dissociation between the roles of FMRP in ongoing neural function and in developmental dysregulation, at least for PFC, and that PFC function can be restored in the adult FX brain. Future experiments will use single-unit recordings in the PFC of Cre-injected cON mice to determine whether the post-development rescue restores E/I imbalance, or whether a different compensatory mechanism allows for rescued PFC function.

**Disclosures: J.J. Siegel:** None. **R.A. Chitwood:** None. **J.M. Ding:** None. **R. Gray:** None. **B.V. Zemelman:** None. **D. Johnston:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

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**Topic:** A.07. Developmental Disorders

**Support:** CIHR MOP 125888

Fragile X Research Foundation of Canada

**Title:** Chronic minocycline treatment improves hippocampal NMDA receptor function, dendritic atrophy and memory processing in Fmr1 knockout mice

**Authors: *S. YAU**[1], M. VETRICI[2], A. TRUESDELL[3], J. CHIU[3], C. CHIU[3], B. R. CHRISTIE[3]
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**Abstract:** Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability, and is the leading known single-gene cause of autism spectrum disorder. FXS patients display varied behavioral deficits ranging from mild to severe cognitive impairment, mood disorder to language problem. Current medications targeting specific FXS symptoms are limited and there is huge unmet need for developing cure for FXS. Minocycline, which can alleviate social behavioral deficit and improving verbal functioning in FXS patients, is currently the only prescribed and targeted treatment for FXS patients. Previously we have demonstrated dentate gyrus region-specific deficits in NMDA receptor-dependent synaptic plasticity in the hippocampus of Fmr1 knockout (KO) mice. Here we tested whether chronic treatment with minocycline improves deficits in hippocampal dentate gyrus (DG)-dependent cognitive learning and memory via promoting *N*-methyl-D-aspartate receptor (NMDAR)-dependent functional and structural plasticity in the DG. Our results showed that chronic minocycline treatment significantly reversed impairments in hippocampal-dependent cognitive tasks in FXS mice, including novel object recognition and categorical spatial tasks. The behavioral benefits were accompanied by significant enhancement in NMDA receptor function in the dentate granule cells and increases in synaptic contents of PSD-95 and NMDAR subunits NR2B and NR2A in synaptoneurosome fractions. Minocycline treatment also enhanced dendritic length and branching of dentate granule cells in the DG of FXS mice. These findings indicate that minocycline treatment could reverse deficits in hippocampal synaptic and structural plasticity, as well as learning and memory performance in FXS mice, providing important therapeutic basis of pro-cognitive effect of minocycline in FXS.

**Disclosures: S. Yau:** None. **M. Vetrici:** None. **A. Truesdell:** None. **J. Chiu:** None. **C. Chiu:** None. **B.R. Christie:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

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**Program#/Poster#:** 556.10/B49

**Topic:** A.07. Developmental Disorders

**Support:** U01MH105971

R01MH096946

**Title:** Cellular X chromosome inactivation ratio densities amongst defined brain regions predicts female behavioral penetrance of fragile x syndrome in mice

**Authors: *E. SZELENYI**[1], D. FISENNE[1,2], Y. KIM[3,1], K. UMADEVI VENKATARAJU[1], P. OSTEN[1]
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**Abstract:** During female embryogenesis, somatic cells epigenetically silence one of the two inherited X-chromosomes in a process known as random X-chromosome inactivation (XCI). XCI is vital for brain function and cognition, with many X-linked genetic mutations leading to mental retardation syndromes, such as fragile X syndrome (FXS). The cumulative XCI status amongst cells within defined brain regions has been proposed to influence the severity of X-linked disease phenotypes in heterozygous females, but this hypothesis has yet to be experimentally tested in an unbiased, brain-wide fashion. Here, we quantify XCI dynamics across the entire mouse brain at single-cell resolution, both in wild type mice and in the FXS mouse model. We use an X-linked knock-in allele (*MeCP2-GFP*) to fluorescently label active XC (XCa) cells with known maternal or paternal parent-of-origin and computationally quantify *MeCP2-GFP+* XCa densities through serial two-photon tomography. This analysis demonstrated a statistically significant ~12.5% higher paternal XC-inactivation (XCi) across hemispheres and all brain regions, though with a notable variability (ratio range = 25/75 – 75/25%) between individual brains. Next, we determined whether the same bias of higher paternal XCi persists in the FXS mouse model and whether this affects the penetrance of behavioral phenotypes in FXS heterozygous female mice. We show that the average maternal XCa/paternal XCi bias observed in wild-type mice persists in heterozygous mice carrying either the maternal and paternal *FMR1* knockout (KO) allele. Notably, the modest difference in the estimated whole-brain *FMR1* KO XCa cell density, ~60% in maternal *FMR1* KO inheritance and ~40% in paternal *FMR1* KO inheritance, resulted in the manifestation of behavioral phenotypes only in the maternal *FMR1* KO heterozygous mice, including modest alterations in exploratory behavior and spatial memory, and a more pronounced deficit in social behavior. Remarkably, we detected highly significant correlations between the healthy XCa cell densities per brain areas and the severity of behavioral phenotypes: high mutant FMR1 KO / healthy cell ratio in sensorimotor and arousal linked brain areas predicted the presence of deficits in the open field exploration, and high mutant FMR1 KO / healthy cell ratio in regions linked to a social-spatial encoding network predicted social behavior deficits. These data therefore suggest that similar regional changes in the distribution of affected / healthy XCa cells may account for the broad variability of behavioral manifestations in female heterozygous patients with X-linked diseases.

**Disclosures: E. Szelenyi:** None. **D. Fisenne:** None. **Y. Kim:** None. **K. Umadevi Venkataraju:** None. **P. Osten:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.11/B50

**Topic:** A.07. Developmental Disorders

**Support:** ETH Career Seed Grant No. SEED-42 16-1

**Title:** Inhibiting mGluR5 activity by Mavoglurant rescues circuit specific long-range connectivity in Fmr1 knockout mice: A rs-fMRI study

**Authors: *V. ZERBI**[1], M. MARKICEVIC[1], M. RUDIN[2], N. WENDEROTH[1]
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**Abstract: Motivation.** Previous work has demonstrated that neuroimaging biomarkers which capture functional connectivity of the brain can be used to define a specific and robust endophenotype in *Fmr1*$^{-/y}$ mice[1], a well-established animal model of human Fragile-X Syndrome. However, it is currently unknown whether this macroscopic measure of brain connectivity is sufficiently sensitive to detect changes caused at the molecular level. Here we inhibit the activity of the metabotropic glutamate receptor-5 (mGlutR5) via AFQ056/Mavoglurant, a drug that is assumed to normalize excitatory/inhibitory neural signaling imbalances in *Fmr1*$^{-/y}$ mice. In this experiment, we employ resting-state-fMRI (rs-fMRI) to test (2) whether Mavoglurant re-establishes brain connectivity -at least partly- within some of the affected circuits in *Fmr1*$^{-/y}$ mice and (2) whether connectivity within specific circuits correlates with social behavioral.
**Experimental setup.** Untreated and treated adult *Fmr1*$^{-/y}$ mice (18 mg/kg/day of Mavoglurant by food pellets for 3 weeks prior the testing) as well as treated and untreated wildtype mice were evaluated with a three-chambered test to assess sociability and social novelty preference. One week later, we performed MRI scanning to evaluate functional connectivity changes. The datasets are acquired on a 7T scanner equipped with a mouse brain cryogenic coil, using well-established protocols to sedate and monitor mice. Rs-fMRI is cleaned from artifacts and analyzed using several established methods, including independent component analysis, total correlations and BOLD dynamics[2,3].
**Preliminary results and impact.** Preliminary rs-fMRI data indicate the presence of abnormal connectivity in Fmr1$^{-/y}$ mice, specifically for circuits that are associated with sensorial processing; for some, but not all of these circuits Mavoglurant had an effect of restoring functional connectivity. In line with previous findings, we observed that Fmr1$^{-/y}$ mice exhibited altered social interaction. However, at the group level, Mavoglurant did not rescue abnormal social behavioral. Further analyses will identify candidate brain circuits that are associated with

alterations in social behavior. Data from our experiment show that rs-fMRI connectivity is sufficiently sensitive to pick up changes at the molecular level, specifically the pharmacological inhibition of mGluR5 activity. However, our results also show that the effects of Mavoglurant are anatomically specific suggesting that behavioral benefits might be restricted to narrow functional domains.

1. Haberl, MG et al., Science advances (2015)
2. Zerbi, V et al., NeuroImage (2015)
3. Sethi, SS et al., Chaos (2017)

**Disclosures: V. Zerbi:** None. **M. Markicevic:** None. **M. Rudin:** None. **N. Wenderoth:** None.

**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.12/B51

**Topic:** A.07. Developmental Disorders

**Support:** PsiChi Undergraduate Research Grant

Asprey CCAS Student Research Enhancement Award

Vassar College Internal Funds

**Title:** Intranasal oxytocin fails to increase sociability in male offspring of fmr1 deficient dams

**Authors: *K. NEWHALL**[1], A. KOO[2], B. ZUPAN[3,1]
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**Abstract:** The *fmr1* knock-out (KO) mouse model of Fragile X syndrome (FXS) and autism exhibits a number of disorder-associated phenotypes including abnormal sociability. Previous data have shown that maternal *fmr1* genotype is a marker for male offspring social behavior, independent of their own *fmr1* genotype. Specifically, KO and wild-type (WT) adult male mice born to *fmr1* heterozygous (H) dams and *fmr1* WT sires on the FVB strain show increased sociability compared to WT mice born to WT dams. The neuropeptide oxytocin (OT) modulates affiliative social behaviors, and *fmr1* KO mice on the C57 strain, which exhibits reduced sociability, show reduced number of OT+ cells in the paraventricular nucleus (PVN) of the hypothalamus. In contrast, increased expression of OT in the PVN and supraoptic nuclei (SON) of the hypothalamus of valproic acid-treated rats, another rodent model of autism, were associated with increased sociability. We therefore asked whether maternal *fmr1* haploinsufficiency-dependent increase in offspring sociability in the FVB strain is associated with increased OT+ cells in the PVN as well as altered sensitivity to sociability-modulating effects of intranasal (IN) OT administration. We administered 0.3IU OT or saline 15 minutes

prior to sociability testing using a modified single chamber sociability task. Mice were grouped by offspring and maternal genotype, and tested on a within-subject counter-balanced design for drug treatment. We found that relative to saline, OT administration in WT mice from WT dams [WT(WT)] increased sociability, but had no effect or even reduced social interaction time in KO and WT mice from H dams, respectively. Furthermore, avoidant behavior was reduced following OT administration only in the WT(WT) group. Preliminary examination of immunolabeled OT+ cells in the PVN and SON regions has found no differences in cell number across groups, suggesting that lack of responsivity to exogenous OT in H dam offspring is not mediated by reduced OT cell numbers. Additionally, published data shows no evidence of activation of endogenous OT signalling prior to 30 minutes post-IN OT administration in mice, so it is unlikely that our data reflect altered signaling of PVN OTergic neurons. Rather, we hypothesize that the reduced responsivity to sociability-increasing effects of IN OT in offspring of *fmr1* deficient dams may reflect abnormal OT receptor expression and/or function.

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**Poster**

**556. Fragile X: Disease Predictors and Treatments**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 556.13/B52

**Topic:** A.07. Developmental Disorders

**Title:** Functional deficiency in Fragile X neurons derived from human induced pluripotent stem cells

**Authors: *A. ZHANG**[1], H. KJELDSEN[2], M. MASTERS[2], M. J. BOLAND[3], A. SZÜCS[4], K. L. NAZOR[5], M. SZYMANSKI[2], Y. WANG[1], J. LORING[1]
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**Abstract:** Fragile X Syndrome (FXS) is the leading monogenic cause of intellectual disability and autism spectrum disorder. It is caused by expansion of a trinucleotide repeat in the 5'UTR of the Fragile X Mental Retardation-1 (FMR1) gene. We recently reported that induced pluripotent stem cells (iPSCs) derived from FXS patients exhibit profound neurogenic defects early in development, accompanied by genome-wide changes in DNA methylation and gene expression (Boland, 2017). To understand how these early phenotypes affect neuronal function in FXS neurons, we performed single cell patch clamp and microelectrode array (MEA) recording in control and FXS neurons (day 80 of culture). Our patch clamp recording revealed that FXS and control neurons share similar intrinsic properties, but FXS neurons received much less synaptic input**.** We applied a novel analysis, Near-Field Electromagnetic Holography analysis (Kjeldsen, 2015), with our MEA recording which evaluate spike activity and patterns of directional

propagation which maps networks of activity in space and time. Complement to patch clamp result, we detected more energy flow but less efficient synaptic transmission in FXS neurons using MEA recording. Notably, our MEA recording also revealed an impaired ephaptic transmission, a form of neural communication that can influence the synchrony of electric waves in the brain and could contribute to epilepsy. We are currently incorporating neuronal subtype and morphology in our modeling algorithm. Additionally, we will integrate immunocytochemistry, transcriptome, methylome, and functional analysis to dissect the molecular pathways underlying the functional phenotypes in FXS neurons. Our aim is to predict the structural-functional properties of neurons in underlying networks and their variance as they are revealed by this new mapping technique. Our long-term goal is to leverage our results to develop a phenotypic assay that is amenable to high-throughput therapeutic drug screening.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.01/B53

**Topic:** A.07. Developmental Disorders

**Support:** The Jerome Lejeune Foundation Grant #1483

**Title:** Developmental excitatory to inhibitory GABA switch (DEIGS) is delayed in Ts65Dn mice, a genetic model of Down syndrome

**Authors: L. LYSENKO**[1,2], J. KIM[1], F. MADAMBA[1], A. A. TYRTYSHNAIA[3], A. RUPARELIA[1], *A. M. KLESCHEVNIKOV[1]
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**Abstract:** Down syndrome (DS) is the most frequent genetic cause of developmental abnormalities leading to intellectual disability. One notable phenomenon affecting the formation of nascent neural circuits during late developmental periods is the so called ‘developmental excitatory to inhibitory GABA switch’ (DEIGS). We examined DEIGS properties in DS using primary cultures and acute hippocampal slices from Ts65Dn mice, a genetic model of DS. Cultures of DIV3-DIV13 Ts65Dn and 2N (control) neurons were loaded with FURA-2AM, and polarity of GABA action was assessed using local applications of GABA. In 2N cultures, the number of GABA-activated cells dropped from ~100% to 20% between p3-p13 reflecting DEIGS. In Ts65Dn cultures, the timing of this switch was delayed by few days. Next,

microelectrode recordings of multi-unit activity (MUA) were performed in CA3 slices during bath application of the GABAA agonist isoguvacine. MUA frequency was increased in p8-p12 and reduced in p14-p22 slices reflecting the switch of GABA from excitatory to inhibitory mode. The timing of this switch was delayed in Ts65Dn by ~ 2 days. Furthermore, giant depolarizing potentials (GDPs), a form of primordial neural activity, were observed in p12-p14 Ts65Dn but not in 2N slices. Finally, levels of NKCC1 and KCC2 chloride transporters were measured in the hippocampus of p12 Ts65Dn and 2N pups. Levels of NKCC1 were unaltered, while levels of KCC2 were reduced in Ts65Dn. Thus, DEIGS timing is delayed in Ts65Dn mice. These changes may affect nascent neural circuits formation and contribute to the synaptic and neuronal abnormalities in DS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.02/B54

**Topic:** A.07. Developmental Disorders

**Support:** Indiana Clinical and Translational Sciences Institute, NIH Grant UL1TR001108

IUPUI Research Support Funds Grant

**Title:** Characterization of brain region-specific Dyrk1a protein levels and kinase activity during development of Ts65Dn Down syndrome mice to guide pharmacotherapy by Dyrk1a inhibition

**Authors: M. STRINGER**[1], J. M. LACOMBE[2], *C. R. GOODLETT[3], R. J. ROPER[2]
[1]Psychology, [2]Biol., [3]IUPUI, Indianapolis, IN

**Abstract:** Down syndrome (DS) is caused by three copies of human chromosome 21 (Hsa21) and results in phenotypes including intellectual disability. Ts65Dn mice, the most extensively studied DS model, have three copies of ~50% of the genes on Hsa21 and display many phenotypes associated with DS, including cognitive deficits. *DYRK1A* is a dosage-sensitive gene found in three copies in humans with Trisomy 21 and in Ts65Dn mice, and is involved in CNS development. Overexpression of *DYRK1A* is hypothesized to cause many of the cognitive and developmental deficits observed in DS, and has been touted as a target for drug development in DS. Studies testing Epigallocatechin-3-gallate (EGCG), a putative inhibitor of Dyrk1a, have yielded inconclusive results and there is no direct evidence that EGCG itself produces any therapeutic outcomes via Dyrk1a inhibition. Definitive evidence that excessive expression/activity of Dyrk1a directly contributes to specific phenotypes in DS mouse models is

limited, and there is no direct evidence that pharmacological inhibition of Dyrk1a *in vivo* causes enduring improvement in DS cognitive phenotypes. In part, this reflects the remarkably limited knowledge of the temporal regulation of Dyrk1a expression and activity in different brain regions across development in DS mouse models. To establish the therapeutic potential of Dyrk1a inhibitors, it is first necessary to determine when and in what brain regions excessive Dyrk1a is evident and directly associated with enduring aberrant functional development. The goal of this study is to provide the first systematic quantification of Dyrk1a protein levels and kinase activity at key postnatal (P) ages (P12, P30, P42 and P65) in Ts65Dn mice, at ages of translational relevance to clinical applications in humans (birth, early adolescence, late adolescence, young adult). Contrary to expectation, Western blot analyses indicated a surprising reduction of Dyrk1a protein in cerebellum and no difference in cerebral cortex or hippocampus in young adult Ts65Dn as compared to euploid controls mice, and high performance liquid chromatography assays in the same tissue indicated kinase activity was not elevated in any of the three brain regions of trisomic mice. Ongoing studies continue to test the hypothesis that temporal differences in Dyrk1a protein and kinase activity will be evident in specific brain regions at earlier postnatal periods. Stages when excessive Dyrk1a is evident in one or more brain regions should identify candidate periods to test the therapeutic efficacy of Dyk1a inhibition in DS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 557.03/B55

**Topic:** A.07. Developmental Disorders

**Support:** P30GM110768

**Title:** Human iPSC-based organoid and chimeric mouse models reveal OLIG2-dependent abnormal production of GABAergic neurons in down syndrome

**Authors: *R. XU**, P. JIANG
UNMC, Omaha, NE

**Abstract:** Down syndrome (DS), characterized by the triplication of human chromosome 21 (HSA21), is the most common genetic cause of developmental delay and intellectual disability. Over-inhibition or overproduction of GABAergic neurons significantly contributes to the cognitive deficit of DS. However, deciphering the genotype-phenotype relationships in DS with mouse models is challenging, due to the incomplete trisomy of orthologous genes on HSA21 and

in some animal models, the overexpression of additional genes that are not syntenic to HSA21 genes. Previous studies have reported that *Olig1* and *Olig2*, which are triplicated in DS, play critical roles in regulating GABAergic neuron differentiation in mice. However, their functions in regulating differentiation of human GABAergic neurons are largely uncertain. Here, we established human induced pluripotent stem cell (iPSC)-derived 3-dimensional brain organoid and chimeric mouse brain models to recapitulate the differentiation of GABAergic neurons in humans. First, we generated human iPSC-derived brain organoids, containinga large population of OLIG2-expressing ventral forebrain neural progenitors. Using this model, we found increased expression of OLIG2 but not OLIG1 in DS forebrain organoids. Moreover, compared to the control organoids, these DS organoids overproduced GABAergic neurons, particularly the calretinin-expressing GABAergic neurons. Mechanistically, we found that the overexpression of OLIG2 upregulated the expression of transcription factors that were reported to regulate development and specification of GABAergic neurons, such as *LHX8*. The upregulated expression of *LHX8* could be corrected by *OLIG2* knockdown in DS organoids. Furthermore, we engrafted the DS hiPSC-derived ventral forebrain neural progenitors into the neonatal mouse brains and found similarly abnormal production of GABAergic neurons *in vivo*. These data demonstrate that OLIG2 plays a critical role in GABAergic overproduction in DS based on our established human iPSC-derived organoid and chimeric mouse brain models. These results suggest a potential therapy for DS by regulating the expression of OLIG2 to manipulate GABAergic neuron production.

**Disclosures:** **R. Xu:** None. **P. Jiang:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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Alzheimer's Association

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Linda Crnic Seed Grant

Simon's Foundation SFARI 27444

**Title:** Examining the effects of the $GABA_A$ antagonist pentylenetetrazol on seizure activity and EEG spectral power in the Dp16 mouse model of down syndrome

**Authors: *D. J. PETERSON**[1], J. LEVENGA[2], H. WONG[3], P. CAIN[4], C. HOEFFER, Jr.[5]
[1]Univ. of Colorado - Boulder, Boulder, CO; [3]Inst. for Behavioral Genet., [2]Univ. of Colorado Boulder, Boulder, CO; [5]Integrated Physiol., [4]Univ. of Colorado, Boulder, Boulder, CO

**Abstract:** Down syndrome (DS) is a neurodevelopmental disorder that is caused by the presence of an extra copy of human chromosome 21 (Hsa21). Currently, DS is one of the most common genetic causes of intellectual disability in the clinical population. While health management has significantly improved the clinical prognosis and quality of life in the DS population, there are currently no available drug treatments to ameliorate the cognitive impairments associated with DS. In addition, recent findings have shown that people with DS develop symptoms consistent with early onset Alzheimer's disease (AD). Further, Both AD and DS have been associated with abnormal EEG activity and seizures. In DS, seizures seem to peak at two ages; the first peak occurs in the first two years of age, while the second peak occurs during adulthood. The second peak may be connected with the symptoms of AD. The goal of the present study was to test the hypothesis that the Dp(16)1Yey/+ mouse model of DS develops epilepsy during aging. Dp16 mice have a direct duplication of the entire Mmu16 region that is conserved in HSA21. In the current study, we examined the effects of pentylenetetrazol (PTZ), a $GABA_A$ antagonist on seizure activity and EEG spectral power in freely moving mice. Aged (12-16 months) Dp16 and WT control mice were surgically implanted with preamplifier headmounts containing two EEG and one EMG electrodes. PTZ was then administered via intraperitoneal (i.p.) injections every other day in increasing dosages and seizure activity was scored visually using the revised Racine scale. We found that Dp16 display phenotypic differences in both the response to PTZ and spectral power following PTZ administration. We expect that old Dp16 mice have increased power in beta and gamma frequencies due to excessive inhibition by the GABAergic circuit reported in DS model mice, while spiking frequency may be reduced. This work will be important to better understand circuit dysfunction during aging and its role in seizure activity in DS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 557.05/B57

**Topic:** A.07. Developmental Disorders

**Title:** Incomplete developmental excitatory-inhibitory GABA shift alters neuronal network dynamics in Down syndrome

**Authors: *M. ALBERTI**[1,2], S. NASKAR[1], I. COLOMBI[1,2], M. PARRINI[1], S. MAGARA[1], M. NANNI[1], V. PASQUALE[1], M. CHIAPPALONE[1], A. CONTESTABILE[1], L. CANCEDDA[1,3]
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**Abstract:** Down syndrome (DS) is a genetic disorder caused by the presence of an extra copy of chromosome 21 and it is mainly characterized by a wide range of cognitive disabilities. Studies on DS mouse models have shown that learning and memory deficits are likely determined by neurodevelopmental alterations arising during brain development and extending into adulthood. Among the many abnormalities found in the brains of DS mice, altered GABAergic inhibition is believed to largely impinge on cognitive functions. Indeed, we have recently found that the expression of the chloride importer NKCC1 is increased in the brains of both the Ts65Dn mouse model of DS and individuals with DS. Accordingly, we have found that increased intracellular chloride concentration renders $GABA_A$-mediated currents depolarizing in the adult brain of Ts65Dn mice. Here, we show that NKCC1 upregulation is also replicated *in vitro* in primary neuronal cultures from Ts65Dn mice. As a consequence, we found that the GABA developmental excitatory-inhibitory shift -assessed by calcium-imaging experiments- is largely incomplete in trisomic neurons. Accordingly, patch-clamp recordings in mature trisomic cultures indicate the occurrence of a high percentage of neurons showing depolarizing GABA signaling. In agreement with a depolarizing action of GABA, multi-electrode array (MEA) experiments also show that neural response to GABAergic drugs are profoundly altered also at the network level in mature neuronal cultures. Interestingly, treatment of Ts65Dn neurons with the FDA-approved NKCC1 inhibitor Bumetanide rescues intracellular chloride accumulation in trisomic neurons and restores inhibitory GABA signaling at both the cellular and network levels. Finally, consistent with a depolarizing GABA action in Ts65Dn mice, the positive $GABA_A$ allosteric modulator Diazepam demonstrates a paradoxical anxiogenic effect in trisomic mice, which is reversed by Bumetanide. Our findings demonstrate that NKCC1 upregulation drives depolarizing $GABA_A$ signaling in trisomic neurons, leading to altered neuronal-network activity and behavioral abnormalities in DS mice.

**Disclosures:** **M. Alberti:** None. **S. Naskar:** None. **I. Colombi:** None. **M. Parrini:** None. **S. Magara:** None. **M. Nanni:** None. **V. Pasquale:** None. **M. Chiappalone:** None. **A. Contestabile:** None. **L. Cancedda:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.06/B58

**Topic:** A.07. Developmental Disorders

**Support:** Fondecyt (Chile) #1130241

Fondos ICM-ECONOMIA (Chile) #P09-022-F

**Title:** RCAN1 knock-down restores exocytosis in a neuronal cell line derived from the cerebral cortex of a trisomy 16 mouse, an animal model of Down syndrome

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**Abstract:** Down Syndrome (DS, trisomy 21) is a condition that entails an excess dosage of genes localized in chromosome 21, where genes mapped to the DS critical region are particularly relevant. Among those genes is the Regulator of Calcineurin 1 (RCAN1), whose overexpression disturbs neurotransmitter release, and impairs synaptic plasticity, learning and memory. The relative contribution of RCAN1 in a context where other genes of the DS critical region are also overexpressed has yet to be elucidated. In the present work, we aimed to explore the contribution of RCAN1 to exocytosis. We used the CTb cell line, derived from the brain cortex of a trisomy 16 mouse, a model for DS, to study the contribution of RCAN1 to exocytosis. Single exocytotic events were imaged at the evanescent field of cells expressing the vesicular acetylcholine transporter fused to the pH-sensitive green fluorescent protein pHluorin, and subsequently measuring the fluorescent signal with total internal reflection fluorescence microscopy. We found that, as compared with CNh cells (a cell line established from the cerebral cortex of a normal littermate), CTb cells not only overexpress RCAN1 but display alterations in Ca2+-induced exocytosis, manifested by a reduced number of exocytotic events and a slower time-dependent kinetics. CNh and CTb cells displayed respectively 34 ± 4.4 and 23 ± 1.9 exocytotic events during a period of 3 min of recording ($p<0.05$). Decay time values for such events were 2.4 ± 0.2 s and 3.4 ± 0.3 s for CNh and CTb cells, respectively, being significantly slower in the trisomic cells ($p<0.05$). We further demonstrated that RCAN1 knockdown restored this altered exocytosis to levels comparable to those of the euploid CNh line. Our data support the critical contribution of RCAN1 to neurotransmission dysfunction in the DS condition.

**Disclosures: J. Vázquez-Navarrete:** None. **X. Baéz-Matus:** None. **M. Acuña:** None. **A.M. González-Jamett:** None. **S. Brauchi:** None. **A. Martínez:** None. **P.A. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pablo Caviedes. **A. Cárdenas:** None.

**Poster**

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**Program#/Poster#:** 557.07/B59

**Topic:** A.07. Developmental Disorders

**Support:** NINDS F31 NS 100391

NIH R21 NS094091

Protein Folding Disease Initiative from University of Michigan

**Title:** Functional interaction between Down syndrome cell adhesion molecule and Amyloid precursor protein in axon development

**Authors: *M. VELING**[1], G. R. STERNE[2], B. YE[3]
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**Abstract:** The genes that drive different aspects of developmental defects in Down syndrome (DS) are largely unknown. A potential driver of neurodevelopmental defects is Down syndrome cell adhesion molecule (Dscam), which is located in the Down Syndrome critical region of chromosome 21. Our previous studies have demonstrated that increased levels of Dscam causes axon terminal overgrowth in *Drosophila*. Moreover, we found that Dscam requires Abelson tyrosine kinase (Abl) to promote this axon terminal overgrowth. Interestingly, Amyloid Precursor Protein (APP), which is often overexpressed in the brains of DS patients, has also been shown to promote axonal growth in *Drosophila*. This raises the possibility that overexpression of these two genes, as occurs in DS, exacerbates the developmental defects that are caused by overexpression of each gene alone. We used genetic and biochemical approaches to test this possibility. Our results suggest that Dscam and *Drosophila* homolog of APP (Appl) function in parallel pathways that interact with each other to promote axon terminal growth. This study elucidates how two genes function together to contribute the developmental defects in DS. (M.V. and G.S. contributed equally to this work.)

**Disclosures:** **M. Veling:** None. **G.R. Sterne:** None. **B. Ye:** None.

**Poster**

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**Support:** NIH R01, NS086933-01

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Simon's Foundation SFARI 2744

**Title:** Disrupted sleep architecture in a mouse model of Down syndrome

**Authors: *P. CAIN**[1], D. J. PETERSON[2], J. LEVENGA[2], C. A. HOEFFER[3]
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**Abstract:** Down syndrome (DS) results from the triplication of the 250 or so genes on human chromosome 21 (HSA21). Sleep disruption is a well-documented phenotype in approximately 50% of Down syndrome individuals. These disruptions may appear as night terrors or sleep walking during slow-wave sleep or REM behavior disorders or nightmares during REM sleep. There are mouse models of DS, including Ts65Dn mice, which are trisomic for a segment of mouse chromosome 16 orthologous to a region containing over 150 genes of HSA21 along with genes found on a segment of mouse chromosome 17. The sleep disruption observed in Ts65Dn mice may be attributed to the additional copies of these non-orthologous genes. Dp(16)1Yu mice (Yu et al., 2010), derived on a C57BL/6J strain, duplicate only the portion of mouse chromosome 16 that is homologous to HSA21 and thus are more comparable to DS. Among observed phenotypes are heart defects, learning and memory deficits comparable to DS symptoms. Sleep disruption has not been investigated in Dp(16) mice.
In this study, old (16 mos) Dp16+/+ mice were surgically implanted with EEG and EMG electrodes and EEG activity recorded. Sleep and motor activity were recorded across two 24 hr periods with sleep states (Wake, NREM, REM) scored and activity confirmed via video recording. We observed disrupted sleep demonstrated by increased activity as compared to wild-type conspecifics. In addition, we found that compared to wild-type, Dp(16) mice display significant differences in the relative EEG power distributed among oscillation frequencies in both sleep and awake states. The results we see are consistent with sleep disturbances in DS. These differences in relative power reflect underlying differences in neuronal activity at the network level and thus are causative agents rather than merely symptoms.

Yu, T., Li, Z., Jia, Z., Clapcote, S. J., Liu, C., Li, S., ... & Carattini-Rivera, S. (2010). A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. *Human molecular genetics*, ddq179.

**Disclosures: P. Cain:** None. **D.J. Peterson:** None. **J. Levenga:** None. **C.A. Hoeffer:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Topic:** A.07. Developmental Disorders

**Support:** NIH K99 AG044469

NIH R00 AG044469

Alzheimer's Association NIRG-15-362799

Wake Forest ADCC Pilot Grant

Wake Forest CTSI Pilot Grant

**Title:** Glucagon-like peptide cleavage produce rescues long-term potentiation in a mouse model of Down syndrome

**Authors: *S. M. DAY**[1], W. YANG[2], X. ZHOU[2], T. MA[2]
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**Abstract:** Down syndrome (DS) is one of the most common forms of intellectual disabilities and is due to the trisomic repeat of chromosome 21 (HSA21). Impaired cognitive ability is a hallmark of DS pathology, and there are currently no pharmacological treatments that have effectively ameliorated the memory deficits associated with DS. Long-term potentiation (LTP) is a major form of synaptic plasticity and a cellular model of memory and is impaired in DS model mice.
We have previously reported that the glucagon-like peptide-1 (GLP-1) cleavage product, GLP-1 (9-36) enhances LTP in wild-type mice and restores LTP in mouse models of Alzheimer's disease (AD). Here, we show that GLP-1 (9-36) rescues LTP deficits in DS model mice. We also show that GLP-1 (9-36) treatment decreases mitochondrial superoxide within hippocampal CA1 neurons. Together these data provide evidence that GLP-1 (9-36) may improve memory in DS model mice by decreasing oxidative stress within CA1 hippocampal neurons.
This study provides a potential mechanism through which GLP-1 (9-36) may improve memory

in DS model mice. In the future, we will further elucidate the molecular mechanisms through which GLP-1 (9-36) influences learning and memory in mouse models of DS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 557.10/B62

**Topic:** A.07. Developmental Disorders

**Title:** Upregulation of NKCC1 chloride importer impairs GABAAR-mediated inhibition and memory in Down syndrome

**Authors: *M. PARRINI**[1], S. NASKAR[1], M. ALBERTI[1], M. NANNI[1], G. RONZITTI[2], F. MINGOZZI[2], A. CONTESTABILE[1], L. CANCEDDA[1,3]
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**Abstract:** Down syndrome (DS) is caused by the triplication of human chromosome 21 and it represents the most frequent genetic cause of intellectual disability. The Ts65Dn mouse model of DS exhibits the main cognitive disabilities of the human syndrome and it is characterized by learning and memory deficits in several behavioral tasks. Previous studies have shown that altered GABAergic transmission through chloride-permeable $GABA_A$ receptors ($GABA_ARs$) largely contributes to cognitive impairment inTs65Dn mice. Specifically, we have recently found that intracellular chloride accumulation shifts the chloride reversal potential ($E_{Cl}$) toward more positive values and $GABA_AR$-mediated signaling from hyperpolarizing to depolarizing in the adult brain of Ts65Dn mice. Moreover, intracellular chloride accumulation is paralleled by increased expression of the chloride importer NKCC1 (Na-K-Cl cotransporter) in the brains of both trisomic mice and individuals with DS. Importantly, pharmacological inhibition of NKCC1 with the FDA-approved drug Bumetanide restores learning and memory in Ts65Dn mice. In order to validate NKCC1 as a molecular target for cognitive deficits in DS and open the possibility for a future gene-therapy approach to treat the disease, we have here investigated whether normalization of NKCC1 activity could rescue cognitive deficits in Ts65Dn mice. In particular, we have developed and optimized a RNA-interference approach to knockdown NKCC1 expression. Our results show that reducing the expression of NKCC1 restored intracellular chloride concentration and $GABA_AR$-mediated inhibition in trisomic neurons *in vitro*. Most importantly, AAV-mediated neuron-specific NKCC1 knockdown *in vivo* in the hippocampus of adult Ts65Dn animals rescued behavioral performance on different learning and memory tests at levels undistinguishable from those of WT mice. Since the developmental excitatory-inhibitory shift in GABA signaling is fundamental for proper adult brain physiology,

we are currently assessing long-term effects and possible adverse outcomes of NKCC1 downregulation in DS neonatal mice. These findings indicate that NKCC1 upregulation drives intracellular chloride accumulation and depolarizing $GABA_AR$-signaling in trisomic cells, leading to behavioral impairments in DS mice. Moreover, our study identifies a new molecular target for treatments aimed at rescuing cognitive disabilities in individuals with DS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.11/B63

**Topic:** A.07. Developmental Disorders

**Title:** Molecular mechanisms underlying tubulin gene variants in malformations of cerebral cortex development

**Authors: *A. V. DERRICK**[1,1], T. D. CHUSION[2], A. E. FRY[2], J. G. L. MULLINS[1], W. B. DOBYNS[3], M. I. REES[1], S. CHUNG[1]
[1]Swansea Univ. Med. Sch., Swansea, United Kingdom; [2]Inst. of Med. Genet., Cardiff, United Kingdom; [3]Ctr. for Integrative Brain Res., Seattle, WA

**Abstract:** Microtubules play essential roles during neuronal proliferation, migration and post-migrational organisation stages of cerebral cortex development. Microtubule polymers are composed of α and β tubulin heterodimers, forming the dynamic scaffold-like structure essential for cell motility and function. In recent years, a number of genetic variations in neuronal, developmentally-regulated tubulin genes (*TUBA1A, TUBB2B, TUBA8, TUBB4B, TUBB, TUBB2A* & *TUBB3*) have been associated with a spectrum of malformations of cortical development (MCDs) including microcephaly, lissencephaly (LIS) and polymicrogyria-like malformations (PMG). Individuals with LIS and PMG were screened for tubulin mutations by whole-exome sequencing and targeted gene screening. Three novel tubulin gene-variants were identified in *TUBA1A* including two missense changes and one predicted in-frame deletion. Novel missense mutations were also identified in *TUBB2A* (n=2), *TUBB2B* (n=1) and *TUBB* (n=1). The tubulin variants were functionally analysed using *in silico* predictive protein modelling, *in vitro* microtubule polymerisation assays, and confocal immunocytochemistry. Homology models of tubulin mutants predicted the protein conformation consequences of the amino acid residue substitutions. C-terminally FLAG-tagged tubulin expression constructs were generated and transfected into HEK293 cultured cells. Incorporation of tubulin variants into the microtubule network was observed in comparison to wild-type, with reduced microtubule incorporation or altered microtubule stability/dynamics. *In silico* and *in vitro* functional analysis

provides evidence of the potential damaging effects of these variants on microtubule assembly and gives further insight towards *in vivo* causality in MCDs.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 557.12/B64

**Topic:** A.07. Developmental Disorders

**Support:** TGen / Center for Rare Rare Childhood Disorders

**Title:** A novel, de novo FBXO28 frameshift in a patient presenting with the predominant form of Chromosome 1q41-q42 deletion syndrome

**Authors: *C. BALAK**[1], N. BELNAP[1], K. RAMSEY[1], A. SINIARD[1], S. SZELINGER[1,2], M. RUSSELL[1], R. RICHHOLT[1], M. DE BOTH[1], W. JEPSEN[1], I. PIRAS[1], M. NAYMIK[1], S. RANGASAMY[1], I. SCHRAUWEN[1], D. CRAIG[1,3], V. NARAYANAN[1], M. HUENTELMAN[1]
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**Abstract:** Chromosome 1q41-q42 deletions have recently been associated with a distinguishable neurodevelopmental disorder in early childhood (OMIM 612530). Features of this microdeletion syndrome vary moderately depending on the location and specific genes encompassed by the each deletion. Despite this, a predominant phenotype has emerged that includes developmental delay, intellectual disability, epilepsy and unique facial features including a depressed nasal bridge, gingival hyperplasia and widely-spaced teeth. It is also frequently accompanied with CT/MRI abnormalities, hypo/hypertonia, nail hypoplasia, digit abnormalities or contractures and less often congenital anomalies including diaphragmatic hernias and cardiac defects. To date, no definitive gene(s) have been directly attributed to the predominant 1q41-q42 microdeletion phenotype as even the smallest deletions encompass a minimum of 6 genes. Nevertheless recent reports have proposed DISP1, FBXO28 and TP53BP2 to play roles in 1q41-q42 pathogenesis through studies of smallest region of overlap and single gene murine models. Here we present the first report of a single-gene loss-of-function event in a 3 year-old female patient with global developmental delay, intellectual disability, seizures, distinct facial features and other traits highly reflective of the overriding 1q41-q42 microdeletion phenotype. Through Whole Exome Sequencing we identified a de novo, heterozygous c.972_973delACinsG (p.Arg325GlufsX3) 2 base pair genomic event in FBXO28 in the female proband. The resulting frameshift leading to a

premature stop codon three amino acids downstream has not been reported in any genomic reference database including ExAC and gnomAD. This frameshift variant is the first report of single-gene, base-pair level event causing the hallmark features of the predominant 1q41q42 deletion syndrome phenotype. Additionally, it establishes FBXO28 as a novel monogenic neurodevelopmental disease gene. In light of this new evidence we propose a more accurate name of FBXO28 Haploinsufficiency Syndrome in describing the phenotype displayed in our patient and others harboring FBXO28 deficiencies in the future.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.13/B65

**Topic:** A.07. Developmental Disorders

**Support:** Foundation for Angelman Syndrome Therapeutics Integrative Research Environment

**Title:** Phenotypic rescue with neuroprotective therapeutics in an Angelman syndrome mouse model

**Authors: *H. A. BORN**[1], A. T. DAO[1], L. A. MARTINEZ[1], A. N. CARTER[2], A. T. LEVINE[2], E. J. WEEBER[4], D. J. SEGAL[5], A. E. ANDERSON[3]
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**Abstract:** Angelman Syndrome (AS) is a monogenetic neurodevelopmental disorder that affects approximately 1 in 15,000 births. In addition to cognitive and motor deficits, epileptic seizures and abnormal EEG activity are commonly found in AS. Currently, there is no treatment for AS available, and the seizures associated with AS are resistant to most anti-epileptic drugs. The most common genetic cause of AS is the deletion or mutation in the maternally imprinted Ube3a gene, encoding ubiquitin ligase (Ube3a), leading to absence or dysfunction of the Ube3a protein. In our studies, we have investigated the effect of mouse strain background on the AS behavioral profile, electroencephalography (EEG) activity, and seizure threshold in a mouse model of AS (Ube3a gene maternal deletion). We found strain-dependent differences in the AS phenotypes. The AS C57Bl/6J mice displayed hypoactivity, impaired motor coordination, learning and memory deficits, and abnormal EEG activity. The AS 129 mice exhibited poor performance on the wire

hang and contextual learning and memory tests and lower threshold to audiogenic seizures. These findings indicate that the mouse genetic background modifies the behavioral, EEG, and seizure threshold phenotype in the AS mice. Using these strain-specific phenotypes for the AS mice we are screening novel therapeutics, including artificial transcription factors (ATF) to restore Ube3a brain levels and testing whether these manipulations reverse the behavioral, EEG, and seizure phenotypes in the AS mouse model. We have also begun work screening the effect of different neuroprotective compounds from Neuren Pharmaceuticals (NNZ-2566), Anavex (A2-73), and Mitochon (MP101) on AS phenotypes. Our initial data suggests different compounds selectively rescue specific phenotypes in the AS and wildtype littermate mice. These studies will serve as preclinical data for additional translational studies in larger mammals and ultimately humans with AS.

**Disclosures:** **H.A. Born:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neuren Pharmaceuticals, Anavex Life Sciences Corp., Mitochon Pharmaceuticals Inc. **A.T. Dao:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neuren Pharmaceuticals, Anavex Life Sciences Corp., Mitochon Pharmaceuticals Inc. **L.A. Martinez:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neuren Pharmaceuticals, Anavex Life Sciences Corp., Mitochon Pharmaceuticals Inc.. **A.N. Carter:** None. **A.T. Levine:** None. **E.J. Weeber:** None. **D.J. Segal:** None. **A.E. Anderson:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neuren Pharmaceuticals, Anavex Life Sciences Corp., Mitochon Pharmaceuticals Inc..

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Program#/Poster#:** 557.14/C1

**Topic:** A.07. Developmental Disorders

**Support:** XDB0202003

**Title:** The angelman syndrome protein Ube3a regulates the assembly of PP2A

**Authors: *J. WANG**, Q. Z. XIONG
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**Abstract:** Disruption or duplication of maternally inherited 15q11-13, the chromosomal location where UBE3A resides, results in neurodevelopmental diseases including Angelman syndrome(AS) or Autism. In most causes, AS is resulted from the deletion, disruption or mutation of the maternal UBE3A allele, which encodes a HECT domain ubiquitin E3 ligase and its deficiency. Although a few targets of Ube3a have been identified in brain, they could not

explain all the phenotypes of the syndrome. Several studies demonstrated that phosphorylation-level of several proteins is dysregulation in AS mice model. Recent studies indicated Ube3a can be phosprylated by PKA at residue T485 and inhibited its activity, of which a de novo autsim linked missense mutation leads to autism. These findings indicate that Ube3a are related to protein phosphorylation homeostatsis in brain.Reversible phosphorylation of proteins is tightly and precisely regulated by relative activities of protein kinases and phosphatases. Protein phosphatase 2A(PP2A) is one major of the Ser/Thr phosphatases, which is essential for different aspects of celluar progresses, including cell cycle, cell differentiation, cell fate determination and multiple signal pathways. The underlying basis of diversity functions is the PP2A complex structure and a plethora of regulations. PP2A holoenzymes are hetertrimers formed by a catalytic C subunit, a scaffolding A subunit and a variety of regulatory B subunits that are divided into four non-homologous families: PR55/B(B55), PR61/B'(B56), PR48/PR72/PR130/B'' and PR93/PR110/B'''.In brain, impaired funciton of PP2A is associated with several neuronal diseases. However, it remains largely unknown about how these regulating factors together affect the PP2A activity *in vivo*.Herein, we show, using coimmunoprecipitation and mass spectrum methods, that Ube3a specifically interacts with PP2A heterotrimers containing regulatory B subuint PPP2R2A/B55α, which belongs to PR55 family members. In Angelman syndrome model mice, the assembly of PP2A increased, thereby enhancing the activities of PP2A during postnatal three to six weeks. Furthermore, using SILAC mice technology, we show that PTPA, the protein phosphatase 2A(PP2A) activator, increased in AS model mice brain, which contribute to the assembly of PP2A holoenzymes. Downregulation of PTPA can partially prevent the motor learning deficiency in AS model mice.These findings descirbe a molecular regulation that contributes to some certain pathologic mechanisms behind the neurodevelopmental disease Angelman syndrome.

**Disclosures:** **J. Wang:** None. **Q.Z. Xiong:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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NICHD grant T32-HD040127

**Title:** Delta rhythmicity is a reliable EEG biomarker in Angelman syndrome

**Authors: *M. S. SIDOROV**[1], H. DEN BAKKER[2], G. M. DECK[3], M. DOLATSHAHI[3], R. L. THIBERT[3], L. M. BIRD[4], C. J. CHU[3], B. D. PHILPOT[1]
[1]Cell Biol. and Physiol., [2]Univ. of North Carolina, Chapel Hill, NC; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Rady Children's Hosp., San Diego, CA

**Abstract:** Clinicians have qualitatively described rhythmic delta activity as a prominent EEG abnormality in individuals with Angelman syndrome, but this phenotype has yet to be rigorously quantified in the clinical population or validated in a preclinical model. Here we sought to quantitatively measure delta rhythmicity and evaluate its fidelity as a biomarker. We quantified delta oscillations in mouse and human using parallel spectral analysis methods and measured regional, state-specific, and developmental changes in delta rhythms in a patient population. Delta power was broadly increased and more dynamic in both the Angelman syndrome mouse model, relative to wildtype littermates, and in children with Angelman syndrome, relative to age-matched neurotypical controls. Enhanced delta oscillations in children with Angelman syndrome were present during wakefulness and sleep, were generalized across the neocortex, and were more pronounced at earlier ages. Delta rhythmicity phenotypes can thus serve as reliable biomarkers for Angelman syndrome in both preclinical and clinical settings.

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**Poster**

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**Title:** Epigenome wide association study of DNA methylation in Williams syndrome

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**Abstract:** Epigenetic alterations, including DNA methylation, have been implicated in many psychiatric and neurodevelopmental disorders. Williams Syndrome (WS) is rare genetic neurodevelopmental disorder and characterized by multiple symptoms including hypersociability. WS is caused by a 7q11.23 heterozygous deletion containing 26-28 genes, which include genes associated with epigenetic regulation. In this study, we performed epigenome-wide association study (EWAS) to determine the contribution of DNA methylation to WS. We examined a total of 171 subjects, including a discovery set (34 WS and 34 controls) and an independent confirmation set (56 WS and 47 controls). We used the Social Responsiveness Scale-2 (SRS-2), the Hyperacusis Questionnaire (HQ), the revised Fear Survey Schedule for Children (FSSC-R) and the sensory profile (SP) to evaluate behavioral and neuropsychiatric symptoms in WS. DNA methylation profiles were obtained from whole blood samples from a discovery set using the Illumina HumanMethylation 450 K BeadChip. Validation analyses of the epigenome-wide findings performed in a confirmation set using site-specific methylation with pyrosequencing. After accounting for batch effects, the effects of leukocyte subsets and multiple testing, we have identified multiple differentially methylated positions (DMPs) and regions (DMRs) consistently associated with WS in a discovery set. Some of the identified genes with differential methylation (*ANKRD30B*) have previously been associated with WS and we replicate those findings in a confirmation set. Pathway analysis revealed significant enrichment of our DMPs in cardiac-adrenergic signaling and tRNA splicing. To our knowledge, this study is the largest EWAS performed in WS. Our findings provide insights into the molecular basis and pathophysiology of the various phenotypes of WS.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 557.17/C4

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant MH106934

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NIH Grant MH109904

Ricerca Corrente, Italian Ministry of Health

Pioneer Award DP1AG047744-01

**Title:** The Williams syndrome/7q11.23 gene DNAJC30 links mitochondria to neural development and behavior

**Authors: *A. T. TEBBENKAMP**[1], L. VARELA[2], J. CHOI[1], M. I. PAREDES[1], A. GIANI[1], D. FRANJIC[1], A. M. M. SOUSA[1], Z.-W. LIU[2], M. LI[1], M. KOCH[2], K. SZIGETI-BUCK[2], A. TOBIAS[2], Z. LI[1], Y. I. KAWASAWA[3], C. D. PASPALAS[1], P. PRONTERA[4], G. MERLA[5], A. F. T. ARNSTEN[1], T. L. HORVATH[2], N. SESTAN[1,2]
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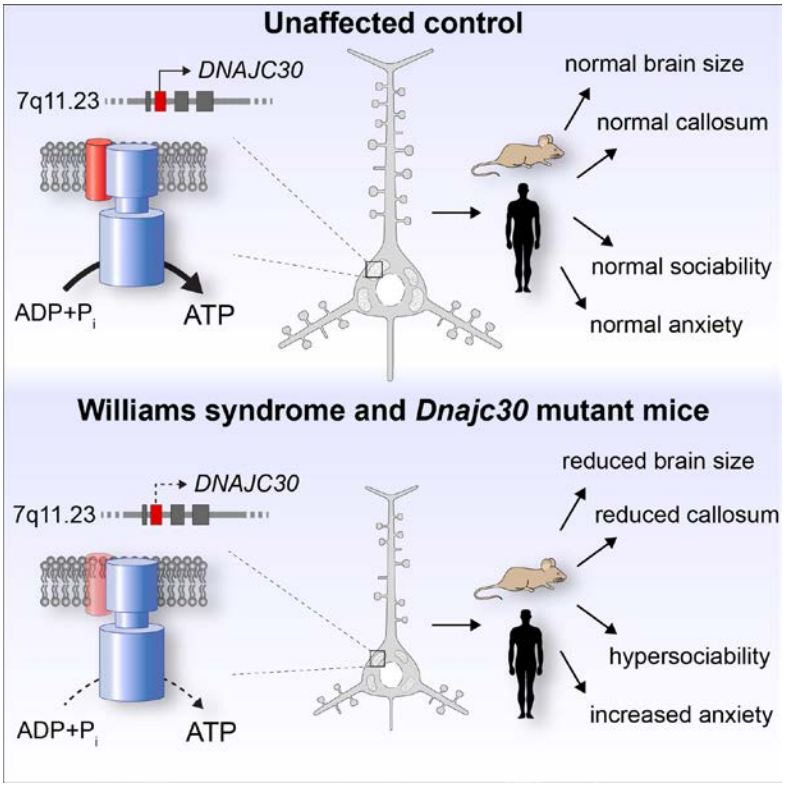
**Abstract:** Despite the known causality of copy number variations (CNVs) to human neurodevelopmental disorders, the mechanisms behind each genes' contribution to the constellation of neural phenotypes remains elusive. Here, we investigated the 7q11.23 CNV, associated with Williams syndrome and autism spectrum disorder, and reveal that Williams syndrome is mediated in part by mitochondrial dysfunction. Dysfunction is facilitated by the 7q11.23 protein DNAJC30, which interacts with ATP synthase. Removal of Dnajc30 in mice resulted in hypofunctional mitochondria, decreased ATP synthesis, and lowered K+ATP channel-mediated membrane potentials in neurons of the cerebral cortex. Dnajc30 knockout mice exhibited Williams syndrome-like features of altered social behavior and anxiety, as well as a smaller corpus callosum, reduced axonal caliber, pyramidal neuron size, and dendritic elaboration, similar to that seen in Williams syndrome patients. On the whole, we reveal a mechanism of how hypofunctional mitochondria stunt neural circuit development and cause several hallmarks of neurodevelopmental disorders.



**Disclosures:** **A.T. Tebbenkamp:** None. **L. Varela:** None. **J. Choi:** None. **M.I. Paredes:** None. **A. Giani:** None. **D. Franjic:** None. **A.M.M. Sousa:** None. **Z. Liu:** None. **M. Li:** None. **M. Koch:** None. **K. Szigeti-Buck:** None. **A. Tobias:** None. **Z. Li:** None. **Y.I. Kawasawa:** None. **C.D. Paspalas:** None. **P. Prontera:** None. **G. Merla:** None. **A.F.T. Arnsten:** None. **T.L. Horvath:** None. **N. Sestan:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.18/C5

**Topic:** A.07. Developmental Disorders

**Title:** ATRX specifies stem cell identity during human brain development

**Authors: *T. SANOSAKA**, R. TOMOOKA, M. CHAI, I. KOYA, T. ANDO, H. OKANO, J. KOHYAMA
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**Abstract:** The prevalence of human diseases caused by mutations in chromatin remodeling genes underpins the importance of chromatin structure in gene regulation. One such disease, Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome, is caused by mutations of *ATRX* gene. This syndrome is characterized by mental retardation with severe developmental delay, craniofacial and urogenital abnormalities, as well as mild anemia. Previous studies have reported the role of ATRX in α-thalassaemia and cancer; however, the function of ATRX during human brain development remains unknown. In addition, multiple organ defects observed in ATR-X syndrome patients, and the identification of *ATRX* mutation as the sole genetic cause of ATR-X syndrome has raised the question of how disruption of single factor can lead to multiple phenotypes. In the present study, we found that ATRX regulates stemness and neurogenic property of human neuroepithelial (NE) cells. Dysregulation of ATRX results in the loss of NE identity and the aberrant acquisition of neural crest properties. We also generated chromatin landscapes of ATRX-bound genomic regions and found that ATRX displayed correlation with repressive heterochromatin (H3K9me3), as reported previously. In addition to its well-established repressive role, we found that a small but significant fraction of ATRX-bound regions was associated with active histone marks. Such an association was undetectable in other somatic cells, indicating the dual function of ATRX is exclusive to neural lineage. Thus, the function of ATRX as a transcription activator in the regulation of cell identity during human brain development certainly warrant further study.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Topic:** A.07. Developmental Disorders

**Support:** NIH/NIMH 1R01MH106623-01A1

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NIH K12 IRACDA

**Title:** Identifying mechanisms underlying intellectual disability linked to β-catenin disruptive mutations

**Authors: *R. WICKHAM**, J. ALEXANDER, A. PIRONE, L. EDEN, P. ZAMAN, S.-X. JIN, L. FEIG, M. JACOB
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**Abstract:** Intellectual disability (ID, IQ<70) affects 2-3% of the United States population. Despite its prevalence, the underlying pathophysiology is poorly defined, so effective pharmacological treatments are lacking. Several ID-linked human gene mutations have been identified, including disruptive mutations in the *CTNNB1* gene, encoding the β-catenin protein. β-catenin, through its roles in synaptic adhesion complexes and as a mediator of Wnt-target gene expression, is required for proper brain development, synaptic function, and plasticity. Human and mouse genetic studies suggest that the period of peak synaptogenesis in the brain may be a critical window for pathophysiological changes that can lead to ID. However, the pathophysiological changes caused by β-catenin loss in the brain during this window are unknown; their identification is essential to advance the design of effective therapeutic interventions to ameliorate ID. To gain insights into the pathological consequences of β-catenin loss-of-function in the developing brain, we have generated a novel mouse model with β-catenin conditionally knocked-out (β-cat cKO) during peak synaptogenesis in glutamatergic neurons. Our preliminary studies show that β-cat cKO mice display memory impairments, compared with wild-type littermates. We have also found several novel molecular changes in the hippocampus, including reduced levels of two β-catenin binding partners critical for synaptic adhesion and stability, N-cadherin and α-catenin. Consistent with these changes, preliminary findings suggest decreases in synaptic spine density and maturity on hippocampal CA1 pyramidal neurons in the β-cat cKO. Moreover, preliminary data indicates hippocampal TBS-LTP is also reduced at CA3/CA1 synapses, suggesting reduced synaptic plasticity required for learning and memory. As an unanticipated change, the β-cat cKOs exhibit increases in γ-catenin, a homologue of β-catenin with partial overlapping function. γ-catenin's neural specific role is unknown, and normally γ-

catenin levels are very low in neurons. Our studies will elucidate γ-catenin's neural functions, with particular emphasis on the composition of the cadherin based synaptic adhesion complex and Wnt-target gene expression. Together, these studies are providing new insights into molecular changes that can lead to intellectual disabilities

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

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**Topic:** A.07. Developmental Disorders

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International Foundation of CDKL5 (IFCR, USA)

**Title:** Mice lacking cyclin-dependent kinase-like 5 manifest autistic and ADHD-like behaviors

**Authors: *W.-L. LIAO**, C.-L. JHANG, W.-J. CHUANG
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**Abstract:** Neurodevelopmental disorders frequently share common clinical features and appear high rate of comorbidity, such as those present in patients with attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD). While characterizing behavioral phenotypes in the mouse model of CDKL5 disorder, a neurodevelopmental disorder caused by mutations in the X-linked gene encoding cyclin-dependent kinase-like 5 (CDKL5), we found that these mice manifest behavioral phenotypes mimicking multiple key features of ASD, such as impaired social interaction and communication, and increased stereotypic digging behaviors. These mice also displayed hyper-locomotion, increased aggressiveness and impulsivity, as well as deficits in motor and associative learning, resembling primary symptoms of ADHD. Furthermore, through brain region-specific biochemical analysis, we uncovered that loss of CDKL5 disrupts dopamine synthesis and the expression of social communication-related key genes in the corticostriatal circuit. Thus, our findings suggest that CDKL5 dysfunction contributes to the comorbid features of ASD and ADHD, and therapeutic development targeting the dopamine pathway and the corticostriatal circuit may ameliorate shared symptoms in ASD and ADHD.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Program#/Poster#:** 557.21/C8

**Topic:** A.07. Developmental Disorders

**Title:** Dasotraline is a monoamine reuptake inhibitor not a releasing agent as revealed by tetrodotoxin (TTX) sensitivity in microdialysis in the nucleus accumbens of freely-moving rats

**Authors: D. J. HEAL**[1], R. S. KULKARNI[1], L. PINDER[1], *H. L. ROWLEY[1], T. DEATS[2], S. C. HOPKINS[2], K. S. KOBLAN[2]
[1]Renasci Ltd, Nottingham, United Kingdom; [2]Sunovion Pharmaceuticals Inc, Marlborough, MA

**Abstract:** Dasotraline is a novel drug that has been shown in clinical trials to be effective in managing ADHD in adults (Koblan et al, 2015, Neuropsychopharm 40**:**2745; Hopkins et al, 2016, Clin Drug Invest 36**:**137). Dasotraline acts as a potent inhibitor of human DA transporters (DAT; dopamine uptake $IC_{50}$ 3 nM) and NE transporters (NET; norepinephrine uptake $IC_{50}$ 4 nM), and a weaker inhibitor of human serotonin transporters (SERT; serotonin uptake $IC_{50}$ 15 nM). We have used the sodium channel blocker, TTX, to investigate whether dasotraline evokes monoamine release in the CNS. d-Amphetamine was the reference comparator.
A single 2.0mm microdialysis probe was stereotaxically implanted into nucleus accumbens (ACB) (AP +2.2mm, ML ±1.5mm, DV -8.0mm relative to bregma) of isofluraneanaesthetised male, Sprague Dawley rats (300±50g). After ≥16hr recovery, 15min microdialysate samples (1.2µl/min artificial CSF (aCSF)) were taken from freelymoving rats for 2hr after administration of dasotraline, d-amphetamine or vehicle. TTX was reversedialysed via the probe starting 15min before drug or vehicle injection and was maintained throughout the experiment. Dopamine (DA), DOPAC and HVA were measured by ALEXYS$^{TM}$ hplc-ecd. All results are reported as mean±SE, n=7-9.
Basal DA efflux (6.11±0.29fmol/5µl; 0-2hr) in ACB was rapidly decreased by reverse dialysis of TTX (1µM) reaching a nadir of 85.6% vehicle control at 135min ($p<0.001$). TTX also markedly reduced extracellular DOPAC (≤57.9%; $p<0.001$) and HVA (≤28.5%; $p<0.001$). Under control conditions, dasotraline (10mg/kg ip) produced a gradual increase in DA efflux that peaked at 770±137% of baseline at 120min ($p<0.001$). Dasotraline produced a concomitant fall in DOPAC (nadir = 36.2% at 75min; $p<0.001$) but not HVA. dAmphetamine (3mg/kg ip) produced a rapid increase of DA efflux in ACB with a peak of 3015±634% of baseline at 30min ($p<0.001$). There were concomitant decreases in DOPAC and HVA ≤70.7% ($p<0.001$) and ≤36.2% ($p<0.001$), respectively. Under conditions of reversedialysis of TTX into ACB, dasotraline administration failed to significantly increase DA efflux above vehicle control values. In contrast, DA release by damphetamine was unaltered in the presence of TTX with a maximum increase of 2437±705% at 30min ($p<0.001$). Analysis of the data in 1hr time-bins confirmed no significant

differences between the damphetamineinduced DA efflux in the presence or absence of TTX. These findings lead to 3 important conclusions. Dasotraline is a DA reuptake inhibitor. Dasotraline is not a DA releasing agent. Dasotraline will be devoid of damphetamine-like stimulant properties.

**Disclosures:** **D.J. Heal:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **R.S. Kulkarni:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **L. Pinder:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **H.L. Rowley:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **T. Deats:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **S.C. Hopkins:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **K.S. Koblan:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.22/C9

**Topic:** A.07. Developmental Disorders

**Support:** NIH R01 MH083807

**Title:** Molecular genetic etiology of an ADHD-like phenotype in a selectively bred mouse model

**Authors: *A. M. SOROKINA**[1], P. MAJDAK[2], M. C. SAUL[2], J. V. GOGOLA[2], J. S. RHODES[3]
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [3]Psychology, [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

**Abstract:** Despite the prevalence of Attention-Deficit/Hyperactivity Disorder (ADHD) in our society, and our readiness to dispense pharmaceutical interventions, much of the underlying etiology remains unknown. To help untangle the genetic underpinnings of ADHD, we bred mice from the same starting population either selectively for high home cage activity (High-Active), or randomly (Control). The High-Active mice also display motor impulsivity ameliorated by therapeutic doses of amphetamine, another core feature of ADHD. Previous work has implicated altered striatal function in ADHD, but the molecular mechanisms are not known. The goal of this study was to identify gene expression differences in the striatum of our High-Active line as compared to the Control line. RNA was extracted and sequenced from the entire striatum of 10 High-Active and 10 Control mice, then analyzed via statistical analysis and Weighted Gene Co-Expression Network Analysis (WGCNA). WGCNA uncovered a network of correlated genes involved in cell structure, monoamine regulation, and *Wnt* signaling that are differentially expressed between the High-Active and Control lines, as well as human ADHD-linked genes

such as latrophilin 3. Future work will first focus on quantifying differential gene expression by looking at protein expression and localization. Then, using this data, we will focus on identifying potential novel medications that can help ameliorate negative behavioral aspects of ADHD through targeted action at the molecular level.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Program#/Poster#:** 557.23/C10

**Topic:** A.07. Developmental Disorders

**Title:** Dasotraline - Evaluation of its dopamine reuptake characteristics in comparison to stimulants and non-stimulants by microdialysis in the nucleus accumbens of freely-moving rats

**Authors: H. L. ROWLEY**[1], R. S. KULKARNI[1], L. PINDER[1], *D. J. HEAL[1], T. DEATS[2], S. C. HOPKINS[2], K. S. KOBLAN[2]
[1]RenaSci Ltd, Nottingham, United Kingdom; [2]Sunovion Pharmaceuticals Inc, Marlborough, MA

**Abstract:** Dasotraline is a novel DAT, NET and SERT uptake inhibitor that is being developed as a new treatment for ADHD. In cell-lines transfected with human transporters, dasotraline is a potent uptake inhibitor of dopamine (DA; $IC_{50}$ 3nM) and NE ($IC_{50}$ 4nM), and a weaker inhibitor of serotonin ($IC_{50}$ 15 nM). We have used microdialysis to compare dasotraline's effects on DA efflux against methylphenidate, d-amphetamine, phentermine and bupropion.
Microdialysis probes (2mm) were implanted into nucleus accumbens (ACB) (AP +2.2mm, ML ±1.5mm, DV -8.0mm relative to bregma) of isofluraneanaesthetised male, Sprague Dawley rats (300±50g). After ≥16hr recovery, 20min samples (1.2µl/min artificial CSF (aCSF)) were taken from freelymoving rats for 4hr after administration of dasotraline [1-10], methylphenidate [1-10], d-amphetamine [0.13], phentermine [1-9], bupropion [10-50] or vehicle. Doses in square brackets are [mg/kg ip]. DA, DOPAC and HVA were measured by ALEXYS™ hplc-ecd. All results are reported as mean±SE, n = 6-10.
Basal DA efflux was 5.29±0.22fmol/5µl (0-4hr). Dasotraline produced dose-dependent gradual increases in DA efflux peaking at 160-240min. Peak increases as % baseline: 144±21% [1], 204±38% [3] and 926±269% [10] (all $p<0.01$). Dasotraline produced concomitant falls in DOPAC (≤33.3%; $p<0.001$) but not HVA. The stimulants, damphetamine, phentermine and methylphenidate, dosedependently evoked rapid increases in DA efflux that peaked at 40min and declined rapidly thereafter. There was no dose-effect ceiling with the stimulants. Peak increases: damphetamine = 3393±399% [3], phentermine = 924±196% [9] and methylphenidate = 487±105% [10] (all $p<0.001$). dAmphetamine and phentermine decreased DOPAC (≤70.7%;

p<0.001 and ≤56.7%; p<0.001) and HVA (≤42.2%; p<0.001 and ≤27.2%; p<0.001). Methylphenidate reduced DOPAC (≤32.4%; p<0.001) but not HVA. The nonstimulant, weak dopamine reuptake inhibitor, bupropion, increased extracellular DA with maximum effects at 20-40min. Peak increases: 228±37% [10], 552±78% [30] and 441±104% [50] (all p<0.001). There was a clear dose-effect ceiling for bupropion. Bupropion produced concomitant falls in DOPAC (≤29.1%; p<0.01) but not HVA.
The stimulants produced rapid, large increases in DA efflux with no dose-effect ceiling. Bupropion rapidly increased DA efflux, but its effect was dose-limited. Dasotraline evoked slow increases in DA efflux in ACB. Dasotraline is clearly different from the DA stimulants and bupropion. The results indicate that at pharmacologically relevant doses (1-3mg/kg), dasotraline produces only relatively small increases in synaptic DA concentrations in vivo.

**Disclosures:** **H.L. Rowley:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **R.S. Kulkarni:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **L. Pinder:** A. Employment/Salary (full or part-time):; RenaSci Ltd. **D.J. Heal:** A. Employment/Salary (full or part-time):; RenaSci Ltd. 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.; Sunovion Pharmaceuticals Inc. **T. Deats:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **S.C. Hopkins:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **K.S. Koblan:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** A.07. Developmental Disorders

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National Research Foundation of Korea (NRF)-2016R1D1A1B02010387

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**Title:** The Atxn7 overexpressing mice: A potential animal model of the hyperactive endophenotype of attention-deficit/hyperactivity disorder (ADHD)

**Authors: *I. I. DELA PENA**[1], J. DE LA PENA[1], I. DELA PENA[2], H. KIM[1], C. BOTANAS[1], R. CUSTODIO[1], M. KIM[1], J. RYU[3], B.-N. KIM[4], D. HAN[5], J. CHEONG[1]
[1]Uimyung Res. Inst. For Neuroscience, Sahmyook Univ., Seoul, Korea, Republic of; [2]Pharmaceut. Sci., Loma Linda Univ., Loma Linda, CA; [3]Sch. of Life Science, Kyungpook Natl. Univ., Daegu, Korea, Republic of; [4]Psychiatry and Inst. of Human Behavioral Medicine, Seoul Natl. Univ. Col. of Med., Div. of Child & Adolescent Psychiatry, Seoul, Korea, Republic of; [5]Dept. of Psychiatry, Chung Ang Univ. Med. Sch., Seoul, Korea, Republic of

**Abstract:** Attention-deficit/hyperactivity disorder (ADHD) is a complex neurodevelopmental disorder characterized by inappropriate levels of hyperactivity, impulsivity, and inattention. Determining specific genetic risk variants for each symptom dimension of ADHD may aid in the identification of the biological risk factors of the disorder. In the present study, we explored the potential genetic underpinnings of the hyperactive phenotype of ADHD. To this end, we examined differentially expressed genes (DEGs) in the prefrontal cortex (PFC) of SHR/NCrl, an animal model of ADHD, compared with its genetic control, the Wistar Kyoto (WKY/NCrl) rat and the Wistar rat, strain used to represent the “normal” heterogeneous population. Relative to WKY/NCrl and Wistar controls, SHR/NCrl showed hyperactivity in the open-field test. Treatment with the ADHD drug, amphetamine (AMPH) reduced hyperactivity in SHR/NCrl. Meanwhile, AMPH increased locomotor activity in WKY/NCrl and Wistar rats. Gene expression analysis found 21 common upregulated and 36 downregulated genes in the PFC of drug-naïve SHR/NCrl when compared with WKY/NCrl and Wistar rats. Of these DEGs, expression levels of *Atxn7* which are involved in transcription, respectively, were downregulated following AMPH treatment in SHR/NCrl. qRT-PCR analyses verified expression patterns of these genes in the PFC of drug-naïve and AMPH-treated SHR/NCrl. We then proceeded in generating Atxn7 overexpressing transgenic (Atxn7 TG) mice to clarify whether changes in *Atxn7* gene expression in the brain correlate with hyperactive behavior. Behavioral screening results showed that the Atxn7 TG mice are hyperactive, with no alterations or impairment in other behaviors. qRT-PCR and immunofluorescence confirmed that *Atxn7* gene and ATXN7 protein is overexpressed in various brain areas of the Atxn7 TG mice. Furthermore, treatment with atomoxetine (ATO) reduced the hyperactive behavior in Atxn7 TG mice. Although further studies are warranted, our findings suggest that the *Atxn7* gene might play an important role in the pathophysiology of ADHD and that the Atxn7 TG mice can be used as a specific animal model of the hyperactive phenotype of this disorder.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

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**Topic:** A.07. Developmental Disorders

**Support:** National Brain Tumor Society

Childhood Brain Tumor Foundation

NIH Grant K08NS073793

NICHD DC-IDDRC 1U54HD090257

**Title:** Age-dependent neurological deficits induced by molecularly targeted drugs are reversible

**Authors: *J. SCAFIDI**[1], J. RITTER[2], J. EDWARDS[2], B. M. TALBOT[2], V. GALLO[2]
[1]Dept of Neurol. and Ctr. for Neurosci. Res., [2]Ctr. for Neurosci Res., Children's Natl. Med. Ctr., Washington, DC

**Abstract:** Newly developed molecularly targeted anti-cancer drugs target signaling pathways critical for normal brain development. While their use in the pediatric population is being considered, the age-dependent effects of these agents on the developing brain are unknown. The objective of this study was to define the cellular and behavioral effects of these targeted therapies when administered at different developmental stages. The agents chosen target pathways commonly altered in pediatric brain tumors and that are also crucial for cellular growth, proliferation, and migration during normal brain development: i) the receptor tyrosine kinase family, such as epidermal, vascular, and platelet-derived growth factor receptors; and ii) the downstream intracellular mechanistic target of rapamycin. In this study, naïve mice at different ages received a short-course of molecularly targeted drugs acting on these pathways: i) gefitinib, which inhibits the epidermal growth factor receptor; ii) sunitinib malate, which inhibits both the vascular and platelet-derived growth factor receptors; or iii) rapamycin, which inhibits the mTOR pathway. Cellular and behavioral characteristics involving the white matter and hippocampus were determined, as these regions undergo rapid development during childhood and are important for sensorimotor and cognitive function. We found that all three targeted therapeutic agents produced the greatest detrimental effects on the white matter and hippocampus when mice were treated at an early age (postnatal day [P]12 to P17), compared with administration at a later age (P17 to P22). Interestingly, there was no evidence of cellular or behavioral deficits when mice were treated with any of these drugs in adulthood. We then wanted to determine whether the detrimental effects of these agents could be reversed by an intervention known to promote plasticity - environmental enrichment. We chose the group

treated at an early age with these therapeutic agents (P12-17) and they underwent enrichment for 8-12 hours per day until P30. Behavioral tests of white matter and hippocampal function clearly demonstrated significant improvements in behavioral performance compared to treated mice that did not undergo enrichment. Interestingly, these improvements with a short course of enrichment were long-lasting and still evident at 2 months of age. Our findings that molecularly targeted drugs have age-dependent and reversible effects on the brain are of great significance, as these drugs are being tested in pediatric patients.

**Disclosures: J. Scafidi:** None. **J. Ritter:** None. **J. Edwards:** None. **B.M. Talbot:** None. **V. Gallo:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.26/C13

**Topic:** A.07. Developmental Disorders

**Title:** Postnatal erythropoietin repairs executive function deficit detected with touchscreen in adult rats with CNS injury that mimics preterm birth

**Authors: L. L. JANTZIE**[1], A. OPPONG[2], F. CONTEH[2], *S. ROBINSON[2]
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**Abstract:** Children born preterm are prone to executive function deficits. A persistent challenge has been to develop highly translatable cognitive tests that accurately replicate human tests of executive function. Touchscreen operant chambers use the same testing paradigms as human of executive function tests, and thus have the potential to speed translation of effective interventions from preclinical models to clinical trials. Erythropoietin (EPO) is a promising, safe, neuroreparative agent for perinatal brain injury currently being tested in clinical trials. We hypothesized that touchscreen paradigms can test the ability of neonatal EPO to repair deficits in cognition, and executive function, including cognitive flexibility. We used an established model of prenatal injury that mimics chronic deficits of children born preterm, including impaired gait and social interaction in adult rats. On embryonic day 18 (E18), pregnant Sprague-Dawley rats underwent laparotomy and transient systemic hypoxia-ischemia (TSHI) via occlusion of uterine arteries. Shams had anesthesia with laparotomy only. On postnatal day 1 (P1), injured rats of both sexes were randomized to treatment with EPO (2000U/kg ip P1-P5) or vehicle. Rats were weaned on P21, and began mild food restriction on P28. On P35, rats commenced training for reward retrieval in touchscreen operant chambers, with one testing session per day. Rats that passed training then underwent visual discrimination (VD) testing, and if successful, continued on to a reversal (REV) learning task to test cognitive flexibility. Passing criteria was set *a priori*

at 80% for 2 consecutive days with 60 trials per day. Two-way ANOVA with Bonferroni correction was used to compare differences for parametric variables. Results show the majority of rats pass training (sham, n=16, 100%; TSHI-veh, n=22, 92%, TSHI-EPO, n=24, 100%), which shows that young rats with late gestation injury can be trained in touchscreen paradigms. The majority of sham (81%), TSHI-veh (59%) and TSHI-EPO (75%) rats also passed VD, demonstrating that rats with prenatal CNS injury can perform VD. However, on REV, TSHI-EPO (58%) rats passed, while only 45% of TSHI-veh rats passed (p=NS), compared to sham (63%). The number of trials correct was higher for TSHI-veh (133±15), compared to shams (83±11, p=0.03) or TSHI-EPO (87±10, p=0.03). Notably, when different learning stages during REV were assessed, it was clear that TSHI rats had a perseverative reversal deficit. No differences attributable to sex were apparent in early analyses. Together, these results show for the first time that touchscreen can detect repair of executive function in rats with perinatal brain injury.

**Disclosures: L.L. Jantzie:** None. **A. Oppong:** None. **F. Conteh:** None. **S. Robinson:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.27/C14

**Topic:** A.07. Developmental Disorders

**Support:** NCTR/FDA

**Title:** Desflurane-induced neuronal damage in infant monkey after prolonged exposure

**Authors: *F. LIU**[1,2], Q. YIN[1], S. LIU[1], C. M. FOGLE[1], T. A. PATTERSON[1], M. G. PAULE[1], J. P. HANIG[2], W. SLIKKER, Jr.[1], C. WANG[1]
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**Abstract:** It has been a public concern as to whether general anesthesia has any adverse effects on developing brain. Desflurane is frequently used to maintain general anesthesia for pediatric surgeries. In the present study, postnatal day (PND) 5 or 6 rhesus monkeys were randomly assigned to a control group (room-air; n=3) and a desflurane-exposure group which was exposed to 5.7% desflurane in medical grade air (n=3) for 8 hours. Four hours after completion of the exposure the animals were euthanized and brain tissue were collected for histochemical analyses, including Fluoro-Jade C staining and immunohistochemical staining of Bax and Iba1 (ionizing calcium-binding adaptor molecule 1). Fluoro-Jade C staining indicated an increased number of Fluoro-Jade C-positive cells in layers II, III, IV and V of the frontal cortex after an eight-hour exposure to desflurane. Higher expression of Bax, a pro-apoptotic protein, was observed in the

brain of exposed group, suggesting elevated apoptosis is one of the consequences of prolonged desflurane exposure in the developing monkey brain. In addition, increased Iba1 expression indicated the involvement of microglia activation in desflurane-exposed infant monkey brain. These findings suggest that an 8-hour exposure to a clinically-relevant concentration of desflurane could cause neuronal damage in infant monkey brain, which may at least be partially apoptotic in nature. The role of microglial activation in desflurane-induced neuronal degeneration merits further studies.
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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.28/C15

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant HD080910

**Title:** Adeno-associated virus mediated expression of a functional human SLC6A8 in a mouse model of Creatine Transporter Deficiency

**Authors: *K. C. UDOBI**[1], M. K. PERNA[2], N. R. DELCIMMUTO[3], M. R. SKELTON[4]
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**Abstract:** Creatine (Cr) transporter (Crt) deficiency (CTD) is characterized by a lack of brain Cr, severe intellectual disability, and aphasia. To date there is no treatment for CTD. Unlike the other Creatine Deficiency Syndromes involving mutations in Cr synthesis enzymes, CTD involves a mutation in the Crt, preventing treatment by Cr supplementation. Faced with this barrier, there is a need for the development of novel and creative treatment strategies. Adeno-associated viral vector 9 (AAV9) has recently been shown to penetrate the blood-brain barrier via intravascular (IV) administration and efficiently targets cells of the central nervous system, making it a good candidate for diffuse gene delivery. The purpose of this study is to determine the efficacy of an AAV9 expressing a functional copy of the human Crt (AAV9-hCrT) in transducing cells from a knockout out mouse model of CTD (*Slc6a8*$^{-/y}$). In this study, the efficacy of AAV9-hCrT was tested both *in vitro* and *in vivo*. Cultured fibroblasts and primary hippocampal neurons from *Slc6a8*$^{-/y}$mice were treated with AAV9-hCrT. Cr uptake and SLC6A8 gene expression were increased in cells treated with AAV9-hCrt. *Slc6a8*$^{-/y}$mice were then treated with AAV9-hCrt via IV administration. Increases in SLC6A8 transcript and Cr levels were

observed in the brain of *Slc6a8*$^{-/y}$mice treated with AAV9-hCrt. These findings lay the foundation for the use of gene therapies for the treatment of CTD.

**Disclosures: K.C. Udobi:** None. **M.K. Perna:** None. **N.R. Delcimmuto:** None. **M.R. Skelton:** None.

**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.29/C16

**Topic:** A.07. Developmental Disorders

**Support:** STAR GRANT

**Title:** Sex differences in the 20HETE induction and toxicity in primary cortical neurons after oxygen-glucose deprivation

**Authors: *N. E. MOHAMMED**[1], R. KOEHLER[2], Z. YANG[3]
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**Abstract:** 20-Hydroxyeicosatetraenoic acid 20HETE, an arachidonic acid metabolite from cytochrome P450 (CYP) 4A and 4F, contributes to ischemic brain injury in adults and the newborn. Our previous work has indicated that 20-HETE can be induced in oxygen-glucose deprivation (OGD) neurons and directly aggravate neuronal injury. In addition, mRNA of Cyp4a12a, a male-specific 20-HETE synthesis enzyme, exists in mixed-sex mouse primary cortical neurons. Thus, it is crucial to determine whether 20-HETE plays different roles in male or female neurons after OGD, an *in vitro* model of ischemia. Here, we tested the hypothesis that there are sex differences in the 20HETE production and toxicity in primary cortical neurons after OGD. The sex of E15 embryo or 1-day old pups of C57/bl6 mice were identified by their internal sex organs. Primary mouse cortical neurons or astrocytes were cultured from male or female embryo or 1-day old pups and further confirmed cells' sex stratification by PCR amplification of Jarid1c (X chromosome-specific) and Jarid1d (Y-chromosome-specific). Quantitative real-time PCR results showed that Cyp4a10 mRNA presented in both male and female neurons on 7 days *in vitro*. However, only Cyp4a12a mRNA could be found in male neurons. mRNA of Cyp4a12b and Cyp4a14 were not detected in neither male or female neurons. 1-h OGD increased levels of Cyp4a10 and Cyp4a12a mRNA at 1 and 3 h recovery. For astrocyte, Cyp4a10 mRNA was also existed in female and male cells. However, only Cyp4a12a was existed in males and Cyp4a12b was found in females. Cyp4a14 was not detected in both male and female astrocytes. 1-h OGD did not induce the level of astrocyte CYP4A mRNA. 20-HETE measurement with ELISA assay

on primary cortical neurons or astrocytes showed a significant increase in 20HETE release in culture medium of male neurons. However, no significant 20HETE induction could be found in female neurons or male or female astrocytes. CellTiter Blue (CTB) cell viability assay results indicated ~40% of neuronal death at 24 h after 1-h OGD. 10 µM of HET0016 protected OGD male neurons rather than female neurons, which is consistent with the effects of 20-HETE antagonist 20-6,15-HEDGE (50 µM). However, 20HETE analog 20-5,14HEDGE (10 µM) could not only reverse the protection of HET0016 in OGD male neurons, but lead to more severe injury in both male and female neurons. Moreover, CTB assay results suggested that 20-5,14HEDGE application could directly lead to neuronal injury without OGD in both sex. Therefore, we conclude that induced 20HETE in male neurons exerts detrimental effects after OGD.

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**Poster**

**557. Neurodevelopmental Disorders: Models and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 557.30/C17

**Topic:** A.07. Developmental Disorders

**Title:** A novel preclinical model of chronic pain from cerebral palsy

**Authors: *A. Y. OPPONG**[1], T. R. YELLOWHAIR[2], J. KIM[1], L. L. JANTZIE[2], S. ROBINSON[1]
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**Abstract:** Children, adolescents and adults with cerebral palsy (CP) are prone to chronic pain. The most common cause of preterm birth in the USA is chorioamnionitis from varying combinations of bacterial inflammation and hypoxia-ischemia. The proportion of patients with CP who suffer chronic pain increases from adolescence to adulthood. The frequency and severity of chronic pain in patients with CP is disproportionate to the progression of musculoskeletal deformity, which suggests that patients with CP may have altered nociception. Discovery of more effective interventions for patients with CP plus chronic pain requires an adequate preclinical model to study the cellular and molecular mechanisms of pain, and their potential reversibility. Similar to other developing CNS circuits that are disrupted by perinatal injury, dorsal spinal cord nociceptive (DSCN) circuits undergo perinatal maturation, and are thus vulnerable to early CNS insults. We used an established rat model of CP from chorioamnionitis in which adult rats have impaired gait and cognition that mimics children born very preterm. We hypothesized adult rats that suffer a prenatal injury that mimics CNS injury from very preterm birth would exhibit a nociceptive phenotype as young adult rats. Using an established preclinical

model of CP, chorioamnionitis was induced on embryonic day 18. Rat pups were born at term (E22, ~30 weeks human gestation). Adult rats of both sexes were tested for mechanical (von Frey up-down) and thermal sensation (tail immersion and Hargreaves) from juveniles (P30) to adults (P90). Lumbar dorsal spinal cord was assayed with immunoblotting. Groups were compared with two-sided t test, with $p<0.05$ significant. Rats exposed to prenatal injury showed sustained hindpaw allodynia at P30, P40, P60 and P90 compared to shams (n=12-19, all $p<0.01$). Injured rats initially at P30 exhibited hyposensitivity to thermal tail immersion compared to shams ($p<0.01$), which normalized by P40. Notably, Hargreaves testing revealed injured rats were hypersensitive at P90 compared to shams ($p=0.005$), which suggests both thermal and mechanical hindpaw sensation are abnormal at P90 in rats with prenatal injury. The chemokine CXCL1 is implicated in calpain-mediated degradation of co-transporter KCC2 in neuropathic pain. Rats with prenatal injury had elevated serum CXCL1 and lumbar cord KCC2 degradation products compared to shams (both $p<0.01$). Together, these results for the first time present a preclinical model that replicates both the impaired gait and nociception of patients with CP. Multiple mechanisms contribute to deficits in patients with CP, and we predict these mechanisms alter DSCN circuits too.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 558.01/C18

**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** Extracellular domain mutations increase memantine potency for GluN2A subunit containing NMDA receptors

**Authors: D. BLEDSOE**[1], B. LAUBE[2], B. G. KLEIN[3,4], *B. M. COSTA[1,4]
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**Abstract:** N-methyl D-aspartate receptors (NMDAR) play crucial role in normal brain function and pathogenesis of neurodegenerative and psychiatric disorders. Functional tetra-heteromeric

NMDAR contains two obligatory GluN1 subunits and two identical or different non-GluN1 subunits from among six different gene products; four GluN2 (A-D) and two GluN3 (A-B) subunits. Since NMDARs composed of different GluN2 subunits (GluN2A-D) confer varied physiological properties and have different distributions in the brain, pharmacological agents that target NMDARs with specific GluN2 subunits have significant potential for therapeutic applications. In the present work, by amino acid point mutations and electrophysiology techniques, we have studied the role of ligand binding domain (LBD) interactions in determining the effect of well-characterized pharmacological agents including agonists, competitive antagonists, channel blockers and allosteric modulators. Remarkably, memantine blocked the mutant GluN1/2A receptors with significantly higher potency than the wild type GluN1/2A receptors. In addition, similar changes were observed with GluN2B subunit-containing receptors. However, memantine potency remained unchanged with the mutant GluN1/2C or 2D subunit-containing receptors. These results indicate that GluN2A and GluN2B receptors are more sensitive to LBD interactions. Further, they readily translate any subtle LBD modifications all the way downstream to the extracellular vestibule of channel pore, where memantine binds, to adopt a conformation that locally resembles GluN2C or 2D subunit-containing NMDA receptors. Further studies on NMDA receptor LBD to transmembrane domain signal propagation mechanisms will help develop GluN2 subunit selective biomolecules that can be used for the treatment of neurological and psychiatric disorders.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 558.02/C19

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NICHD Intramural grant awarded to Chris J. McBain

**Title:** NMDAR-mediated excitation-transcription coupling in hippocampal interneuron subtypes revealed by RNA sequencing

**Authors: *V. MAHADEVAN**, R. CHITTAJALLU, K. A. PELKEY, X. YUAN, S. HUNT, D. ABEBE, C. J. MCBAIN
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**Abstract:** Decades of research has established that inhibitory interneurons, which coordinate circuit excitability and information processing in the brain, are amongst the most heterogeneous cell types in the nervous system. It is now clear that flawed interneuron development and

function precipitates neurological disorders such as epilepsy, schizophrenia and autism. Additionally, wealth of evidence points to the role of interneuron-specific NMDA-type ionotropic glutamate receptor (NMDAR) dysfunction recapitulate the phenotypes of several neurological disorders. In the present study we apply cell-type specific, next-generation RNA sequencing to examine the role of activity-dependent gene expression programs in interneurons. We perform this by selectively ablating GluN1 subunit of NMDARs in the medial ganglionic eminence (MGE)-derived, hippocampal interneurons, that gives rise to predominantly parvalbumin and somatostatin cohorts. We identify several classes of genes including transcription factors, cytoskeletal modifiers and regulators of neuronal excitability, differentially expressed in the MGE-derived interneurons lacking GluN1. We will use a combination of molecular, cellular and electrophysiological approaches to validate the consequence of abnormal gene regulation following the loss of GluN1, based on our sequencing data. Overall, our findings indicate NMDAR signaling as a critical regulator of excitation-transcription coupling in hippocampal interneurons, and allow us to design rational therapeutic strategies to rescue interneuron deficits underlying neurological disorders.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 558.03/C20

**Topic:** B.02. Ligand-Gated Ion Channels

**Title:** Influence of species differences at NMDA receptors pharmacology

**Authors: *D. C. BERTRAND**[1], S. BERTRAND[1], E. NEVEU[1], K. KAMBARA[1], M. A. ACKLEY[2]
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**Abstract:** NMDA receptors play critical roles in normal physiological function within the CNS and disruption of their function can have profound detrimental consequences on CNS function. For example, downregulated NMDA receptor function in anti-NMDA encephalitis provides further evidences about the determinant role of these receptors in psychiatric and neurological illness.
Development of novel molecules acting at the NMDA receptors necessitate the identification of pharmacologically active compounds at human NMDA receptors but also their characterization in different animal models that are used in preclinical efficacy and safety studies. Nowadays the availability of genomes from multiple organisms and the possibility of recombinant expression offer new possibilities to assess the characterization of a given receptor across different species.

In the present study we have examined the functional properties of NDMA receptors reconstituted by expression of the GluN1a and GluN2A subunits from human, dog, rat and mouse and compared their pharmacological properties and modulation by two synthetic oxysterol NMDA receptor allosteric modulators, SGE-301 and SGE-550.
Sequence alignments of the human, dog, rat and mouse GluN1 and GluN2A indicate that while some regions of the receptors are highly conserved notable differences can be observed in other segments of the proteins. To evaluate the possible importance of such variation on the receptor properties it is therefore critical to examine the functionality of the different receptors and to compare the pharmacology of compounds of interest.
Expression of NMDA receptors in *Xenopus* oocytes offered an efficient way to assess their functional properties using two electrode voltage clamp. Determination of the concentration activation curve to glutamate revealed the high similitude in sensitivity to the natural agonist and showed no major differences in the time course of Glutamate-evoked currents. However, determination of the pharmacological modulation by oxysterols revealed, differences in their potency across species. SGE-301 was approximately 50-fold more potent at the human receptor than the mouse. Rank order of potency for SGE-301 across species was human > rat > dog > mouse. SGE-550 displayed more modest differences in potency across species, with a maximum of a 5-fold difference between the rat and dog receptors.
Altogether, these data highlight the importance of fully characterizing the activity of novel NMDA receptor modulators across species that will be used in preclinical efficacy and safety models in order to better understand pharmacokinetic-pharmacodynamic relationships

**Disclosures:** **D.C. Bertrand:** None. **S. Bertrand:** None. **E. Neveu:** None. **K. Kambara:** None. **M.A. Ackley:** None.

**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** Stereoselective actions of a positive allosteric modulator of NMDA receptors reflect unique structural determinants of action

**Authors: *R. E. PERSZYK**[1], K. L. STRONG[2], M. P. EPPLIN[2], D. MENALDINO[2], M. J. MCDANIEL[1], H. KUSUMOTO[1], K. K. OGDEN[3], J. ZHANG[1], P. LE[1], D. C. LIOTTA[2], S. F. TRAYNELIS[4]
[1]Pharmacol., [2]Chem., Emory Univ., Atlanta, GA; [3]Neurosci., Scripps Florida, Jupiter, FL; [4]Dept Pharmacol, Emory Univ. Sch. of Med., Atlanta, GA

**Abstract:** The NMDA receptors (NMDARs) are tetrameric assemblies of 2 GluN1 and 2 GluN2 subunits. The tetrahydroisoquinoline **(+)-CIQ** is a positive allosteric modulator (PAM) of GluN2C- and GluN2D-containing NMDARs, increasing the current response to maximally effective concentrations of agonist by 3-fold. Recently we identified a chiral analogue, **IPQ-2**, that is a non-selective PAM of NMDARs. This compound enhances saturated responses of GluN1/GluN2D NMDARs by 3-fold and can enhance the response of GluN1/GluN2B NMDARs by 2-fold. **IPQ-2** increases glutamate potency by 1.5-4 fold for all NMDARs (n=15-28). Separation of **IPQ-2** *R* and *S* enantiomers revealed that ***R*-(+)-IPQ-2** acts like **(+)-CIQ**, potentiating only GluN1/GluN2C and GluN1/GluN2D NMDARs, whereas ***S*-(-)-IPQ-2** can potentiate all NMDARs. In addition, ***S*-(-)-IPQ-2** slowed the current response deactivation time course following removal of glutamate for all NMDARs (n=5-16), in contrast to ***R*-(+)-IPQ-2**, which (like **CIQ**) only slowed the deactivation of GluN1/GluN2C (n=5-15 cells). We utilized scanning alanine mutagenesis to search for structural determinants of action that might explain the subunit selectivity of the enantiomers of **IPQ-2**. We focused on the M1 transmembrane helix as well as the pre-M1 helix, which lies parallel to the membrane and is in contact with the M3 transmembrane helix that forms the channel gate. We evaluated mutations at each position in these regions in GluN1, GluN2B, ad GluN2D. We identified 13 residues in the GluN1 subunit that perturbed **IPQ-2** potentiation of GluN1/GluN2B, including a stretch of 6 consecutive residues in the GluN1 pre-M1. By contrast, no mutations at GluN2B pre-M1 residues influenced **IPQ-2** potentiation, suggesting that structural determinants of the pan-potentiator actions of ***S*-(-)-IPQ-2** may reside in or near GluN1 pre-M1. We next evaluated whether GluN1 mutations also altered the actions of ***S*-(-)-IPQ-2** on GuN1/GluN2D NMDARs. We screened a large number of mutations with racemic **IPQ-2**, and selected a subset of residues at which to evaluate the two enantiomers. We found that 5/6 mutations in GluN1 perturbed the actions of ***S*-(-)-IPQ-2** on GluN1/GluN2D, compared to 2/6 mutations that influenced ***R*-(+)-IPQ-2**. By contrast, we found that 8/8 GluN2D pre-M1/M1 mutations perturbed ***R*-(+)-IPQ-2** effects, compared to 1/8 mutations that altered the actions of ***S*-(-)-IPQ-2**, consistent with structural determinants for ***S*-(-)-IPQ-2** residing in GluN1 and for ***R*-(+)-IPQ-2** residing in GluN2. These results suggest that two distinct allosteric modulatory sites reside in the GluN1 and GluN2 subunits, and show that selectivity among these sites can be tuned by subtle changes in the modulator structure.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** B.02. Ligand-Gated Ion Channels

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CIBERER# CB15/00010

**Title:** Amnesia: Passive transfer from man to mouse by a patient-derived, recombinant monoclonal antibody

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**Abstract:** Objective: The most frequent autoimmune encephalitis syndrome is characterized by anti-NMDAR autoantibodies. Their pathogenic relevance has been implied by *in vitro* analysis and passive transfer of patients' cerebrospinal fluid (CSF) in mice *in vivo*. We aimed to analyze

the intrathecal plasma cell repertoire in anti-NMDAR encephalitis, identify autoantibody-producing clones, and characterize their antibody signatures in recombinant form *in vitro* and *in vivo*.
Methods: Treatment-naive patients were subjected to flow cytometry analysis of the peripheral and intrathecal immune response throughout the disease course. Recombinant human monoclonal antibodies (rhuMab) were cloned and expressed from matching immunoglobulin heavy (IgH) and light (IgL) chain amplicons of clonally expanded intrathecal plasma cells (cePc) and tested for their pathogenic relevance using *in vitro* assays and an *in vivo* mouse model of passive cerebroventricular antibody transfer.
Results: Intrathecal accumulation of B cells and plasma cells corresponded to the clinical course. The presence of cePc with hypermutated antigen-receptors indicated an antigen-driven intrathecal immune response. Consistently, a single recombinant human GluN1-specific monoclonal antibody, rebuilt from intrathecal cePc, was sufficient to reproduce NMDAR epitope specificity *in vitro*. After intraventricular infusion in mice, it accumulated in the hippocampus, decreased synaptic NMDAR density, and caused severe memory impairment *in vivo*.
Interpretation: A CNS-specific humoral immune response is present in anti-NMDAR encephalitis specifically targeting the GluN1 subunit of the NMDAR. Having recovered this defined intrathecal antibody signature in recombinant form proves its pathogenic relevance by passive transfer of disease symptoms from man to mouse and may contribute to understanding the role of the NMDAR in synaptic function, cognition and behavior.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 558.06/C23

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NIDA R01DA020140

**Title:** An *In vitro* characterization of mtor, eef2k/eef2 and bdnf signaling in the antidepressant actions of ketamine and (2r,6r)- hydroxynorketamine

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**Abstract:** Major depressive disorder (MDD) is a devastating psychiatric disorder which afflicts 7% of the adults in the US (SAMSHA). MDD is commonly treated with selective serotonin/norepinephrine reuptake inhibitors (SS/NRIs), but these drugs are fully effective for only 2/3 of patients (Trivedi *et al., 2006*). Moreover, SS/NRIs often require several weeks of treatment to achieve efficacy which can increase the risk of suicide for some patients. Some NMDAR (N-methyl-d-aspartate receptor) antagonists elicit a rapid antidepressant response in humans and animal models. For example, the anesthetic drug, ketamine, is a rapid-acting antidepressant (Berman, *et al.* 2000) that can improve symptoms of depression in as quickly as 1 hr (Zarate, *et al.,* 2006). Ketamine blocks the ion channel pore of NMDA receptors (Orser *et al.*,1997), but the mechanism of rapid antidepression is unclear. 2R,6R, hydroxynorketamine (6-HNK) is a metabolite of ketamine that was recently shown to have rapid antidepressant action in rodent models (Zanos, *et al*., 2016). However, 6-HNK may not block NMDARs and the biochemical signals evoked by ketamine and 6-HNK are not fully distinguished. The rapid antidepressant action of ketamine activates mTOR (mammalian target of rapamycin); eEF2K/eEF2 (Eukaryotic Elongation Factor 2 kinase/ Eukaryotic Elongation Factor 2); and BDNF (brain-derived neurotrophic factor) signaling. In this study organotypic brain slices were obtained from rodents to evaluate the acute and prolonged biochemical actions of ketamine and 6-HNK in hippocampus and cortex. Brain slices were incubated in ketamine or 6-HNK, for 1h and tissue was harvested at 1h and 24h post-treatment. Protein lysates were obtained for each sample and evaluated by western blot. Our findings suggest ketamine and 6-HNK may activate similar biochemical pathways. These data suggest further research is required to more fully distinguish the actions of ketamine and its metabolites.

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**Poster**

**558. NMDA Receptors II**

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** The uncharged form of memantine can access the NMDAR channel through a hydrophobic route

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**Abstract:** NMDA receptors (NMDARs) are a class of ionotropic glutamate receptors present at most excitatory synapses in the brain. NMDARs are highly calcium permeable, and timely calcium influx through NMDARs initiates signaling cascades responsible for many forms of learning and memory. Aberrant activation of NMDARs can also occur, and is implicated in numerous pathologies. A variety of NMDAR inhibitors exist, including open-channel blockers, inhibitors that bind in the pore of NMDARs and impede ion flux. Open channel blockers are typically charged molecules and can only access their binding site in the presence of agonist, when the channel is open. Memantine, a drug approved to treat the symptoms of Alzheimer's disease, is mostly charged at physiological pH, but exists in equilibrium between its charged and uncharged forms. Memantine potently inhibits NMDARs through an open-channel block mechanism. However, a second form of inhibition by memantine has also been described, called second site inhibition (SSI). SSI involves memantine binding in the absence of agonist, and causing inhibition of NMDAR current upon subsequent agonist application. Because SSI was observed following memantine application in the absence of agonist, it was thought that SSI involved memantine binding outside the channel gate on NMDARs. However, our data suggest that SSI involves entry of the uncharged form of memantine into the plasma membrane, from where it can access the channel blocking site. We expressed NMDARs in tsA201 cells and used whole-cell patch clamp electrophysiology to further investigate SSI. By varying pH, and by using a permanently charged trimethyl derivative of memantine synthesized by extensive methylation of memantine, we examined access of uncharged memantine to the NMDAR pore through a hydrophobic pathway. The presence of a hydrophobic pathway through which drugs can pass has not been demonstrated in NMDARs, and reveals a new route of drug access to NMDAR channels.

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**Poster**

**558. NMDA Receptors II**

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** Hemiester analogues of pregnenolone sulfate: A new class of positive modulators of N-methyl-D-aspartate receptors

**Authors: *P. HUBALKOVA**[1,2], B. KRAUSOVA[1], B. SLAVIKOVA[3], M. NEKARDOVA[3], V. VYKLICKY[1], H. CHODOUNSKA[3], E. KUDOVA[3], L. VYKLICKY[1]
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**Abstract:** N-methyl-D-aspartate receptors (NMDARs) play a key role in excitatory synaptic transmission, and their dysfunction underlies some neurological and psychiatric disorders. Receptor hypofunction was implicated in autism, schizophrenia, and various forms of intellectual disability, and compounds with a positive allosteric effect at NMDARs may have a beneficial effect in these diseases. The aim of this study was to characterize the structure-activity relationship of newly synthesized structural analogues of pregnenolone sulfate (PES) - a naturally occurring neurosteroid that has a positive modulatory action at NMDARs.
We designed, synthesized, and electrophysiologically tested PES analogues, pregne-5-ene and androst-5-ene dicarboxylic acid esters, which differ in the length of the hemiester substituent at the C-3-position and have various structure modifications at C-17 and C-20. All newly synthesized steroids potentiate GluN1/GluN2B receptor currents from +8 to +539 % and most of them are more potent modulators of NMDARs than PES, which potentiates currents by +78 %. The structure-function analysis of synthetic steroids indicates the optimal length of the C-3 substituent (hemisuccinate to hemiazelate), depending on the C-17 substituent for the maximal positive modulatory effect at NMDARs.
The most potent modulator 20-oxo-pregnenolone hemiadipate (20-oxo-PE-hAdi) was further tested for activity at GluN1/GluN2A-D receptors. Dose-response analysis indicates significant differences in the degree of maximal potentiation ($E_{max}$) for 20-oxo-PE-hAdi modulation of GluN1/GluN2A (226.3 ± 36.2 %); GluN1/GluN2B (151.0 ± 15.6 %), GluN1/GluN2C (139.6 ± 39.8 %), and GluN1/GluN2D (114.8 ± 8.8 %) with no significant differences in $EC_{50}$ (9.8 to 13.0 µM).
The endogenously occurring neurosteroid PES (100 µM) and its analogue 20-oxo-PE-hAdi (30 µM) were evaluated for activity at native NMDA, AMPA, and GABA receptors. PES and 20-oxo-PE-hAdi potentiated responses mediated by native NMDAR (+55.8 ± 5.6 % and +138.3 ± 11.4 %, respectively), inhibited responses of native AMPA receptors (-32.2 ± 2.2 % and -6.8 ± 1.6 %, respectively), and GABA receptors (-89.2 ± 2.3 % and -82.8 ± 4.7 %, respectively).
We conclude that the newly synthesized hemiester analogues of PES are potent and selective positive modulators of NMDRs and may lead to the development of new therapeutics.

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**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** Intracellular state affects NMDA receptor positive allosteric modulator activity

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**Abstract:** N-Methyl-D-aspartate (NMDA) receptor hypofunction is believed to contribute to the symptoms of schizophrenia and, perhaps, autism. Thus, NMDA receptor positive allosteric modulators (PAMs) are potential therapeutics for the treatment of these conditions. Our laboratories have previously reported the mechanism of action and properties of NMDA receptor PAMs based upon phenanthroic acid and naphthoic acid. In this study, we have identified the differential effect of GluN1- and GluN2-C terminals on potentiation by these PAMs. We also observed that the C-terminal is important for potentiation by other known PAMs. Intracellular $Ca^{+2}$ affected potentiation especially at GluN2A-containing NMDA receptors. Intracellular treatment with a $Ca^{+2}$ chelator diminished the potentiation by PAMs. Phosphorylation state of the receptor also appears to affect PAM activity. PKC activation enhanced the potentiation of PAMs, especially at GluN2B-containing NMDA receptors. These results suggest that the intracellular C-terminal segment of NMDA receptors regulates the activity of NMDAR PAMs and that PAM actions can be dependent upon intracellular factors. Thus, it may be possible to modulate NMDA receptors differentially in different phosphorylation states. These findings may help in the development of new therapeutics for neuropsychiatric disorders.

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**Poster**

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** A gating motif in NMDA receptors targeted by disease mutations

**Authors: *J. AMIN**[1,2,3], A. GOCHMAN[1], X. DONG[4], H.-X. ZHOU[4], L. P. WOLLMUTH[1]
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**Abstract:** NMDA receptors (NMDAR) are glutamate-gated ion channels that have a wide distribution in the nervous system. Recently a variety of *de novo* missense mutations in NMDAR subunits have been identified that are associated with severe neurological disorders, particularly epileptic encephalopathies. These disease mutations often target motifs critical to the process of glutamate-induced channel opening or gating. NMDARs are obligate heterotetramers typically composed of two GluN1 and two GluN2 subunits. The pore domain or central core of the ion channel, which is homologous to an inverted K+ channels, is formed by transmembrane segments M1 and M3 and an M2 pore loop. Eukaryotic iGluRs possess an additional transmembrane segment, the M4 segment. Surprisingly, a number of missense mutations are found in the GluN1, GluN2A and GluN2B M4 segments. Based on on-cell single channel recordings, many of these missense mutations have limited effects on gating but very dramatic effects, including in one instance halting gating, occurred for missense mutations at a highly conserved glycine, positioned at the extreme extracellular end of M4. Even alanine substitutions at this conserved glycine severely restricted channel opening, with these actions subunit dependent: for GluN1, any substitution at the conserved glycine equally restricted gating, whereas for GluN2A, the alanine substitution was severe but less dramatic. Interestingly, this glycine is part of a preserved structural motif in the extracellular M4, termed the NMGV motif. We show that the conserved glycine in GluN1 acts as a gating hinge which allows elements of this motif to interact with the pore lining helix of an adjacent subunit during channel opening. This action creates a bridge between the GluN2 M3 and GluN1 M4 transmembrane helices, stabilizing the open state. The NMGV motif in GluN2 also appears to participate in transmembrane interactions but the conserved glycine does not appear to function as a hinge. These results have strong implications for how such disorders are caused at the ion channel level and highlight an important role for the M4 in NMDA receptor gating.

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**Poster**

**558. NMDA Receptors II**

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**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NINDS intramural Research program (S.W. and K.W.R.)

**Title:** Biochemical characterization of glutamate receptors and associated proteins

**Authors: *S. WON**, K. W. ROCHE
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**Abstract:** Glutamate receptors mediate the majority of excitatory synaptic transmission in the central nervous system. Ionotropic glutamate receptors (NMDA, AMPA, and kainate receptors) are widely expressed throughout the nervous system and responsible for a variety of processes in mammalian brain including neuronal development, excitatory neurotransmission, learning and memory, and synaptic plasticity. NMDARs assemble as heterotetramers containing two obligatory GluN1 and two GluN2 (A-D) or GluN3 (A-B) subunits to form functional receptors. Calcium influx through NMDARs is thought to be critical for induction of synaptic plasticity, a cellular mechanism for learning and memory. AMPARs are composed of four subunits (GluA1-4), which combine to form tetramers, mostly heterotetrameric. In addition, AMPARs conduct the majority of fast synaptic transmission throughout the CNS and their trafficking and synaptic expression is the critical mechanism that underlies much of the plasticity of excitatory transmission. At excitatory synapses, glutamate receptors assemble into multi-protein complexes including NMDARs, AMPARs and a variety of scaffolding proteins such as the membrane-associated guanylate kinases (MAGUKs). Indeed, the MAGUKs interact with many receptors, channels and neuronal adhesion molecules. Through the years, many studies have indicated that synaptic proteins have unique biochemical properties including distinct posttranslational modifications like phosphorylation and palmitoylation, as well as specific interacting proteins. Indeed, many high-profile studies have been aimed at understanding glutamate receptor ‘interactomes’ using mass spectrometry. However, these protein assemblies are entirely dependent on the solubility of the PSD proteins and antibodies used to immunoprecipitate the proteins of interest, and a systematic comparison of synaptic protein solubility has been lacking. We have now characterized the solubility of NMDARs, AMPARs, neuroligins, and MAGUKs in different tissue and culture conditions. We evaluate adult mouse hippocampus, cultured cortical neurons, and expression of synaptic proteins in heterologous cells. Interestingly, we find that NMDAR subunits and PSD-95 are highly insoluble in brain with mild detergent and are sensitive

to temperature and pH. In contrast AMPARs and neuroligins are much more soluble and don't vary much in different cell preparations. These results reveal the complication that various protein complexes containing NMDAR or PSD-95 in neuron exist in different assemblies, which will likely reveal different interactomes depending on preparation and detergent.

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**Poster**

**558. NMDA Receptors II**

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**Title:** Synaptically-induced slow inward currents mediated by pannexin-1 in CA3 pyramidal neurons

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**Abstract:** Pannexins are large pore ion channels widely expressed in the mammalian brain. While pannexins have been involved in a number of pathological conditions, including brain ischemia, inflammation, neurodegeneration and epilepsy, little is known about their physiological role. Remarkably, strong, non-physiological NMDA receptor (NMDAR) stimulation triggers a secondary pannexin-1 (Px1)-mediated inward current in neurons, which has been linked to excitotoxicity and epilepsy. Px1 is highly expressed in the hippocampus where it co-localizes with PSD-95, suggesting a synaptic role. Using pharmacological and genetic tools combined with brain slice electrophysiology in rodents, we found that synaptic activation of NMDARs elicits a Px1-mediated slow postsynaptic current (Px1 EPSC) in CA3 but not CA1 pyramidal neurons. Here, we further characterized these Px1 EPSCs in acute hippocampal slices. Px1 EPSCs were revealed at physiological temperature (35 °C), occurred probabilistically following NMDAR-EPSCs, and produced enough depolarization to elicit action potentials in CA3 pyramidal neurons. In addition, Px1 EPSCs were more robustly elicited by activating mossy fiber to CA3 pyramidal cell synapses than CA3-CA3 synapses. Intracellular loading of the calcium chelator BAPTA (10 mM) or bath application of the NMDAR open channel blocker MK-801 (50 µM) abolished Px1 EPSCs, strongly suggesting that calcium influx

via NMDARs is necessary to elicit these currents. Moreover, a low concentration of the group I metabotropic glutamate receptors (mGluR1/5) agonist DHPG (1 μM) strongly facilitated Px1 EPSCs, whereas mGluR5 antagonism (MPEP, 4 μM) or intracellular loading of the irreversible G protein inhibitory GDP-βS (2 mM) reduced these currents. Activation of other $G_q$ protein-coupled receptors, such as muscarinic receptors M1, facilitated Px1 EPSCs, whereas activation of the $G_s$ protein-coupled receptor A2A had no effect. Our findings indicate that Px1 EPSCs can be regulated by neuromodulators and provide a mechanism for signal amplification in the CA3 area that may be relevant to memory formation and temporal lobe epilepsy.
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**Poster**

**558. NMDA Receptors II**

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**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NIH R15AG045820-01A1

**Title:** Activity-Dependent postsynaptic signaling in hippocampal neurons is altered by transgenic expression of chimeric NMDA receptor GLuN2 subunits

**Authors: *J. P. FOTANG**[1], C. LEONG[2], F. BOURA[2], S. HUSSAIN[2], R. HOPWOOD[2]
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**Abstract:** N-methyl-D aspartate receptors (NMDARs) at excitatory synapses in the hippocampus are central players in the synaptic plasticity required for learning and memory. Two predominant signaling properties of NMDARs that have been independently linked to hippocampal plasticity are calcium conductance into the postsynaptic spine and direct intracellular protein signaling. These properties vary with the composition of the NMDAR such that, compared to NMDARs with GluN2A subunits, NMDARs with GluN2B subunits conduct calcium for a longer period after activation and display greater affinity for the obligatory synaptic plasticity protein, CaMKII. Thus, it is not possible to determine the separate influences of these NMDAR properties by switching the entire GluN2 subunit. To overcome this obstacle, we created GluN2 chimeras, one having the amino (A)-terminus and transmembrane domains (TMDs) of GluN2A fused to the carboxy (C)-terminus of GluN2B (termed ABc) and, vice versa, the other line having the A-terminus and TMDs of GluN2B fused to the C-terminus of GluN2A (termed BAc). These chimeric GluN2 subunits were expressed in transgenic mice using the tet-off expression system with tetracycline transactivator protein (tTA) expression under

transcriptional control of the CaMKII minimal promoter. tTA expression was seen in many forebrain regions, but predominantly in hippocampal pyramidal cells. This project used a three by two design in which half of the animals from each genotype (ABc, BAc, and Wildtype-WT) were exposed to a Y-maze in a novel environment to activate NMDARs in the hippocampus. Control animals were not exposed to the Y-maze. We quantified intracellular signaling through fluorescent puncta counts following immunohistochemistry for plasticity related proteins in the synaptic regions of hippocampal area CA1. Pairs of antibodies were applied (anti-pCaMKII and anti-CaMKII; anti-calmodulin and anti-PSD95). There were no genotype or maze exposure effects on number of puncta for pCaMKII, CaMKII or colocalization of these two proteins. Also, there was no effect of genotype or maze exposure on PSD95-positive puncta. However, the number of calmodulin puncta was reduced by maze exposure in WT and ABc, but not BAc mice and colocalization between PSD95 and calmodulin was reduced after maze exposure only in ABc mice. The pattern of results for calmodulin parallels alterations in learning and memory we observed in these mice when tested previously in the Morris water maze. The findings suggest that alterations in calmodulin, but not CaMKII, allow the NMDAR subunit mutations to impact cognition.

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**Poster**

**558. NMDA Receptors II**

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**Program#/Poster#:** 558.14/C31

**Topic:** B.02. Ligand-Gated Ion Channels

**Title:** NYX-2925 promotes GluN2B-PSD95 colocalization and LTP in rat hippocampal neurons

**Authors: *M. S. BOWERS**[1,2], P. K. STANTON[3], A. L. GROSS[1], R. M. MITCHELL[1], M. A. KHAN[1], R. A. KROES[1,2], J. R. MOSKAL[1,2]
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**Abstract:** Aptinyx has developed a novel class of small molecule N-Methyl-D-Aspartate (NMDA) receptor modulators with broad applicability across neurologic and psychiatric disorders. NMDAR activation was measured using a [$^3$H]MK-801 potentiation assay in membrane extracts prepared from human NR2 subtype-expressing HEK cells. NYX-2925 in the presence of glutamate (50 μM) dose-dependently facilitated [$^3$H]MK-801 binding to all four NR2 subtypes. Moreover, NYX-2925 preferentially activated the NR2B subtype by several orders of magnitude. Given the pivotal role of synaptic NMDAR in mediating long-term potentiation (LTP), the effect of NYX-2925 on LTP was measured at Schaffer collateral CA1 synapses in

hippocampal slices prepared from young adult rats. NYX-2925 enhanced NMDAR-dependent LTP and increased NMDAR current. Therefore, the effect of NYX-2925 on surface expression of NR2B-containing NMDAR within PSD95-positive puncta was quantified using spinning disc confocal microscopy in primary rat hippocampal neurons. NYX-2925 increased colocalization of NR2B and PSD95 in a dose- and time-related manner. Moreover, this effect was dependent on both glutamate and glycine concentration. Increased colocalization was paralleled by NR2B and PSD-95 protein expression. Glutamate (50 μM) alone increased colocalization, but not protein expression. These data suggest that NYX-2925 facilitates synaptic plasticity in part by increasing synaptic NR2B. Thus, the biological effects of NYX-2925 result not only from direct receptor modulation, but also by regulating the trafficking of receptors into and out of the synapse.

**Disclosures:** **M.S. Bowers:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc. **P.K. Stanton:** 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.; Aptinyx, Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Aptinyx, Inc. **A.L. Gross:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc. **R.M. Mitchell:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc. **M.A. Khan:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc. **R.A. Kroes:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc. **J.R. Moskal:** A. Employment/Salary (full or part-time):; Aptinyx, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc..

**Poster**

**558. NMDA Receptors II**

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**Topic:** B.02. Ligand-Gated Ion Channels

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**Title:** Coupling ligand binding to ion channel opening in NMDA receptors

**Authors: *A. GOCHMAN**, J. AMIN, K. CHAN, L. WOLLMUTH
Stony Brook Univ., Stony Brook, NY

**Abstract:** *N*-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that are fundamental to brain function. Highlighting the central role of NMDARs is the recent identification of numerous *de novo* missense mutations in NMDAR subunits that are associated with severe neurological disorders. Many of these missense mutations disrupt NMDAR channel gating, the process of converting glutamate binding to opening of its associated $Ca^{2+}$-permeable ion channel, but fundamental features of the gating process in NMDARs remain unknown. In all glutamate-gated ion channels, the M3 transmembrane segment lines the pore and contains elements of the activation gate. Opening of the ion channel is driven by tight mechanical coupling between glutamate-induced conformational changes in the ligand-binding domain (LBD) and displacement of the M3 helices. Surrounding the M3 helices are additional transmembrane helices, M1 and M4. These outer helices must be displaced for efficient pore opening to occur, but whether they are displaced actively by conformational changes in the LBD or passively through movement of the M3 helices remains unknown. To address the displacement of the outer structures, we inserted glycines into the linkers coupling M1 and M4 to the LBD, the S1-M1 and S2-M4 linkers, respectively, and assayed the effect of these manipulations on NMDAR gating using on-cell single channel recordings. Like comparable insertions in the M3-S2 linker, insertions in S1-M1 dramatically attenuated gating, suggesting that like M3-S2 this region is mechanically coupled to the LBD. In contrast, glycine insertions in the S2-M4 linker strongly potentiated NMDAR gating, suggesting a distinct role for the M4/S2-M4 complex in NMDAR gating. To elucidate segment-specific roles in the gating machinery, we combined potentiating S2-M4 glycine insertions with attenuating M3-S2 glycine insertions. These combination insertions have a higher equilibrium open probability ($P_{open}$) than the M3-S2 glycine insertions alone. Interestingly, the relative degree of potentiation for the combination insertions was much greater than the independent effect of S2-M4 insertions in the wild-type background, suggesting that the M4/S2-M4 complex does not act independently as a potentiator of gating, but rather acts synergistically with other transmembrane segments including M3/M3-S2. Hence, the M4/S2-M4 region appears not to exert its effect on gating independently of the active movement of the core gating elements M1 and M3. Our results have implications for how disease causing missense mutations in the outer structures might impact receptor function.

**Disclosures:** **A. Gochman:** None. **J. Amin:** None. **K. Chan:** None. **L. Wollmuth:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health R01 grant.

**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 558.16/C33

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NIH R01NS036654

NIH F32NS086361

NIH F32-NS078873

**Title:** Single channel properties of triheteromeric GluN1/GluN2A/GluN2C NMDA receptors are distinct from diheteromeric GluN1/GluN2A and GluN1/GluN2C

**Authors: *S. F. TRAYNELIS**[1], A. KHATRI[1], S. A. SWANGER[1], S. BHATTACHARYA[1], K. B. HANSEN[2], H. YUAN[1]
[1]Dept Pharmacol, Emory Univ. Sch. of Med., Atlanta, GA; [2]Dept. of Biomed. & Pharmaceut. Sci., Univ. of Montana, Missoula, MT

**Abstract:** N-methyl-D-aspartate receptors (NMDARs) are ligand-gated ion channels that are tetrameric complexes of two GluN1 and two GluN2 subunits, of which there are four subtypes (GluN2A-D). A large proportion of NMDARs in adult brain contain two different GluN2 subunits, and are referred to as triheteromeric receptors because they comprise three unique subunits (e.g. GluN1/GluN2A/GluN2C). We have utilized a strategy to enrich cell surface triheteromeric receptors by harnessing the interactions of coiled-coil domains fused to the intracellular domains of different GluN2 subunits to mask a C-terminal endoplasmic reticulum retention signal. We assessed the degree of subunit escape for of this system in Xenopus oocytes, and found that about 90% of the current observed in oocytes coinjected with GluN1 and the modified GluN2A/GluN2C cRNAs arose from triheterimeric GluN1/GluN2A/GluN2C receptors. We subsequently transfected HEK cells with GluN1/GluN2A, GluN1/GluN2C, or GluN1/GluN2A/GluN2C. We excised outside-out patches and recorded the unitary currents activated by maximally effective concentrations of glycine (100 μM) and glutamate (1 mM). In patches where double openings were not observed, we were able to statistically verify that the unitary currents arose from a single channel. The unitary currents were idealized by fitting a filtered step response function to each potential open-closed transition, and we analyzed histograms of the duration and amplitude of all fitted unitary currents, as well as the duration of all closed periods by maximum likelihood fitting. The properties of channels from patches obtained from cells expressing modified GluN1/GluN2A (n=3, mean open time 2.83 ms, mean chord conductance 75, 64 pS) or GluN1/GluN2C (n=7, mean open time 0.70 ms, mean chord conductance 48, 30 pS) were similar to those previously published. We also recorded from 7

patches from GluN1/GluN2A/GluN2C-transfected cells that showed both high and low conductance levels (75, 59, 40 chord conductance) with direct transitions among the three observed levels. We concluded that these patches contained a single triheteromeric GluN1/GluN2A/GluN2C receptor, which opened in bursts like GluN1/GluN2A, but had a low open probability (0.02) and mean open time (0.84 ms) similar to GluN1/GluN2C. The properties of the triheteromeric GluN1/GluN2A/GluN2C receptors may allow synaptic signaling to proceed with a reduced charge transfer. Similar work will be required to understand the properties of other triheteromeric receptors in the adult CNS.

**Disclosures: S.F. Traynelis:** A. Employment/Salary (full or part-time):; Emory University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was supported by the National Institute of Neurological Disorders and Stroke (NINDS) of the NIH under award number R01NS036654 to S.F.T.. F. Consulting Fees (e.g., advisory boards); S.F.T. is a consultant of Janssen Pharmaceuticals, Inc., Pfizer Inc, Boehringer Ingelheim Pharma GmbH & Co. KG, and a member of the Scientific Advisory Board for Sage Therapeutics, and a co-founder of. **A. Khatri:** 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 F32-NS078873 to A.K. **S.A. Swanger:** 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 F32NS086361 to S.A.S.. **S. Bhattacharya:** None. **K.B. Hansen:** None. **H. Yuan:** None.

**Poster**

**558. NMDA Receptors II**

**Location:** Halls A-C

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**Program#/Poster#:** 558.17/C34

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NIH R01NS036654

Janssen Pharmaceuticals, Inc.

**Title:** Triheteromeric GluN1/GluN2A/GluN2C NMDA receptors have unique pharmacological properties

**Authors: *S. BHATTACHARYA**[1], A. KHATRI[1], S. A. SWANGER[1], K. B. HANSEN[2], H. YUAN[1], S. F. TRAYNELIS[1]

[1]Pharmacol., Emory Univ., Atlanta, GA; [2]Dept. of Biomed. & Pharmaceut. Sci., Univ. of Montana, Missoula, MT

**Abstract:** N-methyl-D-aspartate receptors (NMDARs) are major excitatory receptors that play distinct roles in the central nervous system. Over the past decades, most studies on NMDARs have focused on diheteromeric receptors that contain two GluN1 and two identical GluN2 subunits. However, four genes encoding GluN2 subunits (GluN2A-D) have been identified, and multiple GluN2 subunits are expressed in almost all neurons. Many native NMDARs are triheteromeric assemblies of two GluN1 and two different GluN2 subunits (e.g. GluN1/GluN2A/GluN2C). Thus, there is a lack of information about the properties of physiologically-relevant triheteromeric NMDARs. In this study, we have adapted a strategy that utilizes the masking of engineered endoplasmic reticulum retention signals to selectively express GluN1/GluN2A/GluN2C receptors, allowing an evaluation of triheteromeric receptor properties. Our data suggest that triheteromeric GluN1/GluN2A/GluN2C receptors have a distinct pharmacological profile compared to GluN1/GluN2A and GluN1/GluN2C with respect to altered EC50 and IC50 values for agonists, endogenous ions, and allosteric modulators. Since receptors that contain both GluN2A and GluN2C are known to be expressed in the cerebellum, we used cerebellar granule cells as a model to study native GluN2C-containing NMDAR. The positive allosteric modulator 1616-19 is highly selective for GluN2C-containing NMDARs and potentiates only diheteromeric receptors that contain two GluN2C subunits; 1616-19 has no effect on triheteromeric receptors that include only one copy of GluN2C. We used 1616-19 to test for the presence of triheteromeric GluN1/GluN2A/GluN2C receptors in granule cells in cerebellar slices. Our data suggest that the GluN2C/D-selective modulator CIQ, which can potentiate triheteromeric GluN1/GluN2A/GluN2C receptors, enhanced the response to pressure-applied NMDA in granule cells (n=6), consistent with surface expression of GluN2C. By contrast, 1616-19 had no effect on granule cell NMDAR responses in slice preparations (n=8), suggesting cell surface GluN2C was mostly in the form of triheteromeric NMDARs rather than diheteromeric GluN1/GluN2C receptors. This study advances our understanding of the NMDAR identity in cerebellar neurons, and raises the idea that the GluN2C subunit preferentially associates with GluN2A, suggesting that there may be very little diheteromeric GluN1/GluN2C receptors in some neurons.

**Disclosures: S. Bhattacharya:** None. **A. Khatri:** None. **S.A. Swanger:** None. **K.B. Hansen:** None. **H. Yuan:** None. **S.F. Traynelis:** A. Employment/Salary (full or part-time):; Emory University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was supported by the National Institute of Neurological Disorders and Stroke (NINDS) of the NIH under award number R01NS036654 to S.F.T and a grant to Emory from Janssen Pharmaceuticals, Inc. F. Consulting Fees (e.g., advisory boards); S.F.T. is a consultant of Janssen Pharmaceuticals, Inc., Pfizer Inc, Boehringer Ingelheim Pharma GmbH & Co. KG, a member of the Scientific Advisory Board for Sage Therapeutics, and a co-founder of Neu.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.01/C35

**Topic:** B.05. Transporters

**Support:** NSERC discovery grant

**Title:** Region-and activity- dependent regulation of transporter-mediated glutamate uptake

**Authors: *N. F. PINKY**[1], C. M. WILKIE[2], M. P. PARSONS[3]
[1]Biomed. Sci., Mem. Univ. of Newfoundland, St. Johns, NL, Canada; [2]Biomed. Sci., Mem. Univ. of Newfoundland, St. John's, NL, Canada; [3]Biomed. Sci., Mem. Univ., St John's, NL, Canada

**Abstract:** Glutamate is the major excitatory neurotransmitter in the central nervous system and is essential for neuronal survival and rapid cellular communication. However, too much glutamate can have a damaging effect on cell health through a process known as excitotoxicity. Once released into the extracellular space, glutamate must be cleared rapidly in order to prevent excitotoxic damage; this is accomplished through glutamate transporters which are located in high concentrations on astrocyte membranes adjacent to synaptic sites. As excitotoxic cell death is associated with many neurodegenerative diseases, it is important to understand the mechanisms by which the healthy brain efficiently clears extracellular glutamate during neurotransmission. While different types of glutamate transporter exist, it is thought that GLT-1 is responsible for over 90% of total glutamate uptake activity. However, prior work relied heavily on biochemical assays from synaptosome preparations which have recently been shown to overemphasize neuronal glutamate uptake at the expense of astrocytic glutamate uptake. Here, we used a novel optogenetic sensor of extracellular glutamate termed iGluSnFR (intensity based glutamate sensing fluorescence reporter) to visualize, in real-time and on a millisecond timescale, how glutamate clearance dynamics are affected by neural activity in different brain regions *in situ*. By electrically stimulating neural activity in acute brain slices from C57Bl/6 mice, we find that the hippocampus is much more efficient at clearing glutamate compared to the cortex and striatum, particularly during prolonged high-frequency trains of synaptic stimulation. Using a pharmacological approach to quantify the relative contribution of different transporters to total glutamate uptake, our estimates show that while GLT-1 indeed plays an important role in glutamate uptake, approximately 75% of total uptake capacity can be maintained even at high levels of neural activity when GLT-1 is inhibited by a saturating concentration of the GLT-1 blocker DHK. These data suggest a much more important role of non-GLT-1 transporters than previously thought. In all, our data demonstrate that the transporter-mediated regulation of extracellular glutamate dynamics is dependent on both the region being studied and the amount

of neural activity, and therefore highlights the importance of studying glutamate dynamics in real-time *in situ*.

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**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.02/C36

**Topic:** B.01. Neurotransmitters and Signaling Molecules

**Support:** NIH Grant NS065920

NIH Grant NS064025

**Title:** Mapping the spatial extent of climbing fiber-mediated spillover to cerebellar interneurons

**Authors: *K. ABIRAMAN**, A. NIETZ, L. CODDINGTON, L. OVERSTREET-WADICHE, J. I. WADICHE
Univ. of Alabama At Birmingham, Birmingham, AL

**Abstract:** Excitatory synaptic transmission in the nervous system is mediated by glutamate, and is traditionally considered to occur exclusively at synapses. Under conditions of high activity or when released at a high concentration, glutamate spills out of synapses to activate extrasynaptic receptors. While extrasynaptic signaling is thought to augment synaptic transmission in several brain regions, we and others have shown that communication between climbing fibers (CFs) and molecular layer interneurons (MLI) in the cerebellar cortex occurs exclusively via glutamate spillover in the absence of anatomically-defined synapses. Glutamate released at the CF-Purkinje cell (PC) synapse spills out to activate AMPA- and NMDA-type receptors on surrounding MLIs. The actions of extrasynaptic glutamate are controlled by excitatory amino acid transporters (EAATs) that remove glutamate from the extracellular space. Although the cerebellar cortex exhibits highly stereotyped anatomical connectivity and astrocytic transporter expression, it is divided into zones based on differential expression of several proteins including the PC-specific glutamate transporter, EAAT4. We performed simultaneous whole cell voltage clamp recordings and confocal imaging of PCs and MLIs to monitor CF activation and assess spillover responses from a single CF. Post-hoc neuronal reconstruction show that the degree of dendritic overlap between the PC-MLI pairs correlates with the magnitude of spillover current in MLIs. Ongoing experiments seek to understand the spatial spread of spillover mediated MLI activation in zones of high and low EAAT4 expression. Together these results help define the anatomical and molecular factors governing spillover transmission at the CF-MLI synapse.

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**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.03/C37

**Topic:** B.05. Transporters

**Support:** CHDI A-7815, A-12467

DFG Exc 257/1

**Title:** Release/uptake mismatch in corticostriatal synaptic connections due to astrocyte dysfunction in mice suffering from Huntington's disease (HD)

**Authors: A. DVORZHAK**[1], A. WÓJTOWICZ[2], *R. GRANTYN[2]
[2]Exptl. Neurol., [1]Univ. Med. Charité, Berlin, Germany

**Abstract:** Previous studies in mouse models of HD reported reduced spine densities in striatal projection neurons (SPNs) as well as decreased expression of the astrocytic glutamate transporter EAAT2 in striatal tissue. To what extent both changes are linked to each other has remained unclear. It is possible that the loss of glutamatergic synapses initiates an adaptive response of astrocytes resulting in smaller/fewer glutamate transporter clusters. Alternatively, insufficient clearance of synaptically released glutamate may lead to alterations in corticostriatal or thalamostriatal synaptic transmission up to the loss of overstimulated contacts. We addressed these possibilities by comparing 1 year old wildtype (WT) and symptomatic HD mice (Q175 hom). Quantification of EAAT2 immunofluorescence (IF) revealed a significant decrease in the integral EAAT2-IF localized next to vGluT1+ but not vGluT2+ terminals in HD, suggesting the existence of a disease-related mismatch between glutamate release and glutamate uptake at the level of individual corticostriatal synapses. In line with this finding, sulphorhodamine-labelled astrocytes in slices from HD mice exhibited smaller glutamate-induced sodium transients as recorded after loading SBFI-AM. They also showed a slower decay of glutamate transporter currents (GTCs) and decreased single-cell GTC maxima in response to increasing concentrations of photolytically applied glutamate. The idea of a glutamate release-uptake mismatch was further confirmed by the analysis of unitary corticostriatal responses. Corticostriatal afferents were visualized by YFP fluorescence after stereotaxic injection of a variety of adenoviral constructs. Individual axons were submitted to optogenetic or electrical microstimulation. The AMPAR- and NMDAR-mediated components of the corticostriatal unitary EPSCs (uEPSCs) were separated by setting the holding potential to - 70 or +40 mV or by using respective receptor blockers. In line with the observed reduction in the density of corticostriatal glutamate release sites, uEPSC

maxima were found to be smaller in HD. At the same time the average release probability was significantly higher, likely as a consequence of increased presynaptic excitability. On the postsynaptic side, the most prominent disease-related alterations were the slowing of the uEPSC decay and the increase in the ratio of NMDA/AMPA components in the postsynaptic response. Together, these results support the hypothesis of an HD-related release/uptake mismatch in the corticostriatal pathway. Whether this is also a cause of synapse pruning remains to be determined.

**Disclosures: A. Dvorzhak:** None. **A. Wójtowicz:** None. **R. Grantyn:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.04/C38

**Topic:** B.07. Synaptic Transmission

**Support:** State University of New York at Albany

State University of New York at Albany Research Foundation

**Title:** Neuronal glutamate transporters control dopaminergic signaling in the striatum

**Authors: M. DE**, J. P. MCCAULEY, III, S. BELLINI, *K. E. FLEMING, L. Y. D'BRANT, *M. A. PETROCCIONE, A. SCIMEMI
Univ. At Albany, Albany, NY

**Abstract:** The neuronal glutamate transporter EAAC1 is abundantly expressed in the basal ganglia nucleus of the striatum, yet its role in regulating the functional properties in this brain region remain largely unknown. The striatum plays a crucial role in regulating the execution of stereotyped motor behaviors and reward. In humans, mutations in the gene encoding EAAC1 are associated with the onset of neuropsychiatric disorders like obsessive compulsive disorders. Here we ask how EAAC1 controls glutamatergic signaling in the striatum. Mice that do not express EAAC1 display increased anxiety and abnormal motor behaviors controlled by striatal circuits. This general phenotype suggests that EAAC1 might be involved in regulating excitatory synaptic transmission onto D1 and D2 dopamine receptor expressing medium spiny neurons, which together represent more than 95% of all neurons within the striatum. Consistent with this hypothesis, electrophysiology recordings indicate that EAAC1 limits activation of group I metabotropoic glutamate receptors (mGluRI) and controls D1 dopamine receptor expression. Conversely, blocking mGluRIs rescues D1 dopamine receptor expression. These results indicate that EAAC1 can powerfully regulate excitatory transmission in the striatum and identify new

molecular mechanisms by which loss of EAAC1 might contribute to the onset of OCD-like behaviors.

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**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.05/C39

**Topic:** B.05. Transporters

**Support:** R01AA019458

**Title:** Modulatory effects of Ampicillin-Sulbactam on glial glutamate transporters and metabotropic glutamate receptor 1 as well as reinstatement to cocaine seeking behavior

**Authors: A. M. HAMMAD**, F. ALASMARI, Y. ALTHOBAITI, *Y. SARI
Pharmacol., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

**Abstract:** The glutamatergic system has an important role in drug dependence including cocaine. Studies reported that chronic exposure to cocaine dysregulates the glutamatergic system. Previous findings revealed that glial glutamate transporter-1 (GLT-1), cystine/glutamate exchanger (xCT) and metabotropic glutamate receptors 1 (mGluR1) in the central reward brain regions mediate cocaine reinstatement. Moreover, treatment with ceftriaxone, a β-lactam antibiotic, known to upregulate GLT-1 reduced cocaine cue-induced reinstatement seeking. In this study, we investigated the reinstatement behavior to cocaine (20 mg/kg, i.p) seeking using a conditioned place preference (CPP) paradigm in male alcohol-preferring (P) rats. We further investigated the effects of Ampicillin/Sulbactam (AMP/SUL) (200 mg/kg, i.p), a β-lactam antibiotic, on cocaine-induced reinstatement. We studied also the expression of glial glutamate transporters GLT-1, xCT and glutamate/aspartate transporter (GLAST) as well as mGluR1 expression in nucleus accumbens (NAc) core and shell and dorsomedial prefrontal cortex (dmPFC). We found that AMP/SUL treatment reduced reinstatement to cocaine seeking. This effect was associated with the decrease in locomotor activity. Moreover, GLT-1 and xCT were downregulated in NAc core and shell but not in the dmPFC following cocaine-primed reinstatement. However, cocaine upregulated mGluR1 expression in the NAc core but not NAc shell or dmPFC. Importantly, AMP/SUL treatments normalized GLT-1 and xCT expression in the NAc core and shell; however, the drug normalized mGluR1 expression in the NAc core only. Additionally, AMP/SUL upregulated GLT-1 and xCT in the dmPFC as compared to water naïve group. These findings demonstrated that glial glutamate transporters and mGluR 1 in the

mesocorticolimbic brain regions could be potential therapeutic targets for cocaine-seeking behavior

**Disclosures:** **A.M. Hammad:** None. **F. alasmari:** None. **Y. Althobaiti:** None. **Y. Sari:** None.

**Poster**

**559. Amino Acid Transporters**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.06/C40

**Topic:** B.05. Transporters

**Support:** Atatürk Üniversity BAP 2009/120

TUBİTAK 113S083

ÖYP Grant

**Title:** Effects of ceftriaxone with/without excitatory amino acid transporter blockers on GBM cell line

**Authors: *K. A. NALCI**[1], A. HACIMUFTUOGLU[2], U. OKKAY[2], A. TAGHIZADEHGHALEHJOUGHI[2], N. TASPINAR[2], M. TASPINAR[3]
[1]Med. Pharmacol., Atatürk Üniversitesi, erzurum, Turkey; [2]Med. Pharmacol., Atatürk Üniversitesi, Erzurum, Turkey; [3]Med. Biol., Yüzüncü Yıl Üniversitesi, Van, Turkey

**Abstract:** Introduction
The aim of this study is to increase the amount of intracellular glutamate by activating the target eAAT2 receptor and thus to establish a new therapeutic approach to cancer.
Methods
Glioblastoma multiforme cell lines exposured with EAAT1, EAAT2, EAAT3 blockers and ceftriaxone. With Real-Time PCR Slc1a1, Slc1a2 and Slc1a3 gene expressions had been analyzed. MTT test had been done. Statistical Analyses were done.
SLC1A1

| **Groups** | **SLC1A1** | | | | |
|---|---|---|---|---|---|
| **Control** | 1.00 | ± | 0.185 | | |
| **Ceftriaxone** | 5.26 | ± | 0.190 | * | |
| **Ucph-101** | 1.00 | ± | 0.164 | | |

| | | | | | |
|---|---|---|---|---|---|
| **L-Cysteine** | 0.47 | ± | 0.031 | | |
| **TFB-TBOA** | 0.04 | ± | 0.010 | * | |
| **Ucph-101+ Ceftriaxone** | 7.16 | ± | 0.100 | * | |
| **L-Cysteine + Ceftriaxone** | 0.68 | ± | 0.106 | | ## |
| **TFB-TBOA+ Ceftriaxone** | 8.80 | ± | 0.069 | ** | # |
| **Ucph-101+ L-Cysteine + TFB-TBOA+ Ceftriaxone** | 20.5 | ± | 1.280 | ** | ## |

* p<0.05 statistically significant compared with control# p<0.05 statistically significant compared with ceftriaxone
** p<0.001 statistically significant compared with control ## p<0.001 statistically significant compared with ceftriaxone
SLC1A2

| **Groups** | **SLC1A2** | | | | |
|---|---|---|---|---|---|
| **Control** | 1.00 | ± | 0.103 | | |
| **Ceftriaxone** | 7.29 | ± | 0.450 | ** | |
| **Ucph-101** | 1.06 | ± | 0.121 | | |
| **L-Cysteine** | 0.66 | ± | 0.036 | | |
| **TFB-TBOA** | 0.48 | ± | 0.045 | | |
| **Ucph-101+ Ceftriaxone** | 16.8 | ± | 1.680 | ** | # |
| **L-Cysteine + Ceftriaxone** | 1.66 | ± | 0.425 | | ## |
| **TFB-TBOA+ Ceftriaxone** | 0.37 | ± | 0.306 | | ## |
| **Ucph-101+ L-Cysteine + TFB-TBOA+ Ceftriaxone** | 37.6 | ± | 2.750 | ** | ## |

*,**,#,## =Same as SLC1A1 table attachement.
SLC1A3

| **Groups** | **SLC1A3** | | | | |
|---|---|---|---|---|---|
| **Control** | 1.00 | ± | 0.156 | | |
| **Ceftriaxone** | 0.71 | ± | 0.040 | | |
| **Ucph-101** | 0.29 | ± | 1.510 | * | |
| **L-Cysteine** | 31.4 | ± | 2.460 | ** | |
| **TFB-TBOA** | 0.21 | ± | 0.057 | | |
| **Ucph-101+ Ceftriaxone** | 0.49 | ± | 0.006 | | |
| **L-Cysteine + Ceftriaxone** | 3.70 | ± | 0.476 | * | ## |
| **TFB-TBOA+ Ceftriaxone** | 2.44 | ± | 0.020 | | ## |
| **Ucph-101+ L-Cysteine + TFB-TBOA+ Ceftriaxone** | 0.92 | ± | 0.654 | | |

* p<0.05 statistically significant compared with control# p<0.05 statistically significant compared with ceftriaxone
** p<0.001 statistically significant compared with control ## p<0.001 statistically significant compared with ceftriaxone

**Results and Discussion**

Taken together, this study suggest that Ceftriaxone, or rationally designed and pharmacologically enhanced drugs that act in a similar way as glutamate transporter inducer, may serve as a therapeutic drug for ameliorating and potentially annihilating the cancer cells via inducing EAAT2.

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**Poster**

**559. Amino Acid Transporters**

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**Support:** Aase og Ejnar Danielsens Fond

NIH NS066019

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NIH MH105846

NIH EY13079

**Title:** The glutamate transporter GLT-1 expressed in neurons is important for glutamate homeostasis and synaptic energy metabolism

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**Abstract:** Glutamate is the major excitatory neurotransmitter in the brain. Clearance of glutamate after synaptic release is achieved primarily by the transporters GLT-1 and GLAST that are highly expressed in astrocytes. However, GLT-1 is also expressed in axon terminals. Using a conditional GLT-1 knockout in which GLT-1 is deleted in neurons using synapsin-Cre (synGLT-1 KO), we recently demonstrated significant glutamate uptake mediated by neuronal GLT-1 in crude synaptosomes from the whole forebrain [Petr et al. *J Neurosci* (2015) 35: 5187]. In the present study, we found reduced glutamate uptake into synaptosomes isolated from the cortex (50±7%, n=6; $p<0.05$), hippocampus (16±4%, n=3; $p<0.05$), and striatum (45±3%, n=3; $p<0.01$) of synGLT-1 KO mice compared to controls. Our goal here was to investigate by NMR spectroscopy, HPLC, and GC-mass spectrometry the importance of GLT-1 expressed in neurons for synaptic energy metabolism and glutamate homeostasis.
SynGLT-1 KO and control littermates were subjected to three types of experiments to assess

energy metabolism at a cellular (1), mitochondrial (2) and synaptic (3) level: (1) *in vivo* metabolic $^{13}$C-labeling with [1-$^{13}$C]glucose and [1,2-$^{13}$C]acetate to prime neuronal and astrocytic metabolism, respectively; (2) measurement of oxygen consumption and ATP production in isolated mitochondria to assess energy metabolism; and (3) metabolic $^{13}$C-labeling of purified synaptosomes with [U-$^{13}$C]glucose and [U-$^{13}$C]glutamate to characterize synaptic neuronal energy metabolism. In addition, we performed an electron microscopic assessment of mitochondria in the neocortex of synGLT-1 KO and control littermates.
The key significant findings in cerebral cortex include: (1) reduction in aspartate level (57%; p=0.04) paralleled by an increased glutamate level (6%; p=0.03) in the synGLT-1 KO, and absence of change in neuronal and astrocytic metabolism based on products of [1-$^{13}$C]glucose and [1,2-$^{13}$C]acetate metabolism, respectively; (2) unchanged oxygen consumption, but increased ATP production (77%; p=0.01) measured in isolated synGLT-1 KO mitochondria; (3) changes in synaptosomal $^{13}$C-labeling of TCA cycle intermediates following metabolism of [U-$^{13}$C]glucose and [U-$^{13}$C]glutamate. Ultrastructural analysis indicated that the occurrence of mitochondria within astrocyte processes immediately adjacent to and within 5 micrometer from excitatory synapses was doubled in tissue of synGLT-1 KO cortex, relative to controls ($p<0.05$; n=3). These results collectively suggest important roles for neuronal GLT-1 in synaptic glutamate homeostasis and energy metabolism.

**Disclosures: P.A. Rosenberg:** None. **L.F. McNair:** None. **Y. Sun:** None. **K.D. Fischer:** None. **J.D. Nissen:** None. **J.V. Andersen:** None. **N. Nyberg:** None. **M.C. Hohnholt:** None. **B.I. Aldana:** None. **C.J. Aoki:** None. **U. Sonnewald:** None. **H.S. Waagepetersen:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.08/DP02/C42 (Dynamic Poster)

**Topic:** B.05. Transporters

**Title:** Modulation of the excitatory amino acid transporter 2 by spider venoms

**Authors: *A. TORA**, M.-L. RIVES, A. D. WICKENDEN
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**Abstract:** The excitatory amino acid transporter 2 (EAAT2) or sodium-dependent glutamate transporter 2 (SLC1A2) plays an essential role in glutamate clearance from the synaptic cleft, therefore regulating glutamatergic neurotransmission. Numerous neurological diseases, such as mood disorders, addiction, Huntington's disease and amyotrophic lateral sclerosis (ALS) have been linked to impairments of glutamate transporter function and/or expression, making this transporter an emerging therapeutic target. Spider venoms represent an incredible source of biologically active compounds, notably due to diversity within the Araneae order, which

comprises more than 46,000 species. Spider toxins, like argiotoxins from Araneus *gemma* and the joro spider toxin from the Nephilia *clavata* have been shown to block the activity of ionotropic glutamate receptors. Regarding glutamate transporters, previous studies have indicated that EAAT2 function can be enhanced by Parawixin-1, a substance present in the venom of the Brazilian spider Parawixia *bistriata* (Fontana et al., Br J Pharmacol. 2003; 139:1297-309). In this study, we evaluated the EAAT2 modulatory effects of a focused library of spider venom fractions. Using functional assays (fluorescence-based membrane potential measurements, glutamate uptake assays and electrophysiology), we screened a venom library containing 207 fractions derived from 12 different species of orb weaving spiders (VenomTech, UK). Our results showed that venoms from Eresidae, Ctenidae, Lycosidae and Sparassidae families exhibit modulatory activity on EAAT2. These findings may provide insights to the development of new pharmacological tools as well as compounds of therapeutic interest targeting the glutamate transporter family.

**Disclosures:** **A. Tora:** A. Employment/Salary (full or part-time):; Janssen R&D Johnson&Johnson. **M. Rives:** A. Employment/Salary (full or part-time):; Janssen R&D Johnson&Johnson. **A.D. Wickenden:** A. Employment/Salary (full or part-time):; Janssen R&D Johnson&Johnson.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.09/DP03/C43 (Dynamic Poster)

**Topic:** B.05. Transporters

**Support:** Wellcome Trust Principal Fellowship (101896)

European Research Council Advanced Grant (323113-NETSIGNAL)

FP7 ITN (606950 EXTRABRAIN)

Russian Science Foundation grant (15-14-30000)

**Title:** Subcellular distribution of astroglial receptors monitored with super-resolution microscopy

**Authors: *J. P. HELLER**[1,2], K. ZHENG[2], D. A. RUSAKOV[2]
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**Abstract:** Astrocytes play an active role in shaping and maintaining neuronal circuits. In addition to their long-established role in extracellular potassium buffering and glutamate uptake,

these cells can also regulate the activity of local synaptic circuits through secretion and clearance of neurotransmitters. Whilst the molecular signal exchange between astroglia and synapses occurs in a highly heterogeneous microenvironment on the nanoscale, the spatial subcellular distribution of the underlying molecular machineries remains poorly understood.
We have therefore employed super-resolution microscopy techniques such as dSTORM (direct stochastical optical reconstruction microscopy) to visualise the 3D positions of neurotransmitter receptors and transporters in astrocytes. This imaging technique relies on the sequential activation, imaging and bleaching of a sparse subset of fluorescent molecules. Thus, images can be obtained with sub-diffraction resolution by localising individual activated molecules in each frame. Using dSTORM, we were able to localise cytoskeletal proteins as well as clusters of receptors and transporters in astrocytic and neuronal membranes in fixed cultured cells and in brain slices. Moreover, through multi-colour imaging, the positional relationship between synapses and astroglial receptors and transporters can be assessed. We are currently attempting to determine whether the astroglial coverage of excitatory as well as inhibitory synapses is altered in different conditions compatible with long-term synaptic potentiation or depression.

**Disclosures:** **J.P. Heller:** None. **K. Zheng:** None. **D.A. Rusakov:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.10/C44

**Topic:** B.05. Transporters

**Support:** R01AA019458 from from the National Institutes on Alcohol Abuse and Alcoholism

fund provided by The University of Toledo

**Title:** Effects of clavulanic acid treatment on GLT-1, xCT and mGluR2/3 expression on co-abuse of methamphetamine and ethanol in alcohol-preferring rat model

**Authors: *Y. ALTHOBAITI**[1,2], F. ALSHEHRI[1], A. HAKAMI[1], A. HAMMAD[1], Y. SARI[1]
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**Abstract:** Evidence shows a high rate of co-abuse of methamphetamine (METH) and ethanol. In this study, we investigated the reinstatement to METH using conditioned place preference (CPP) in alcohol-preferring (P) rats as an animal model of alcoholism. Among other neurotransmitters, glutamate is implicated in mediating relapse to several drugs of abuse. Glutamate homeostasis is maintained by number of glutamate transporters, such as glutamate transporter type 1 (GLT-1), cystine/glutamate transporter (xCT), and glutamate aspartate transporter (GLAST). In addition,

group II metabotropic glutamate receptors (mGluR2/3) were found to be implicated in relapse to different drugs of abuse. Ceftriaxone, β-lactam antibiotic, has been previously shown to be effective in improving glutamate homeostasis and preventing relapse to drugs of abuse. Here, we tested the effect of clavulanic acid (CA), a non-antibiotic β-lactam compound, on reinstatement to METH seeking, ethanol intake, the expression of glial glutamate transporters, and mGluR2/3 in the nucleus accumbens (NAc) shell and core as well as the dorsomedial prefrontal cortex (dmPFC). Chronic exposure to ethanol decreased the expression of GLT-1 and xCT in the NAc shell, but not the NAc core or dmPFC. CA treatment attenuated the reinstatement to METH seeking, decreased ethanol intake and restored the expression of GLT-1 and xCT in the NAc shell. CA treatment increased the expression of mGluR2/3 in the NAc shell and dmPFC. These findings suggest that CA has the potential to modulate the expression of glial glutamate transporters and mGluR2/3 receptors, which consequently attenuate reinstatement to METH seeking and decrease ethanol intake.
**Key words:** Methamphetamine, glutamate, GLT-1, conditioned place preference, mGluR2/3, xCT, GLAST, reinstatement, clavulanic acid

**Disclosures:** **Y. Althobaiti:** None. **F. Alshehri:** None. **A. Hakami:** None. **A. Hammad:** None. **Y. Sari:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.11/C45

**Topic:** B.05. Transporters

**Support:** Stiftelsen Olle Engkvist Byggmästare

**Title:** Characterization and functional relevance of SLC38 (SNAT) homologues in D. Melanogaster

**Authors: *T. AGGARWAL**, M. M. ERIKSSON, R. FREDRIKSSON
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**Abstract:** Glutamine, glutamate and GABA are important amino acids for our body which are regulated by an important mechanism known as Glutamate/GABA-glutamine cycle working between neurons and astrocytes. This is controlled by several members of membrane bound transporter proteins known as solute carriers (SLCs) which are vital for transport and cellular homeostasis. Imbalance and dysregulation in the cycle and transport can result in cellular stress, cell death and hence neurodegeneration.
SLC38 is one the transporter family also referred to as sodium-coupled neutral amino acid transporters (SNATs). In *D. Melanogaster*, there are two SNATs, one possible orthologous to

SLC38A10 (SNAT10) and one that are ancestral to SLC38 (SNAT 1-6,7,8,11). SLC38 family in *D. Melanogaster* is unexplored, and here we investigate the functional characterization and functional relevance of SLC38 transporters in cellular metabolism and bioenergetics. We have used UAS/Gal4 system to check for expression and we observed that SLC38A10 is expressed in brain and salivary gland and SLC38 only in salivary gland. To characterize the role of SLC38 transporter, we are using RNAi lines for ubiquitous knockdown to be compared to controls. We performed qPCR to check for gene expression, sugar assay to measure important sugars, DAMS (drosophila activity monitoring system) to analyze starvation resistance, circadian rhythm, locomotion activity, survival assay, and tissue staining to check for neuronal loss. We are analyzing the neurotransmitter levels in the brain to check for any upregulation or downregulation of specific neurotransmitter. We have observed significant changes in SLC38 knockdowns compared to controls. This will help us to functionally characterize and further explore any links between the role of SLC38, Glutamate/GABA-glutamine cycle and any neurodegenerative phenotype.

**Disclosures: T. Aggarwal:** None. **M.M. Eriksson:** None. **R. Fredriksson:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.12/C46

**Topic:** B.05. Transporters

**Title:** Modulation of anion channel gating in Excitatory Amino Acid Transporters by c-terminal domains

**Authors: A. M. KARA**[1], A. D. GONZALEZ[2], J. GARCIA-OLIVARES[1], *D. TORRES-SALAZAR[1], S. G. AMARA[1]
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**Abstract:** Excitatory Amino Acid Transporters (EAATs) are responsible for clearing glutamate following its synaptic release, and thus they facilitate the precise transmission of excitatory signals within the CNS. Failure to maintain tight control of extracellular glutamate concentrations can lead to overstimulation of glutamate receptors and neuronal cell death due to excitotoxicity. EAATs serve two functions: they couple the transport of glutamate to the inward movement of sodium ions and they also allow chloride permeation through a substrate-gated anion selective channel. In recent years, our understanding of the substrate transport mechanism has significantly advanced. However, the mechanism and molecular determinants for channel gating and anion permeation are only now emerging. Studies combining molecular dynamics (MD) simulations and electrophysiological techniques have provided evidence for a structural coupling controlling the equilibrium between the transport cycle and anion channel opening, the

latter occurring “outside” the transport cycle in intermediate conformations. We have constructed a computational model of EAAT4 including the C-terminus, which suggests that interactions between the C-terminus and a conserved region of transmembrane domain 3 (TM3) influence the structural coupling between transport and anion channel activity. We tested this hypothesis using electrophysiological recordings in *Xenopus* oocytes expressing different point mutations in TM3 (K119D, R123D, and R127D) and in the C-terminus (E523, E528, and E530) or using transporters with different C-terminal truncations. Our preliminary results indicate that truncation of the full C-terminus (Q521X) disrupts anion channel gating and significantly reduces glutamate transport, indicating that the C-terminal domain and its potential interaction with TM3, may play a critical role in modulating the structural coupling that control the equilibrium between substrate transport and anion channel opening. Our results provide additional insight into the complexity of structural conformations that link substrate binding and the anion channel gating.

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**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

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**Topic:** B.05. Transporters

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NICHD Grant U54 HD086984

Foerderer Foundation

**Title:** Astroglial glutamate transporters and reversed-mode $Na^+/Ca^{2+}$-exchange contribute to neurovascular coupling

**Authors: *J. G. JACKSON**[1], H. TAKANO[2], D. A. COULTER[3], M. B. ROBINSON[4]
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**Abstract:** Cerebral blood flow is increased to match energetic demands imposed by neuronal activation. This process, termed neurovascular coupling, provides the basis for the signal detected with functional MRI. Although the mechanism (s) underlying neurovascular coupling have been studied for two decades, the field is still highly controversial. Astrocytes have fine processes that contact synapses and endfeet that ensheath the vasculature, making them ideal anatomic mediators of this effect. Some have shown that increases in astrocytic $Ca^{2+}$ are

sufficient to increase blood flow and observed $Ca^{2+}$ increases that precede the increase in arteriole diameter, while others have not observed $Ca^{2+}$ responses with the appropriate temporal characteristics. Most excitatory activity is mediated by glutamate and most glutamate is cleared into astrocytes by $Na^{+}$-dependent transporters. A decade ago three groups demonstrated that blocking glutamate uptake attenuates the neurovascular response (Gurden et al 2006; Petzold et al 2008; Schummers et al 2008), but the effects of glutamate transporter substrates have not been examined and the mechanisms underlying transporter-mediated increases in blood flow have not been explored. We hypothesized that glutamate uptake-mediated arteriole dilation might depend on the $Na^{+}/Ca^{2+}$ exchanger. To test this hypothesis, we used two-photon *in vivo* microscopy to measure arteriole diameter in anesthetized mice, of either sex. As others have reported, either electrical stimulation or focal application of glutamate transiently increased arteriole diameter. Focal application of a glutamate transporter substrate (L-(-)-threo-3-hydroxyaspartic acid; THA), at a concentration (100 µM) that does not activate ionotropic glutamate receptors (Erreger et al 2007), increased arteriole diameter to 113 ± 2% of control (n=6 mice). In preliminary experiments, the increases in arteriole diameter induced by either electrical stimulation or THA were blocked by an inhibitor of reversed-mode $Na^{+}/Ca^{2+}$-exchange (1 µM; YM244769). These results suggest that activation of glutamate transporters is sufficient to evoke arteriole dilation *in vivo* and suggest a mechanism by which transporter-mediated dilation may occur.

**Disclosures:** **J.G. Jackson:** None. **H. Takano:** None. **D.A. Coulter:** None. **M.B. Robinson:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.14/C48

**Topic:** B.01. Neurotransmitters and Signaling Molecules

**Support:** NSF Grant 1547693

NSF Grant 1632891

**Title:** Nano- and micro-scale composite materials for tracking modification of brain cell growth over time

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**Abstract:** Glutamate (Glu), a major excitatory neurotransmitter, is taken up by normal brain astrocytes whereas brain tumor cells release more Glu or fail to take it up, causing a positive

feedback loop which can injure neurons. We hypothesized that secretion of Glu or failure of uptake by brain tumor cells can be inhibited by delivering anti-cancer drugs to remove this dysfunctional component of the normally homeostatic system. To verify this, we measured Glu uptake by normal brain astrocytes and brain tumor cells (CRL-2303) from American Type Culture Collection (ATCC) using EnzyChrom$^{TM}$ Glutamate Assay kit and spectrophotometric measurements. Preincubation of cells with Glu for 5 hours at 37°C and 5% $CO_2$ showed uptake in normal brain astrocytes but not by brain tumor cells. Glu (100 uM) was taken up by astrocytes in a cell density-dependent manner, whereas glioma cell cultures lacked such relationship. For 200,000, 100,000, and 50,000 cells per mL, astrocytes showed Glu uptake of 67.7, 41.7, and 15.9% respectively. In contrast, under the same conditions, glioma cells showed 10.1, 8.2 and 2.5% respectively. Our data indicated that this type of brain tumor cells have minimum or no Glu clearing capacity from the extracellular environment compared to normal brain astrocytes. These dynamics could be important for measuring functional effects of potential anti-cancer drugs. For example, camptothecin (CPT), an apoptotic anti-cancer drug, but poorly soluble, was encapsulated here in the lumen of halloysite nanotubes (HNTs) by using their saturated solutions in organic solvents and delivered to the tumor cells. In comparison to free CPT, HNTs-loaded with CPT at submaximal killing concentrations (1 ug/mL), sustained suppression of glioma cell growth *in vitro* after the initial burst of inhibition. In contrast, normal brain astrocytes treated with submaximal apoptotic treatments demonstrated much less recovery after release from the apoptotic stimulus. After determining recovery from apoptosis for the two cell culture types separately, feasibility of mixed glioma/astrocyte cultures was demonstrated using beta-galactosidase staining, which is specific for the glioma cells (modified to carry the expression marker). Mixed cultures of glioma cells and astroctyes better mimic the *in vivo* environment which is heterogeneous, and is anticipated to also reflect altered Glu uptake regulation both before and after anti-cancer drug treatments. Thus, cell killing effects and regulation of Glu uptake together may give added insight to long-term efficacy of potential apoptotic applications for healthy and diseased brain cell systems.

**Disclosures: U. Kansakar:** None. **R. Yendluri:** None. **Y. Lvov:** None. **N.H. Nguyen:** None. **M.A. DeCoster:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.15/C49

**Topic:** B.05. Transporters

**Support:** NIH Grant R01AA019458

**Title:** Targeting glial glutamate transporters for the treatment of cannabinoids dependence in rat model

**Authors: *A. Y. HAKAMI**, F. S. ALSHEHRI, Y. S. ALTHOBAITI, Y. SARI
Univ. of Toledo, Toledo, OH

**Abstract:** Several studies have reported the involvement of CB1 receptors in mediating the effect of many drugs of abuse based on pharmacological manipulations of CB1 receptors. Previous studies on cannabinoids, using conditioned place preference (CPP) paradigm, have reported conflicting results regarding their rewarding effects involving certain neurotransmitter systems. However, less is known about the role of glutamate neurotransmission on cannabinoid dependence. Glutamate homeostasis is maintained by several glial glutamate transporters, such as glutamate transporter 1 (GLT-1), cystine/glutamate transporter (xCT) and glutamate aspartate transporter (GLAST). Studies from our laboratory demonstrated that β-lactam compounds can restore glutamate homeostasis in alcohol-preferring rats, in part, through upregulation of GLT-1 and xCT expression. In this study, we tested the effects of chronic treatment with CP 55940 (20 μg/kg) on the CPP paradigm and glial glutamate transporters. Moreover, we investigated the effect of Ampicillin/Sulbactam (AMP/SUL), β-lactam compounds, on the reinstatement to CP 55940; we also determined the effects of these β-lactams on glial glutamate transporters. We found that our established CPP paradigm showed reinstatement to CP 55940 and this effect was associated, in part, with downregulation of xCT expression in the brain reward regions. Importantly, AMP/SUL treatment attenuated reinstatement to CP 55940 and restored the expression of xCT in the brain reward regions. These findings demonstrated that AMP/SUL attenuated reinstatement to CP 55940 through upregulation of xCT. This study suggests that AMP/SUL may be potentially used for the treatment of cannabinoid dependence.

**Disclosures:** **A.Y. Hakami:** None. **F.S. Alshehri:** None. **Y.S. Althobaiti:** None. **Y. Sari:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.16/C50

**Topic:** B.05. Transporters

**Support:** Grant 2G12MD007592 from the National Institute on Minority Health and Health Disparities

**Title:** Characterization and modelling of a putative glycine transporter in *Drosophila melanogaster*

**Authors: *A. B. LOPEZ**[1], F. FRATEV[2], A. SILVA[3], S. SIRIMULLA[2], M. MIRANDA[3]
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**Abstract:** Glycine is the simplest amino acid, but also serves as a major inhibitory neurotransmitter in the vertebrate central nervous system. Although glycine transporters (GlyT1 and GlyT2) and receptors have been established as critical components of inhibitory neurotransmission in mammals, they are poorly studied in invertebrate models such as *Drosophila melanogaster*. Published work by other groups, using sequence alignments of mammalian transporters against the fly genome, predicts the presence of a single glycine transporter. In this work, we used protein modelling and biochemical assays to characterize this putative glycine transporter (Dmel GlyT2). Based in dynamic simulations, we build a 3D model and localized the possible binding cage for glycine and sodium ions, providing evidence that this is a glycine transporter with equivalent organization to the mammalian GlyT2. To obtain additional biochemical evidence of a glycine transporter, we will express this protein in mammalian cells and analyze for substrate specificity and kinetic parameters. The results should provide strong evidence of the presence of glycinergic components in invertebrates.

**Disclosures: A.B. Lopez:** None. **F. Fratev:** None. **A. Silva:** None. **S. Sirimulla:** None. **M. Miranda:** None.

**Poster**

**559. Amino Acid Transporters**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 559.17/C51

**Topic:** B.05. Transporters

**Support:** DFG Fr 1784/18-1

**Title:** Glycine transporter 2 (GlyT2) surface expression is reduced by the calcium-dependent secretion activator 1 (CAPS1)

**Authors: S. X. MARZ**[1], M. JONES[1], C. FECHER-TROST[2], M. JUNG[3], R. T. ALEXANDER[4], *E. FRIAUF[1]
[1]Univ. of Kaiserslautern, Dept of Biol., Kaiserslautern, Germany; [2]Saarland University, Exptl. and Clin. Pharmacol. and Toxicology, Homburg, Germany; [3]Saarland University, Med. Biochem. and Mol. Biol., Homburg, Germany; [4]Univ. of Alberta, Dept. of Physiology, Canada Dept. of Pediatrics, Edmonton, AB, Canada

**Abstract:** Glycine is an important inhibitory neurotransmitter in the CNS of vertebrates. The neuronal glycine transporter 2 (GlyT2) is localized in presynaptic terminals and mediates glycine

uptake from the synaptic cleft, thus contributing to glycine homeostasis. GlyT2 is considered a potential target for treatment of pain and hyperekplexia. As GlyT2 activity can be regulated by protein-protein interactions, it is essential to investigate the interactome of GlyT2. To do so, we performed coimmunoprecipitations (co-IPs) of GlyT2 with hindbrain lysate and subsequent mass spectrometry (in triplicate). Forty-five putative GlyT2-interacting proteins were exclusively identified in GlyT2 co-IPs of wildtype animals. Among these, the calcium-dependent secretion activator 1 (CAPS1) was identified with 7, 14 and 5 exclusive unique peptides. Co-IPs with endogenous protein followed by immunoblotting verified the binding of CAPS1 to GlyT2. Moreover, GlyT2 efficiently bound to CAPS1 in reverse co-IPs, suggesting a physical interaction of GlyT2 and CAPS1 *in vivo*. The protein-protein binding site was assessed via a peptide spot array. To this end, peptides corresponding to the mouse GlyT2 amino-terminus, carboxy-terminus and parts of CAPS1 were spotted onto a membrane and incubated with hindbrain lysate, followed by antibody labeling. We found binding of CAPS1 to the conserved GlyT2 carboxy-terminus. Immunocytochemistry and immunoblotting of HEK293 cells revealed higher GlyT2 expression when CAPS1 is co-expressed, suggesting a positive regulation. Interestingly, GlyT2 appears to be localized more intracellularly in the presence of CAPS1. Indeed, biotinylation of surface proteins in HEK293 cells indicated a lower relative amount of biotinylated GlyT2 when co-expressed with CAPS1. Demonstration of a functional interaction between GlyT2 and CAPS1 was that glycine uptake was reduced in HEK293 cells co-expressing GlyT2 and CAPS1. The substrate affinity of GlyT2 was unchanged by CAPS1. In contrast, the maximal glycine transport velocity was reduced, confirming a decreased surface expression of GlyT2, which suggests reduced trafficking of the transporter to the plasma membrane or enhanced endocytosis. Taken together, our data provide evidence that CAPS1 interacts with GlyT2 and reduces glycine uptake by decreasing its surface expression. This is in line with the knowledge that CAPS1 is essential for vesicle trafficking, neurotransmitter exocytosis, and the modulation of monoamine uptake and storage by vesicular monoamine transporters.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.01/C52

**Topic:** B.11. Epilepsy

**Support:** NIH Grant NSR01NS064154

**Title:** PADI4/PADI6 gene variations are associated with common forms of human generalized epilepsy

**Authors: *R. J. BUONO**[1], T. N. FERRARO[1], J. P. BRADFIELD[2], H. HAKONARSON[2]
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**Abstract:** Epilepsy is a complex heterogenous disorder with strong heritability. We performed a genome wide association study (GWAS) using single nucleotide polymorphism (SNP) arrays (Illumina HumanHap 550, 610 Quad and OMNIExpress) to identify genetic variants associated with common forms of idiopathic generalized epilepsy (GE) and focal epilepsy (FE). The Philadelphia Cohort contains 2617 patients with common forms of GE or FE of unknown cause. Association tests were performed separately for all epilepsy, GE and FE compared to 8070 controls. All SNPs were tested with a logistic regression using the top 3 eigenvectors and sex as covariates for the 3 phenotypes. SNPtest was used for the association analysis. The cohorts, separated by ancestry, were meta-analyzed using METAL applying an inverse variance-based algorithm. A SNP in the ancestry specific meta-analysis was considered to be genome wide significant with a P-value <= $5x10^{-8}$. Ancestry level meta-analysis statistics were then combined using MANTRA in a trans-ethnic meta-analysis. The number of samples in each analysis was calculated as the effective sample size $N_{eff} = 4/(1/N_{cases}+1/N_{ctrls})$. A SNP in the trans-ethnic meta-analysis was considered to be genome wide significant with a log10(Bayes Factor) >= 6.
No SNPs reached genome wide significance when comparing all cases to controls. Positive association was found in GE patients with four SNPs reaching genome wide significance ($P < 5 \times 10^{-8}$) across the PADI4/PADI6 locus. Four additional SNP markers in this region reached p-values of between $5.3 \times 10^{-8}$ and $8.1 \times 10^{-8}$. Five of these SNPs reached genome wide statistical significance via MANTRA analysis as well. The associated SNPs reside within intronic regions of *PADI4* and *PADI6* with one synonymous
SNP (rs35240185, P424P) and one 3' untranslated region SNP in *PADI4*. Several SNPs reached genome wide significance in the focal cohort including one marker in the ASS1 gene, two markers in the RANBP3 gene and three markers in PIK3C3 with an additional 8 markers in PIK3C3 reaching suggestive significance (Bayes Factor scores 5.65 – 5.88).
Our results demonstrate that genetic variation at 1p36.13 in the *PADI4-PADI6* gene region is associated with GE. These genes are related to processes that regulate early development, cause epigenetic modification of histones and are also implicated in the generation of autoantibodies. FE genes of interest included ASS1 (in the same enzymatic pathway as PADI4/PADI6) RANBP3 and PIK3C3. Further study of these genes may help elucidate causes of epileptogenesis and lead to potential therapeutic targets for common forms of human epilepsy.

**Disclosures: R.J. Buono:** None. **T.N. Ferraro:** None. **J.P. Bradfield:** None. **H. Hakonarson:** None.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.02/C53

**Topic:** B.11. Epilepsy

**Support:** This work was supported by donations to the Center for Rare Childhood Disorders. These include private anonymous donations. TGen also receives support from the State of Arizona.

**Title:** Functional insights of de novo mutations in DNM1 on epileptic encephalopathy and mitochondrial dysfunction

**Authors: *L. LLACI**, G. MILLS, J. DODSON, E. FRANKEL, V. NARAYAN, B. GERALD, R. C4RCD, S. RANGASAMY, V. NARAYANAN
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**Abstract:** Epileptic encephalopathies (EE) (MIM 308350) are severe childhood brain disorders characterized by a variety of severe epilepsy syndromes that differ by age of onset and seizure type. Until recently, the cause of many epileptic encephalopathies was unknown. Whole exome or whole genome sequencing has led to the identification of several causal genes in individuals with epileptic encephalopathy, and the list of genes has now expanded greatly. Studies in our Center for Rare Childhood Disorders and by others have defined de novo missense mutations in the Dynamin 1 gene (*DNM1*, OMIM: 602377) in several patients with early infantile epileptic encephalopathy (OMIM: # 616346). Early onset epileptic encephalopathy, intractable seizures, intellectual disability, developmental delay, and hypotonia are the common neurological deficit described in patients with DNM1. Dynamin 1 (DNM1), with a molecular mass of ~100 kd, functions in GTPase mediated endocytosis, synaptic transmission, and activation of signaling mediated by PKC. DNM1 is highly expressed in neurons, but not widely expressed in other tissues. We describe the phenotypic and genetic spectrum in a cohort of patients (n=5) with early infantile epileptic encephalopathy caused by the DNM1 mutation. Past research has provided insights into dynamin-1 structure and function; however it is still unclear how the de novo missense mutation in DNM1, a core component of postsynaptic endocytosis machinery leads to early epileptic encephalopathy. To understand the pathogenesis of DNM1 epileptic encephalopathy we studied the patient-derived fibroblasts and have developed a zebrafish model of DNM1 mutations. Our results from the zebrafish model with morpholino knockdown and chemical inhibition of DNM1 protein revealed the presence of smaller head (microcephaly) and body size. We detected a hyperactive phenotype and whole body convulsion in zebrafish embryos at 72 hrs. We have also observed mitochondrial dysfunction in the fibroblast cells

derived from a patient. The mitochondrial dysfunction was characterized by decreased ATP generation and reduced store capacity. We also observed an impaired endocytosis mechanism revealed through transferrin uptake assay. Our studies demonstrate a DNM1-specific phenotype in patient-derived fibroblasts and in the zebrafish model. Use of such cellular and simple animal models may lead to a better understanding of DNM1-related neurological disease mechanisms and may lead to the identification of novel therapies.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.03/C54

**Topic:** B.11. Epilepsy

**Support:** CURE epilepsy

Savoy Foundation

**Title:** Loss-of-function mutations in the epileptic encephalopathy candidate gene TRIO impair the pre- and post-natal development of cortical GABAergic interneurons

**Authors: *L. EID**, F. CHARRON-LIGEZ, P. RAJU, F. HANSSON, M. LACHANCE, E. ROSSIGNOL
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**Abstract:** Epileptic encephalopathies (EEs) are neurodevelopmental diseases characterized by early-onset epilepsy with cognitive deficits. EE remain of unknown etiology in up to 50% of patients. However, recent data suggest that a subset of genetically determined epilepsies result from defects in the development of inhibitory interneurons (INs) leading to aberrant enhancement of cortical excitability. Recessive mutations in the *TRIO* gene have been recently identified through whole-exome sequencing in patients with EE or isolated intellectual deficiency by our lab and others. *Trio* encodes a guanine nucleotide exchange factor (GEF) that activates the Rho GTPases Rac1, RhoG and RhoA, implicated in fundamental aspects of neuronal development, including cell cycle regulation, neurite outgrowth, nucleokinesis and caudal neurite retraction. However, the roles of *Trio* in the development of INs are unknown. Considering the proposed involvement of *Trio* in neuronal development and the implication of IN disorders in the pathophysiology of EE, we stipulate that *Trio* might be a central regulator of the development and connectivity of cortical INs. To assess this hypothesis, we performed a

targeted repression of *Trio* in migrating INs through *ex-vivo* electroporation of a *Dlx5/6::shRNA-tdTomato* plasmid in the medial ganglionic eminences of e13.5 embryonic mice and cultured organotypic cortical slice that were imaged after 48h using confocal and time-lapse imaging. We show that the targeted repression of *Trio* impairs the morphological development and migratory dynamics of cortical INs. These morphological defects were partly rescued after co-electroporation with a shRNA-resistant version of the full-length *Trio* cDNA, reflecting the requirement for specific TRIO isoforms. Furthermore, the morphological defects can be partially rescued by co-electroporation of the *Trio* shRNA with a constitutively active form of RhoA. To assess the role of *Trio* in the post-natal development of cortical INs, we transfected a *10kb-GAD67::shRNA-tdTomato* plasmid, specific to parvalbumin (PV)-INs, in post-natal cortical organotypic cultures. We show that the post-natal repression of *Trio* decreases the complexity of the dendritic field and reduces the extent of the innervation field of PV-INs on pyramidal cells. Altogether, our data suggest that *TRIO*-associated EE might be due to a defect in cortical INs development, both pre- and post-natally, presumably resulting in cortical hyperexcitability. We will validate these findings *in vivo* using a *Trio* conditional knockout mouse and further assess the contribution of specific Rho GTPAses to the cellular pathological phenotypes observed.

**Disclosures: L. Eid:** None. **F. Charron-Ligez:** None. **P. Raju:** None. **F. Hansson:** None. **M. Lachance:** None. **E. Rossignol:** None.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

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**Program#/Poster#:** 560.04/C55

**Topic:** B.11. Epilepsy

**Support:** NIH NS085389

Clare Boothe Luce - Creighton University

Student Research and Scholarly Activity Award from Creighton School of Medicine

**Title:** The ketogenic diet regulates catalase via the transcription factor peroxisome proliferator activated receptor gamma 2

**Authors: *S. KNOWLES**[1], S. BUDNEY[1], S. MATTHEWS[1], M. DEODHAR[1], K. A. SIMEONE[2], T. A. SIMEONE[3]
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**Abstract:** The ketogenic diet (KD) is an effective therapy for pediatric epilepsy patients that are refractory to conventional anti-seizure drugs, a condition in which people with seizures do not

achieve adequate seizure control with medication. Recently, we have identified the nutritionally-regulated transcription factor PPARγ2 as an important contributor to the anti-seizure effects of the KD. PPARγ2 regulates hundreds of genes involved in adipogenesis, insulin sensitivity, anti-inflammation, antioxidation and mitochondrial function. We performed a RT-qPCR array experiment on a small set of known PPARγ-regulated genes on hippocampal tissue from wild-type (WT) and epileptic Kcna1-null mice. We found that half the genes were regulated by the KD in both genotypes. As oxidative stress has been implicated in the pathology of epilepsy, we focused on one of the genes identified, the antioxidant catalase. We hypothesized that the KD regulates catalase via PPARγ2. Using qPCR and Western blot techniques, we found that the KD regulates mRNA and protein levels of catalase in WT and Kcna1-null hippocampus, but not Pparγ2-null hippocampus. However, catalase activity assays did not reveal any significant differences between mice fed a standard diet or KD. Ongoing experiments will determine the effects of a catalase inhibitor on the KD antiseizure efficacy. In conclusion, as a dietary therapy the KD has wide ranging effects on gene expression. However, the mechanisms contributing to the antiseizure effects are not completely understood. Our results here suggest that KD regulation of catalase is dependent on PPARγ2.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.05/C56

**Topic:** B.11. Epilepsy

**Support:** NIH/NINDS grant NS056304 (JAH)

**Title:** The role of T-cell intracellular antigen-1 in epileptogenesis

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**Abstract:** T-cell intracellular antigen-1 (TIA-1) is an RNA binding protein that contributes to post-transcriptional control of gene expression by silencing translation of target mRNAs. Under conditions of cellular stress, TIA-1 arrests translation by sequestering target mRNAs in stress granules (SGs). It may also serve functions other than mRNA translational regulation via protein-protein interactions. TIA-1 has recently been implicated in several acute and chronic neurodegenerative conditions, including cerebral ischemia and Alzheimer's disease. Because brain damage is an important risk factor for development of epilepsy, a chronic disorder that is

characterized by spontaneous seizures, the purpose of this study was to examine the possibility that TIA-1 functions in epileptogenesis, the process by which the normal brain becomes prone to spontaneous seizures. Although TIA-1 is known to be expressed in the central nervous system, this has not been characterized systematically in the normal brain. Thus, we first examined the constitutive expression profile of TIA-1 mRNA and protein. In comparison to other tissues, brain exhibited the highest level of TIA-1 mRNA expression and this was particularly high in the hippocampal formation and cortex. Protein was concentrated in the nuclei of glutamatergic neurons in all layers of the cortex and dentate gyrus, CA3, and CA1 regions of the hippocampus. Astrocytes express TIA-1 as well albeit at a lower level than neurons. To assess the role of TIA-1 in epileptogenesis, we examined the effect of *TIA1* gene deletion (-/-) on pentylenetetrazole (PTZ)-induced kindling. Mutant mice lacking TIA-1 exhibited accelerated kindling progression and significantly higher kindling incidence relative to their wild-type littermates (+/+). Moreover, a higher mortality rate was associated with convulsions during kindling acquisition in -/- mice relative to +/+. These effects could not be explained by a reduction in the acute seizure threshold as the incidence of acute PTZ-induced convulsions and mortality were not different between +/+ and -/-. Interestingly, whereas a lower kindling dose of PTZ yielded similar kindling incidences, enhanced mortality was still detected in mutant vs. wild-type mice. Together, these results suggest that TIA-1 functions to antagonize the progressive changes in neuroplasticity associated with kindling development. The extent to which this is due to post-transcriptional regulation or via protein-protein interaction is currently under investigation.

**Disclosures: Y. Gong:** None. **J.A. Hewett:** None.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.06/C57

**Topic:** B.11. Epilepsy

**Support:** National Institute of Neurological Disorders and Stroke R01NS065020

**Title:** PTEN deletion from a subset of hippocampal granule cells produces seizures and hippocampal cell loss

**Authors: *S. KHADEMI**[1], C. L. LASARGE[1], I. V. A. LIMA[2], B. E. HOSFORD[1], A. C. P. DE OLIVEIRA[2], S. C. DANZER[1]
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**Abstract:** Abnormal hippocampal dentate granule cells appear in significant numbers in animal models of temporal lobe epilepsy, as well as in patients with the disease. These cells mediate the

formation of recurrent excitatory circuits, and are hypothesized to promote epileptogenesis. Abnormal granule cells can be produced experimentally in mice by deleting phosphatase and tensin homologue (PTEN), an inhibitor of the mammalian target of rapamycin (mTOR) pathway. Indeed, hyperactivation of the mTOR pathway is also implicated in epileptogenesis. Previous studies have shown that PTEN deletion from as little as ≈10% of granule cell population is sufficient to produce epilepsy, but exactly how these cells produce the disease is unclear. Here, we explored whether granule cell-specific PTEN deletion leads to the loss of hippocampal mossy cells and interneurons. Experiments were conducted in animals with low (<10%) and high (>10%) percentages of PTEN knockout (KO) cells, to determine whether abnormal granule cell "load" impacted the phenotype. We found that PTEN KO mice on a C57BL/6 background, age (2.3 m – 3.7 m), with a high load of knockout cells develop spontaneous cortical seizures and exhibit loss of hippocampal mossy cells and neuropeptide Y-expressing interneurons. By contrast, animals with relatively few knockout cells did not exhibit seizures or cell loss. Together, these data reveal that selective deletion of PTEN from a subset of hippocampal granule cells can reproduce a pattern of cell loss common in temporal lobe epilepsy. These findings are consistent with the hypothesis that abnormal granule cells can be a driving force in epileptogenesis.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.07/C58

**Topic:** B.11. Epilepsy

**Support:** FAPESP

CAPES/PROEX Fisiologia

CAPES/PROEX Genética

FAEPA

CNPq

**Title:** Endogenous expression of epigenetically regulated genes in a genetically-selected rat model of epilepsy

**Authors: *A. FERNANDES**[1], H. R. MAGALHÃES[2], A. L. F. DONATTI[3], J. A. C. OLIVEIRA[4], Á. F. L. RIOS[6], C. C. P. PAZ[7], N. GARCIA-CAIRASCO[4], E. S. RAMOS[5]

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**Abstract:** Epilepsy is one of the most studied neuropathologies in neuroscience, however the basic mechanisms for the development of the seizures are not completely understood. The use of animal models is yet a valuable strategy because of the maintenance of the central nervous system complexity concerning the neuroanatomical/neurochemical substrate underlying the epileptic activity. Endogenously epilepsy-prone animals present advantages in comparison to chemically or electrically induced models due to, again, the maintenance of complexity. In this study we aimed to verify differences between naïve Wistar Audiogenic Rat (WAR), an inbred strain derived from susceptible Wistar rats, and resistant Wistars on the expression of genes that undergo epigenetic influences. We selected genes with monoallelic expression: (a) imprinted genes (Igf2, Peg1, Peg3, and Zac1), (b) a gene with monoallelic stochastic expression (Lgi1), and (c) an X-linked gene (Pgk1). RNA was extracted from the hypothalamus and frontal cortex from two adult females of each strain, placenta and brain from three pups of their respective litters. The cDNA was analyzed by real-time PCR. Zac1 and Peg3 showed higher levels of expression in the adult hypothalamus than in the cortex. In the offspring, Igf2 and Zac1 showed higher expression levels in placenta, and Lgi1 and Pgk1 showed higher expression levels in brain tissue. Adult WARs presented higher expression levels of the Igf2, Peg3, Peg1, Lgi1, and Zac1 genes. Only Pgk1 was highly expressed in adult Wistar. In pups, only Peg1 showed higher expression levels in WAR. Zac1 correlated positively with Igf2, Peg1, and Peg3 genes. Our data suggest that Igf2, Zac1 and Pgk1 genes may have significant roles in epilepsy susceptibility observed in the WAR strain, and strengthen the idea of a net of epigenetically regulated genes in epilepsy.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.08/C59

**Topic:** B.11. Epilepsy

**Support:** MOST 104-2314-B-002-078-MY3

**Title:** Cell-type-specific Rbfox3 (NeuN) deletion causes spontaneous seizures through excitation/inhibition imbalance

**Authors: *D.-F. HUANG**[1], R.-N. WU[1], C.-C. LIEN[2], H.-S. HUANG[1]
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**Abstract:** Neuronal nuclei (NeuN), a neuronal nuclear antigen, is widely used to label neurons for over 20 years. Previous study has identified that NeuN corresponds to a RNA binding protein known as RBFOX3 (RNA binding protein fox-1 homolog 3), one of the FOX family proteins. It is an alternative splicing regulator, which is able to enhance or repress the exon usage. In recent studies, the roles of other FOX family members, RBFOX1 and RBFOX2, in the brain have already been investigated. Previous research also found that RBFOX3 mutations were linked to human epilepsy, cognitive impairment, speech disorder, developmental delay, long sleep latency and some autistic features. Although NeuN has been identified as RBFOX3, it is still unclear what physiological roles it plays in the brain. Therefore, we used *Rbfox3* knockout mice model to investigate the roles of RBFOX3 in the brain. The previous findings in our lab indicated that conventional *Rbfox3*$^{-/-}$ mice had increased seizure susceptibility and abnormal synaptic transmission in the dentate gyrus (Wang, H.Y., et. al.. ***Scientific Reports*** (2015)). We also found that RBFOX3 played an important role in adult hippocampal neurogenesis and synaptogenesis (Lin, Y.S., et. al., ***PLOS ONE*** (2016)). The dentate gyrus granule cells of *Rbfox3*$^{-/-}$ mice exhibited increased spine density, excitatory synapse number, and dendritic complexity. Furthermore, to investigate the roles of RBFOX3 in specific type of neurons, we set up the cell type-specific conditional *Rbfox3* knockout mouse. This approach has allowed us to perform a genetic dissection of *Rbfox3*. Cell-type-specific deletion of *Rbfox3* caused spontaneous seizure, premature death, and circuitry hyper-excitability. RBFOX3 in presynaptic and postsynaptic neurons of the dentate gyrus circuitry played different roles in the normal function of synaptic transmission and hippocampal circuit balance. This study can help us more understand the roles of RBFOX3 in hippocampal circuitry, seizure pathogenesis of defects in *Rbfox3*, as well as how the splicing regulators such as RBFOX3 operate in the brain and different types of neurons.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.09/C60

**Topic:** B.11. Epilepsy

**Title:** The Cacng2 gene promoter: Multiple repressive elements and bi-directional organization with a long non-coding RNA

**Authors: *D. A. CARTER**[1], B. P. A. CORNEY[1], C. L. WIDNALL[1], D. J. REES[2], J. S. DAVIES[2]
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**Abstract:** The *Cacng2* gene encodes a brain-specific synaptic protein, one member of the transmembrane AMPA receptor regulatory family (TARP gamma-2; aka Stargazin). While the role of this protein in synaptic function has been extensively investigated, comparatively little is known about the transcriptional regulation of the *Cacng2* gene, which is surprising given evidence of altered expression in pathophysiological states. We have now cloned the *Cacng2* promoter region from rat genomic DNA, and investigated transcriptional regulatory activity in a series of transient transfection studies, comparing activity to the widely used *Synapsin I* (*SynI*) neuronal gene promoter. In two cell lines (rat PC12 pheochromocytoma; mouse HT22 hippocampal), the rat *SynI* promoter had significantly greater transcriptional activity compared with *Cacng2* sequences. Within the *Cacng2* promoter region, we identified three repressive sub-regions: (i) sequences downstream from the TSS; (ii) a distal upstream region that contains a calcium-response factor (CaRF) binding site previously documented as repressive in a genome-wide screen (Pfenning et al [2010] PLos One 5:e10870), and (iii), a more proximal upstream region containing multiple REST/NRSF sites. Deletion of all three repressive regions enhanced promoter activity in both cell lines, but the refined *Cacng2* promoter construct remained weaker than the *SynI* construct. Further analysis of the *Cacng2* locus revealed a bi-directional promoter organization in which the TARP gamma-2-encoding transcript is paired with a long non-coding RNA (lncRNA) transcribed in the opposite direction. RT-PCR analysis demonstrated brain-specific expression of lncRNA transcripts in adult rat, but absence of expression in undifferentiated PC12 and HT22 cells. RNAseq expression data from GTEx (Genotype-Tissue Expression project, NCBI, V6p; 53 tissues) indicates highly correlated neuronal expression between human *Cacng2* mRNA and an annotated upstream lncRNA (ENST00000 430281.1). Differentiation of HT22 cells in serum-free media & dibutyryl cAMP results in detectable expression of both *Cacng2* mRNA and the associated lncRNA transcripts, providing further evidence of co-regulation. However, in this differentiation paradigm, activity of the *Cacng2* promoter constructs was not enhanced. These experiments reveal significant repressive control of *Cacng2*, located within a proximal regulatory region that contains multiple distinct molecular features, each with potential for independent modulation of expression. Co-regulation of *Cacng2* with an associated lncRNA may subserve another level of genetic control.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.10/C61

**Topic:** B.11. Epilepsy

**Title:** High-throughput phenotypic screen to validate candidate epilepsy genes

**Authors: *J. F. ULLMANN**[1], C. M. LACOURSIERE[1], T. JOBST-SCHWAN[2], F. HILDEBRANDT[2], A. PODURI[1]
[1]Epilepsy Genet. Program and F.M. Kirby Neurobio. Center, Dept. of Neur, Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [2]Med., Boston Children's Hosp., Boston, MA

**Abstract:** Advances in genomic technologies, such as exome sequencing, have led to an exponential growth in the discovery of genes associated with epilepsy. While these initial associations are important, it is critical to confirm which genes truly play a role in epilepsy through functional validation. As a result, in this study we performed a phenotype-based screen to validate candidate epilepsy genes. We employed the zebrafish as our model, an excellent vertebrate model amenable to high-throughput whole-organism phenotype-based screening. Moreover, zebrafish are an established model for epilepsy with sensitivity to convulsants and anticonvulsants with robust and stereotypical behavioral and electrographic seizures. In the first part of this project, we generated a master list of all epilepsy-associated genes based on an extensive review of all available information from both research and clinical diagnostic domains. This list was carefully analysed and filtered to only include candidate genes with high likelihood of true association with epilepsy. Next, we optimized our CRISPR/Cas9 genome editing protocol to generate biallelic mutations in the F0 generation. By using synthetic gRNAs, carefully titrating the gRNAs and Cas9 protein, injecting into the 1-cell zygote, and performing deep sequencing, we established a robust protocol for generating F0 zebrafish with null-like phenotypes. We are employing this protocol to knock out over 20 genes from our master list in zebrafish embryos. Injected and control embryos are reared to 5dpf, at which time they undergo automated epilepsy-related phenotyping. Acknowledging that not every animal system will show positive seizure-like results with every gene, we anticipate based on past experiments that at least 1 in 3 candidate epilepsy genes would show a seizure-related phenotype.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

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**Topic:** B.11. Epilepsy

**Support:** NIH R01 GM039561

Michigan State University

**Title:** Movement disorder in GNAO1 encephalopathy associated with GOF mutations

**Authors: *H. FENG**, B. SJÖGREN, Y. YUAN, R. R. NEUBIG
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**Abstract: Rationale:** The heterotrimeric protein $G_o$, whose α subunit is encoded by *GNAO1*, regulates ion channel function, neurotransmitter release, and neurite outgrowth. Mutations in *GNAO1* have been identified in children with either epileptic encephalopathy (EIEE17) or a severe choreoathetotic movement disorder. The mechanism underlying the complex clinical spectrum of these *GNAO1* encephalopathies poorly understood. A *Gnao1* gain-of-function (GOF) knock-in mutant (*Gnao1*$^{G184S/+}$) causes a mild seizure phenotype in C57Bl/6J mice. In the current study, we did a biochemical analysis of 15 human *GNAO1* mutations to correlate function with clinical phenotype. Moreover, we examined electrophysiology and gait in *Gnao1* mutant mouse models with both GOF and loss-of-function (LOF) mutations to validate the genotype-phenotype correlation identified from patient mutations.
**Methods:** Wild-type and mutant $G\alpha_o$ were expressed in HEK293T cells and protein levels tested by western blot (WB). Function was assessed by co-expression with the $\alpha_{2A}$ adrenergic receptor. Concentration response curves for inhibition of cAMP levels by the $\alpha_2$ receptor agonist UK14,304 were determined. *Gnao1*$^{G184S/+}$ (GOF) and *Gnao1*$^{-/+}$ (hetKO) animals of both sexes (age - 8 to 14 weeks) were analyzed by slice electrophysiology for IPSCs and EPSCs in hippocampus and cortex and gait was assessed using a DigiGait Imaging System.
**Results:** In contrast to previous reports, we observed both GOF (3 mutants) and LOF (9 mutants) for cAMP regulation by pathogenic human *GNAO1* mutations. Three distinct mutant alleles in Arg$^{209}$ had relatively normal function (NF) despite obvious pathogenicity. All LOF mutants had low $G\alpha_o$ protein expression as a potential contributor to their reduced function. Intriguingly, *GNAO1* GOF mutations are associated with movement disorder with or without mild seizures while the LOF mutations are associated with severe epilepsy. The *Gnao1*$^{G184S/+}$ GOF mutant mouse followed a similar pattern. We previously reported that it had a mild seizure phenotype (sensitization to pentylenetetrazole kindling but few spontaneous seizures). Hippocampal and cortical slices from these GOF mutant mice showed reduced spontaneous IPSCs but normal EPSCs. Digigait® analysis showed a striking reduction in stride length and increased paw angle variability in GOF mutant mice but not in *Gnao1* hetKO mice.
**Conclusions:** *De novo GNAO1* mutations have both GOF and LOF biochemical function with the former associated with seizures and the latter with movement disorder. This is phenocopied by a GOF mutant mouse. Further studies of human *GNAO1* mutant alleles in mice are warranted.

**Disclosures: H. Feng:** None. **B. Sjögren:** None. **Y. Yuan:** None. **R.R. Neubig:** None.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.12/C63

**Topic:** B.11. Epilepsy

**Support:** NIH R01 HD082373

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NIH R01NS065371

NIH R24NS092989

NIH R37 NS031348

U54 OD020351

**Title:** Mouse model of childhood epilepsy caused by a de novo GRIN2A missense mutation

**Authors: *A. AMADOR**[1], C. BOSTICK[2], J. PETERS[3], H. OLSON[3], A. PODURI[4], H. YUAN[5], W. CHEN[5], S. J. MYERS[7], S. F. TRAYNELIS[6], M. BOLAND[2], D. GOLDSTEIN[2], W. N. FRANKEL[8]
[1]Columbia Univ., New York, NY; [2]Columbia Univ., New York City, NY; [3]Harvard Med. Sch. & Epilepsy Genet. Program, Boston, MA; [4]Epilepsy & Clin. Neurophysiol., Boston Children's Hosp., Boston, MA; [6]Dept Pharmacol, [5]Emory Univ. Sch. of Med., Atlanta, GA; [7]Collabtech, Atlanta, GA; [8]Columbia Univ. Med. Ctr., New York, NY

**Abstract:** Mutations in the gene encoding N-Methyl-D-aspartate receptors (NMDAR) subunit *GRIN2A*/GluN2A have been identified in individuals with various neurological disorders, such as epilepsy, intellectual disability, autism spectrum disorder, and schizophrenia. Indeed, pathogenic *GRIN2A* variants are known to present with different clinical phenotypes, which makes precision medicine a necessity and a goal to improve outcome. In order to study potential therapeutic solutions, we modeled the *de novo* mutation GRIN2A S644G, based on a child with epileptic encephalopathy, including seizures refractory to conventional anti-epileptic medications. *In vitro* heterologous expression showed that the S644G mutation increases glutamate and glycine potency by 10-fold, and strongly enhances channel open probability by 5-fold, indicating a strong gain-of-function effect. S644G knockin mice generated by CRISPR/Cas9 and examined for seizure susceptibility *in vivo* and for abnormal neuronal network features in primary cortical neuron cultures using the microelectrode array (MEA) platform. While video-EEG of heterozygotes did not reveal obvious spontaneous seizures (5 mice recorded for 48 hours each), electroconvulsive threshold (ECT) testing showed significant and intriguing

differences. As expected, heterozygotes were more susceptible to minimal and maximal high frequency ECT, a test that models generalized seizures. Counterintuitively, however, heterozygotes also showed a significantly elevated threshold (i.e. seizure resistance) in the 6 Hz ECT test, designed to model partial or "psychomotor" seizures. In preliminary studies on the MEA platform, S644G heterozygous cortical neurons show increased firing and more network spikes in network bursts compared to wildtype neurons. Although homozygous S644G mice have not yet been examined, and while we expand the phenotyping to morphological analyses and synaptic studies, we are encouraged by these early findings and the prospects of combined *in vivo* and cellular mouse model platforms to reveal interesting and relevant preclinical features suitable for therapy screening.

**Disclosures:** **A. Amador:** None. **C. Bostick:** None. **J. Peters:** None. **H. Olson:** None. **A. Poduri:** None. **H. Yuan:** None. **W. Chen:** None. **S.J. Myers:** None. **S.F. Traynelis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp Inc. F. Consulting Fees (e.g., advisory boards); Janssen Pharmaceuticals, Inc., Boehringer Ingelheim, Pharma GmbH & Co. KG, Sage Therapeutics. **M. Boland:** None. **D. Goldstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pairnomix, Praxis. F. Consulting Fees (e.g., advisory boards); AstraZeneca. **W.N. Frankel:** None.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.13/D1

**Topic:** B.11. Epilepsy

**Support:** R37NS031348

**Title:** Modeling of an epilepsy-related neurodevelopmental disorder caused by de novo GNB1 missense mutations and identification of targeted treatments

**Authors: *S. COLOMBO**[1], M. J. BOLAND[2], W. N. FRANKEL[3], D. B. GOLDSTEIN[3]
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**Abstract:** Heterozygous *de novo* missense mutations in *GNB1*, a beta subunit of GTPases heterotrimeric G proteins (Gα,β,γ) were recently implicated as the cause of a new syndrome that includes neurodevelopmental disability, hypotonia and seizures (Petrovski S. et al., 2016, PMID 27108799). While G proteins are known to mediate G protein-coupled receptor signaling and regulate a variety of downstream effectors, such as $Ca^{2+}$ and $K^{+}$ channels, or PI3K and MAPK

pathways, it is unclear how mutations in ubiquitously expressed *GNB1*, result in a predominantly neurological disorder. In an effort to unravel the specific neuronal functions of *GNB1* affected by the mutations and to find appropriate treatments, we chose both *in vitro* and *in vivo* approaches. As the mutations tend to cluster in a region important for Gβ interaction with Gα and downstream effectors, we first introduced *GNB1* variants in HEK cells to evaluate interactions of mutant proteins with partners, and the activation of downstream pathways in response to GPCR activity, using techniques such as Bioluminescence Resonance Energy Transfer (BRET), co-immunoprecipitation (co-IP) and phosphoprotein assays. While BRET demonstrated that most variants affect interaction of Gβ with Gα, co-IP confirmed that the Gβ-K78R variant affects interaction with several Gα and also revealed a reduced interaction with a GIRK channel. In order to model the human disease, we used CRISPR/Cas9 to knock-in disease variants, beginning with K78R, in mice and in human induced pluripotent stem cells (iPSC) to be differentiated into neurons. Using microelectrode arrays (MEA) to detect extracellular field potentials (a surrogate for action potentials) of neuronal networks, we observed that heterozygous (het) K78R neurons present a bursting defect compared to wild-type. Moreover, K78R het pups present with developmental delay as shown by a significantly smaller size and delay in reaching developmental milestones compared to wild-type littermates. Preliminary tests reveal that K78R het mice have low seizure threshold. We are presently evaluating the presence of spontaneous seizures (video-EEG) as well as neuronal development, morphology and activity. The MEA platform will be used to perform an initial screen of FDA-approved drugs, and top candidates will be tested in mice to assess efficacy and toxicity. Our work will help to understand *GNB1* functions in neurodevelopment and identify appropriate therapies.

**Disclosures: S. Colombo:** None. **M.J. Boland:** None. **W.N. Frankel:** None. **D.B. Goldstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pairnomix, Praxis. F. Consulting Fees (e.g., advisory boards); AstraZeneca.

**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.14/D2

**Topic:** B.11. Epilepsy

**Support:** NIH grant U54-OD020351

R37 NS031348

**Title:** Characterization and therapeutic screening of a gain of function mutation in KCNT1 utilizing multielectrode arrays

**Authors: *C. D. BOSTICK**[1,2], S. COLOMBO[2], M. J. BOLAND[2,3], V. A. LETTS[2], W. N. FRANKEL[2], D. B. GOLDSTEIN[2]
[1]Dept. of Developmental Genet., [2]Inst. for Genomic Med., Columbia Univ. Med. Ctr., New York, NY; [3]Neurol., Columbia Univ., New York, NY

**Abstract:** *De novo* mutations in the C-terminal domain of the KCNT1 sodium activated potassium channel have been implicated in multiple early-onset epileptic encephalopathies including Malignant Migrating Partial Seizures of Infancy (MMPSI) and Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE). KCNT1 channels are known to contribute to a late afterhyperpolarization after repetitive stimulation and regulation of neuronal excitability and adaptation. While studies utilizing heterologous expression systems have demonstrated a gain of function underlies the mechanism of these *de novo* mutations, there still remain many questions about the resulting hyperexcitability pathology seen *in vivo*. To this end we have characterized mouse models including a knock-in (ki) gain of function *Kcnt1* mutation (Y777H) generated by CRISPR/Cas9 in the JAX Center for Precision Genetics based on an individual with moderate ADNFLE. Homozygous ki mice exhibit spontaneous generalized tonic-clonic seizures in video-EEG, as well as a modestly lower seizure threshold. In addition, *in vitro* studies have been conducted on mouse primary cortical neurons utilizing Multielectrode Arrays (MEA). MEA allows noninvasive measurement of neuronal activity, and has recently been adopted to study aberrant neuronal network activity associated with genetic variants. The multi-well format yields the ability for medium throughput screening for therapeutics in the treatment of various neuronal pathologies. Studies on the spontaneous activity of homozygous ki primary cortical neurons demonstrate increased firing and bursting during neuronal network maturation in homozygous neurons as well as increased neuronal activity during burst and network events post network maturation. In addition, preliminary work has demonstrated that Y777H homozygotes have greater evoked responses to electrical stimulation compared to wildtype control. Utilizing this platform, we will screen candidate therapies from an FDA approved library searching for effective targeted treatments to further assess *in vivo*.

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**Poster**

**560. Epilepsy: Genetics - Phenotype Modelling of Human Mutations**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 560.15/D3

**Topic:** B.11. Epilepsy

**Support:** Burroughs Wellcome Fund Career Award for Medical Scientists

NIH NINDS K08

**Title:** Developmental trajectory of interneuron dysfunction in a mouse model of Dravet syndrome

**Authors: *M. FAVERO**[1], E. LOPEZ[2], N. P. SOTUYO[1,3], E. M. GOLDBERG[1,3,4]
[1]Pediatrics, The Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Sch. of Arts and Sci., [3]Neurosci., [4]Neurol., Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Dravet syndrome (DS) is a neurodevelopmental disorder characterized by childhood onset, treatment-resistant, temperature-sensitive epilepsy, accompanied by intellectual disability and autistic traits, hyperactivity, and ataxia, with markedly increased mortality from status epilepticus and sudden unexplained death (SUDEP). DS is due to a dominant *de novo* heterozygous mutation in the gene *SCN1A* encoding the type 1 neuronal voltage-gated sodium (Na+) channel alpha subunit ($Na_V1.1$). Electrophysiological data indicates that $Na_V1.1$ mutations underlying DS lead to loss of channel function. The effect of $Na_V1.1$ mutation has also been studied in various transgenic mouse models of DS (Scn1a+/- mice), and the phenotype largely replicates the human condition. *In vitro* evidence suggests that preferential dependence upon this channel by inhibitory GABAergic interneurons (INs) leads to reduced IN excitability, particularly in parvalbumin (PV) positive (PV+) INs, while it does not appreciably influence the activity of excitatory pyramidal neurons. Thus, the net effect of *Scn1a* mutation on brain networks overall is considered to be disinhibition due to dysfunction of INs relative to excitatory cells. Here, we performed targeted electrophysiological recordings of PV+ and non-PV+ INs as well as excitatory principal cells in acute brain slices of primary somatosensory cortex prepared from $Scn1a^{+/-}$ mice and age-matched wild-type (WT) littermate controls at various ages to determine the developmental trajectory of cell type-specific dysfunction; $Scn1a^{+/-}$ mice were crossed to IN-specific Cre driver lines to label discrete IN subtypes. At post-natal day (P) 18-21 (within days of epilepsy onset) PV+ INs and, to a lesser extent, SST+ INs, in $Scn1a^{+/-}$ mice, exhibit profound impairments relative to WT, with failure of action potential generation and markedly decreased maximal firing frequency. However, at P35-49 (established epilepsy), these defects had partially, albeit incompletely, recovered, despite ongoing seizures in $Scn1a^{+/-}$ mice at this age. Our results indicate that the prominent effect of *Scn1a* mutation on the firing properties of INs may be only transient, restricted to a delimited time window during development. The mechanism of partial normalization during the established phase of epilepsy may be due to compensatory up-regulation of other Na+ channel subtype(s). Such data suggests that other cellular- and circuit-level mechanisms may contribute to the maintenance of chronic epilepsy and epilepsy-associated developmental disability in Dravet syndrome.

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**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.01/D4

**Topic:** B.11. Epilepsy

**Support:** National Research Foundation of Korea (NRF) grant NRF-2017R1A2B4002704

**Title:** Heterogeneity of gene associated with retinoid-interferon-induced mortality-19 (GRIM-19) expression in the adult mouse brain

**Authors: S.-N. HWANG**[1], J.-C. KIM[2], *S. KIM[3,2]
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**Abstract:** Gene associated with retinoid-interferon-induced mortality-19 (GRIM-19) has been identified as a gene inducing apoptosis of various cancer cells such as thyroid, renal and liver cancer cells and as a subunit of the mitochondrial respiratory chain complex I that has a significant effect on ATP production. The brain, unlike the heart or the kidney, is particularly susceptible to ATP deficiency due to its limited energy storage capability and its high rate of oxygen consumption. The possibility is raised that GRIM-19 plays important roles in regulating ATP level and/or cell death caused by neurological disorders. Although there has been a few efforts to examine the expression changes of GRIM-19 under several pathological conditions such as brain ischemia and Parkinson's disease the thorough investigation of the neuroanatomic distribution of GRIM-19 in the normal brain is still needed to understand the physiological and pathophysiological roles of GRIM-19. Thus, the present study investigated the distribution patterns of GRIM-19 in the adult C57BL/6 mouse brain using immunohistochemistry and analyzed the comparison of GRIM-19 immunoreactivity with the density of neuronal cells which was reported in Allen Brain Atlas (www.mouse.brain-map.org). GRIM-19 was ubiquitously expressed throughout the whole brain as our expectation since GRIM-19 is the composition of the mitochondria. In most regions of the brain, the stronger the GRIM-19 immunoreactivity, the denser the NeuN-positive cells and Nissl substance were shown. However, there were a few exceptions. The regions showing the intensive immunoreactivity of GRIM-19 but weak NeuN immunoreactivity as well as weak Nissl staining included the outer plexiform layer of the main olfactory bulb, the molecular layer of the piriform cortex, the nucleus of the lateral lemniscus, the molecular layer of the cerebellum, etc. On the other hand, the granule layer of the main olfactory bulb showed the opposite pattern. Interestingly, the immunoreactivity of GRIM-19 was relatively prominent in the hippocampal CA3 pyramidal layer compared with that of the

hippocampal CA1 pyramidal layer and the dentate granule cell layer. This is the first report showing immunohistochemical distribution of GRIM-19 throughout the whole adult mouse brain and we expect that the findings obtained in this study can provide a clue to study the pathophysiological mechanisms responsible for various mitochondrial dysfunction-related neurological disorders, including brain ischemia.

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

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.02/D5

**Topic:** B.11. Epilepsy

**Support:** Swedish Research Council

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Swedish Fysiographen Society

European Union's Seventh Framework Programme (FP7/2007 2013) (602102, EPITARGET)

**Title:** Cx3cr1 inhibition modulates synaptic integration of adult born hippocampal neurons following an epileptogenic insult in the rat brain

**Authors: *U. AVDIC**[1], I. ALI[2], C. EKDAHL[2]
[2]Clin. Sci., [1]Lund Univ., Lund, Sweden

**Abstract:** The fractalkine-CX3CR1 pathway has been shown to regulate neurogenesis in the dentate gyrus (DG). Neuronal proliferation in the hippocampus is frequently described in temporal lobe epilepsy and microglia activation is associated with an impaired survival of new adult born neurons. If a reduced inflammatory environment, by CX3CR1 inhibition will alter the synaptic integration of these neurons remains to be studied. These mechanisms might be central in driving network hyperexcitability after epileptogenic injuries and may be targeted for therapeutic intervention. Thus, we studied the expression of synaptic proteins in neurons born in an acute epileptic environment with an altered neuroinflammatory milieu. Adult male rats were implanted with electrodes in the right hippocampus and a brain infusion cannula in the lateral ventricles. Following 1w of recovery temporal status epilepticus (SE) was electrically induced in

the hippocampus. Rats were subsequently injected with retroviral vector expressing GFP. Immediately after, brain infusion cannulas were connected with osmotic pumps carrying either anti-CX3CR1 antibody (ab) or vehicle. Morphological evaluations, 7w following SE, revealed subtle changes in dendritic morphology with reduced medial dendrites in SE-CX3CR1 rats. Seizure-induced microglia activation was also reduced in the hilus in SE-CX3CR1 animals. No changes in the expression of scaffolding proteins (gephyrin) and adhesion molecules (NL-2) were detected at inhibitory synapses. Instead, there was a strong reduction of scaffolding proteins at excitatory synapses in treated rats. PSD-95 cluster density was decreased in the molecular layer of the DG. No changes were observed in the excitatory adhesion molecule NL-1. Here we show that the fractalkine-CX3CR1 pathway mediates neuroinflammation on synaptic integration of new neurons born in an epileptic environment, confirmed by altered morphological profiles and altered expression of excitatory synaptic scaffolding proteins. Future studies need to determine whether an altered neuroinflammatory environment during chronic epilepsy leads to a changes in chronic seizure burden in these animals.

**Disclosures:** **U. Avdic:** None. **I. Ali:** None. **C. Ekdahl:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.03/D6

**Topic:** B.11. Epilepsy

**Support:** NIH Grant MH015442

**Title:** Transcriptome analysis of hippocampal CA1 in rat model of early life seizures

**Authors: *H. O'LEARY**, A. M. CASTANO, L. VANDERLINDEN, L. M. SABA, T. A. BENKE
Univ. of Colorado, Denver, Aurora, CO

**Abstract:** RNA sequencing was used to investigate gene expression in a rat model of early life seizures. We aimed to characterize the functional pathways that are up and down regulated following an early life seizure. Rat hippocampal CA1 mRNA was isolated and profiled one week following a single early life seizure, corresponding to a critical developmental period in which AMPA receptor desensitization is decreased. Isoform level results revealed significant differences in the expression of only 7 genes after adjustments for all genes analyzed. Ingenuity pathway analysis revealed an upregulation of the Long term potentiation pathway, thus confirming previous findings at this developmental time point following seizure. Additionally, editing and splice variants were analyzed with a particular interest in ADAR2 editing sites in the

brain. No differences were observed in the editing of GluA subunits, suggesting another mechanism for alteration in AMPA receptor desensitization in this time period.

**Disclosures: H. O'Leary:** None. **A.M. Castano:** None. **L. Vanderlinden:** None. **L.M. Saba:** None. **T.A. Benke:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.04/D7

**Topic:** B.11. Epilepsy

**Support:** NIH NS050229 (Poolos)

**Title:** Time course and isoform specificity of JNK activation during epileptogenesis

**Authors: A. N. PARIKH**, F. A. CONCEPCION, R. D. BOEHM, *N. P. POOLOS
Univ. of Washington, Seattle, WA

**Abstract:** c-Jun N-terminal kinases (JNK1-3) are members of the stress-activated kinase family that are encoded by three genes, *Mapk8-10*. Abnormal JNK activation has been implicated in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. We recently discovered that JNK is hyperactivated in an animal model of chronic epilepsy, and that pharmacological inhibition of JNK exerts an antiepileptic effect (Tai TY et al, *Neuroscience*, 2017). Currently, we do not know the role JNK plays during the development of chronic epilepsy; thus, this project aims to understand the timeline of JNK activation. We measured both activated (phosphorylated; pJNK) and total JNK (tJNK) levels by immunoblotting hippocampal tissue homogenates both from chronically epileptic rats induced via status epilepticus (SE) and from age-matched controls. We measured JNK levels at four time points after SE onset: one hr, when animals are in SE; one day, when animals are seizure-free; one wk, when animals begin to have spontaneous seizures; and 6 wks, when animals are chronically epileptic. Collectively, the JNK isoforms separate into two electrophoretic bands: 54 kDa and 46 kDa. In both bands, we discovered significant increases in pJNK levels at the chronic epilepsy stage (54 kDa: 160 ± 26% of naive levels, $p<0.05$; 46 kDa: 125 ± 17%, $p<0.05$), while there were no significant changes in pJNK levels at the other three earlier time points. We saw no significant changes in the levels of total JNK for either band at all time points. We also measured changes in the fractional activation of JNK by quantifying pJNK/tJNK. In the 54 kDa band, we discovered significant increases in pJNK/tJNK at one hr and one day, no change at one wk, and a significant increase in chronic epilepsy. In the 46 kDa band, there was no change in pJNK/tJNK at one hr, but significant increases were observed at all later timepoints. We then asked how JNK isoforms segregated into the 54 and 46 kDa bands. We determined that JNK1 segregates primarily in the

46 kDa band, JNK2 is present nearly equally in both bands, and JNK3 (which is brain-specific) is predominately in the 54 kDa band. We conclude that in chronic epilepsy there are substantial increases in activated JNK present in the 54 kDa (containing JNK3 and JNK2) and 46 kDa (containing JNK1 and JNK2) bands, consistent with our previous studies. The increase in fractional activation of JNK (pJNK/tJNK) during epileptogenesis suggests upregulation of upstream signaling mechanisms. Further experiments are underway to quantify the activation of JNK isoforms in chronic epilepsy and their alterations during the development of chronic epilepsy.

**Disclosures: A.N. Parikh:** None. **F.A. Concepcion:** None. **R.D. Boehm:** None. **N.P. Poolos:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.05/D8

**Topic:** B.11. Epilepsy

**Support:** CONACyT 239594

**Title:** Pattern of expression of proinflammatory cytokines on a model of acute seizures under time-restricted feeding

**Authors: *J. SANTILLAN-CIGALES**, J. LANDGRAVE-GÓMEZ, V. S. ARRIAGA-ÁVILA, O. F. MERCADO-GOMEZ, S. GONZALEZ-REYES, R. GUEVARA-GUZMAN
Physiol., Univ. Nacional Autonoma De Mexico, FM, Ciudad De Mexico, Mexico

**Abstract:** Introduction: In recent years, experimental research has demonstrated an important role of pro-inflammatory molecules to develop epileptogenic and ictogenic processes. Recently, our research group reported that time-restricted feeding (TRF) has an anticonvulsant effect; however, the precise mechanism that could be involved is still unknown. Objective: To investigate whether TRF is able to exert its beneficial effect by modifying the expression pattern of pro-inflammatory molecules and therefore, has a neuroprotective role after induction of seizures. Methodology: Briefly, TRF consisted of allowing rats to feed for two hours daily during their luminous phase for 20 days; whereas the control group (AL) had free access to food and water 24 hours a day. After the diet period, status epilepticus (SE) was induced by pre-treatment with lithium chloride (LiCl 3 mEq/kg i.p.). 18 h after LiCl, animals were injected with scopolamine (1 mg/Kg s.c.) and 30 min later. animals received a pilocarpine injection (60 mg/kg s.c.) to induce SE for 90 minutes immediately attenuated with diazepam injection (5 mg/Kg). The mRNA expression of pro-inflammatory molecules such as interleukin 1 beta (IL-β), tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6) in hippocampus of each group were

quantified at 3 h, 12 h and 24 h after SE. In addition, coronal sections of brains were obtained to label Iba-1 and TUNEL to evidence changes of activated microglia and apoptosis, respectively. Results: Our preliminary data show that the group with TRF had a decrease of mRNA levels of pro-inflammatory molecules (IL- β, TNF- α, IL-6) compared to that of AL group, after seizure induction. Likewise, changes in the levels of activated microglia were observed in the hippocampus as well as fewer TUNEL-positive cells after SE. Conclusion: Our data demonstrated that TRF exerts a neuroprotective effect by modifying the mRNA expression pattern of pro-inflammatory molecules after induction of seizures.

**Disclosures: J. Santillan-Cigales:** None. **J. Landgrave-Gómez:** None. **V.S. Arriaga-Ávila:** None. **O.F. Mercado-Gomez:** None. **S. Gonzalez-Reyes:** None. **R. Guevara-Guzman:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.06/D9

**Topic:** B.11. Epilepsy

**Support:** CONACyT 239594

**Title:** Time-restricted feeding confers an anti-oxidative effect in rat hippocampus on pilocarpine seizure model

**Authors: *O. F. MERCADO-GOMEZ**[1], V. S. ARRIAGA-ÁVILA[2], J. J. SANTILLAN-CIGALES[2], M. ÁLVAREZ-HERRERA[2], R. GUEVARA-GUZMAN[2]
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**Abstract: Introduction:** Several reports on experimental models and epilepsy patients have demonstrated that oxidative stress has a crucial role in epilepsy because it contributes to the etiophatogenesis of seizures. On the other hand, metabolic-based diets such as ketogenic diet (KD) and calorie restriction (CR) have shown to have an anti-oxidative effect on several models of neurological disease. We recently described that time-restricted feeding (TRF) has an anticonvulsant effect that is mediated by metabolic and epigenetic changes. **Objective:** To investigate whether TRF could have an anti-oxidative effect in *status epilepticus* (SE)-induced hippocampus. **Methodology:** Male Wistar rats weighing 220 g were divided into four groups (Control, TRF, SE, and TRF plus SE). Control animals had free access to food and water. TRF schedule consisted of allowing rats to feed only for two hours daily for 21 days with free access to water. The pilocarpine seizure model consisted of a pre-treatment of lithium chloride (3mEq/kg i.p.). Eighteen hours after LiCl injection, animals were injected with scopolamine (1 mg/kg s.c.) and 30 min later, animals received a pilocarpine injection (60 mg/kg s.c.) to induce SE for 90 minutes and immediately attenuated with diazepam injection (5 mg/kg). Control

animals followed the same procedure without pilocarpine injection. All animals were sacrificed during an acute time-course (3, 8 and 24 h) and their hippocampi were used to measure malondialdehyde (MDA) levels. Moreover, frozen brain slices were obtained to measure superoxide ion in CA1, CA3 and dentate gyrus of hippocampus by fluorescent microscopy using dihydroethidium (DHE) on rats 24 h after pilocarpine injection. **Results:** Preliminary results showed that pilocarpine-injected rats had significant high levels of MDA compared with those of control animals or TRF-treated animals throughout the time-course. Interestingly, rats subjected to TRF and SE-induced showed a slight decrease of MDA levels during the time points of 3 and 8 hours. However, after 24 h post-SE, MDA levels were statistically reduced compared with those of SE-induced rats. Furthermore, animals subjected to TRF and SE had a reduction of fluorescence of DHE in all regions of hippocampus compared with that of pilocarpine-injected rats. **Conclusion:** Our results suggest that TRF has an anti-oxidative effect on pilocarpine seizure model by reducing MDA levels and superoxide ion presence in the hippocampus. Even though we do not know the precise mechanisms which TRF exert this effect, it is important to note that TRF has similar effects that other diets such as KD o CR whereby could be used as a new alternative therapy.

**Disclosures: O.F. Mercado-Gomez:** None. **V.S. Arriaga-Ávila:** None. **J.J. Santillan-Cigales:** None. **M. Álvarez-Herrera:** None. **R. Guevara-Guzman:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.07/D10

**Topic:** B.11. Epilepsy

**Title:** Early life seizures chronically alter the requirement of L-type voltage gated calcium channels to induce mGluR dependent long term depression

**Authors: *P. B. BERNARD**, A. M. CASTANO, T. A. BENKE
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**Abstract:** We have reported that adult rats have chronically enhanced mGluR-mediated long-term depression (mLTD) following a single episode of early life seizures (ELS). Herein we explored the role of calcium influx and release in the expression of mLTD and specifically how ELS impacts the role of calcium signaling in mLTD. We hypothesized that calcium flux through L-type voltage gated calcium channels (LTCCs) is necessary for the induction of mLTD and that these and related processes are chronically altered following ELS.
ELS were induced in Sprague Dawley rats at P7 with kainate (2 mg/kg, s.c.). Hippocampal slices were prepared for electrophysiological recording at P60+. Induction paradigms designed to isolate mLTD were utilized in area CA1 of the hippocampus and LTD was measured using field

excitatory postsynaptic potentials. Isradipine (10 μM), BayK (1 μM), PKI (300 nM) and cyclopiazonic acid (CPA)(30 μM) were used to probe the dependence on mLTD on calcium-related signaling.
Blocking voltage gated calcium channels with isradipine completely blocked mLTD in controls and normalized enhanced mLTD following ELS. BayK, an agonist of voltage gated calcium channels, increased mLTD in controls only; no further increase in mLTD was observed following ELS. CPA, which inhibits the release of calcium from intracellular stores, normalized mLTD following ELS and had no effect on mLTD in controls. In contrast, PKI, which regulates the activity of voltage gated calcium channels, normalized mLTD following ELS, while also reducing mLTD in controls.
Our results indicate that calcium flux through LTCCs is necessary for mLTD under normal conditions. ELS results in chronic alterations in the role of LTCCs in mLTD and related signaling, including release from intracellular stores. Following ELS, chronically enhanced mLTD can be rescued using pharmacological methods to limit intracellular calcium, either via reducing LTCC activity or reducing the availability of intracellular stores. We have previously linked enhanced mLTD with several behavioral deficits later in life, including deficits in social behavior, learning and memory. Future investigations will determine if normalizing calcium signaling in vivo also normalizes behavior.

**Disclosures: P.B. Bernard:** None. **A.M. Castano:** None. **T.A. Benke:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** B.11. Epilepsy

**Support:** NIH-IRACDA NYCAPS K12-GM-102778

NIH T32GM007518

NSF GRFP

**Title:** Cannabidiol as a potential effector of epileptogenesis through microglial modulation

**Authors: *T. R. VICTOR**[1], J. C. NISSEN[1], M. W. ELMES[2], D. G. DEUTSCH[2], S. E. TSIRKA[1]
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**Abstract:** Epilepsy is a chronic disorder characterized by abnormal brain cell activity leading to recurrent, unprovoked seizures. Pharmacological therapies are commonly used for the treatment

of epilepsy, although nearly 30% of patients do not respond to current medications. This highlights the need for new drug targets and treatments. Many recent studies have centered on the use of cannabidiol (CBD) to treat epilepsy. Early use of CBD, a non-psychotropic component of *Cannabis sativa*, is reported to lessen the severity of experimentally induced seizures in animals and to suppress neuroinflammation in culture. Although CBD has been shown to increase intracellular levels of anandamide, an endogenous cannabinoid, its complete mechanism of action is still poorly understood. Our previous studies have shown that microglia, the immune cells of the central nervous system (CNS), are important mediators of seizure severity. Microglial ability to modulate seizures has been linked to the activation of Toll-like receptors (TLRs). TLRs play important roles in pathogen recognition and inflammation. Microglia also act as modulators of neurogenesis and synaptogenesis, contributing to the refinement of functional neuronal circuits. We postulate that CBD modulates the activation of microglial cells to exert beneficial effects in the brain. In this study, we evaluate the effects of CBD on cell death and inflammation in the hippocampus during recovery. Microglial engulfment of newborn cells and the effect of CBD on TLR expression was also studied.

**Disclosures:** **T.R. Victor:** None. **J.C. Nissen:** None. **M.W. Elmes:** None. **D.G. Deutsch:** None. **S.E. Tsirka:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.09/D12

**Topic:** B.11. Epilepsy

**Support:** National Research Foundation of Korea (NRF) grant NFR-2017R1A2B4002704

**Title:** Changes in the expression of gene associated with retinoid-interferon induced mortality-19 (GRIM-19) after pilocarpine-induced status epilepticus in the mouse hippocampus

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**Abstract:** Gene associated with retinoid-interferon induced mortality-19 (GRIM-19) is a multifunctional gene involved in cell death, mitochondrial metabolism and growth regulation. Recently, it has been also reported that GRIM-19 is closely related to inflammation by regulating the expression of pro-inflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor-α and IL-17. Meanwhile, the common pathological features of status epilepticus (SE) include hippocampal neuronal cell death and glial cell activation of which the mechanisms are related to the action of pro-inflammatory cytokines. However, to date, it is not reported about the role of

GRIM-19 in the pathogenesis of SE. Thus, the present study investigated the expression pattern of GRIM-19 and identified cell types expressing GRIM-19 in the mice hippocampus after pilocarpine-induced SE. Male C57BL/6 mice (22-26 g) were treated with pilocarpine (280 mg/kg, intraperitoneally) and monitored to evaluate seizure stage based on Racine scale. After 2 hours of SE, which was defined as a continuous motor seizure of stage 5 (rearing and falling), diazepam was administered to terminate seizure. The mice which received saline instead of pilocarpine served as control. Pilocarpine- or saline-injected animals were perfused at 1, 4, 7 and 14 days after SE onset. To identify the cell types expressing GRIM-19, double immunofluorescence staining using NeuN (a neuronal cell marker), GFAP (an astrocyte marker) and Iba-1 (a microglia marker) antibody was performed. In saline-treated control animals, GRIM-19-positive cells were mainly observed in both pyramidal neurons and dentate granule cells. Four days after SE, neuronal cell death was observed in certain hippocampal areas including CA1 and CA3 regions whereas astrocytes and microglial cells were activated in the entire hippocampus. At that time, compared with control animals, GRIM-19 immunoreactivity was markedly reduced in pyramidal cell layer where neuronal cell death occurs. Furthermore, GRIM-19-expressing cells induced by SE were turned out to be mainly reactive astrocytes in the stratum radiatum of the hippocampus. These results suggest that GRIM-19 may play a role, at least in part, in the reactive astrocytosis following SE, probably through the action of pro-inflammatory cytokines.

**Disclosures:** **J. Kim:** None. **S. Hwang:** None. **I. Kim:** None. **S. Kim:** None.

**Poster**

**561. Epilepsy: Post-Seizure Modifications - Novel Genes and Inflammation**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 561.10/D13

**Topic:** B.11. Epilepsy

**Support:** CONACyT (FCB 576165)

**Title:** Status epilepticus during infancy impairs male sexual behavior during adulthood

**Authors: *F. CHENA BECERRA**[1], V. X. DIAZ-ESTRADA[2], E. VELAZCO-CERCAS[1], I. ZAMORA-BELLO[1], L. BELTRAN-PARRAZAL[3], J. MANZO[3], G. A. CORIA-AVILA[3], L. LOPEZ-MERAZ[3]
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**Abstract:** Epileptic patients suffer from several disorders associated to this illness, including sexual disorders; though it has been difficult to distinguish how epilepsy affects directly on them

rather than the well-known side-effects of antiepileptic drugs. Recent studies in adult rats have demonstrated that *status epilepticus* (SE) affects sexual performance; nevertheless, SE is more common in childhood. To that end, in this study we assessed the effect of SE in infant male rats on their sexual behavior during adulthood. To do this, thirteen-days-old male rat pups (P13) were given an intraperitoneal injection of lithium chloride (3mEq/kg) and SE was induced 20 h later at P14 by a subcutaneous injection of pilocarpine hydrochloride (100mg/kg); control rats were injected with an equal volume of saline (NaCl 0.9%). Rats were weaned at P21 and at P90 sexual behavior was evaluated during five trials (every four days) lasting one hour. After de fifth trial, serum corticosterone levels were measured using an ELISA kit. Data were analyzed by a Fisher test, a two-way ANOVA for repeated measures, or a Student t test. The results show that in the SE group a lower proportion of males ejaculated in comparison with the control group (2/7 and 6/8, respectively; $p< 0.0001$), effect that was observed in session 3 (1/9 and 6/8, respectively; $p= 0.0134$), 4 (1/9 and 6/8, respectively; $p= 0.0134$) and 5 (2/9 and 6/8 $p= 0.0445$). Frequency of mounts ($p= 0.0009$) and intromissions ($p= 0.0077$) was lower in SE rats than in control rats. Latency to mount attempts ($p= 0.016$), mounts ($p= 0.001$), intromissions ($p= 0.008$) and ejaculations ($p= 0.007$) was increased in SE rats when compared with control rats. Serum corticosterone levels show no difference between SE and control groups ($p= 0.46$). In conclusion, one event of SE in infancy disrupts sexual performance during adulthood in male rats without altering the baseline levels of corticosterone.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 562.01/D14

**Topic:** B.11. Epilepsy

**Title:** Antiseizure actions of a ketone body and its analog in a chronic seizure model

**Authors: *N. SADA**[1,2], A. KADOWAKI[1], T. INOUE[1]
[1]Okayama Univ., Okayama, Japan; [2]Kawasaki Med. Sch., Kurashiki, Japan

**Abstract:** A ketogenic diet has been used to treat a drug-resistant epilepsy. The diet treatment increases a ketone bodies (acetoacetate and β-hydroxybutyrate) in the brain. In addition, ketone bodies have inhibitory actions on the central nervous system, but its molecular mechanisms are not understood. Here, we studied effects of acetoacetate on voltage-dependent Ca2+ channels (VDCCs) and excitatory postsynaptic currents (EPSCs) in hippocampal CA1 pyramidal neurons using patch-clamp recordings. We found that acetoacetate inhibited VDCCs and reduced EPSCs

in hippocampal pyramidal cells. More potent EPSC inhibitors were then explored by modifying the chemical structure of acetoacetate, and 2-phenylbutyrate was identified as an acetoacetate analog that inhibited VDCCs and EPSCs more potently. We further found that 2-phenylbutyrate markedly suppressed hippocampal seizures in vivo and its antiseizure effect was stronger than acetoacetate using a chronic seizure model induced by intrahippocampal injection of kainate. In addition, intraperitoneally-injected 2-phenylbutyrate was delivered to the brain and suppressed seizures in vivo, and its brain concentration reached the level enough to reduce EPSCs in brain slices. In conclusion, 2-phenylbutyrate is an acetoacetate analog that inhibits VDCCs and EPSCs in pyramidal cells, suppresses hippocampal seizures in vivo, and has a brain penetration ability. Thus, 2-phenylbutyrate provides a useful chemical structure as a seed compound to develop new antiepileptic drugs originating from ketone bodies.

**Disclosures:** **N. Sada:** None. **A. Kadowaki:** None. **T. Inoue:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 562.02/D15

**Topic:** B.11. Epilepsy

**Title:** Dynamic modulation of intrinsic excitability and sensory inputs processing in cortical neurons during absence seizures

**Authors: *M. WILLIAMS**[1,2], S. LECAS[1,2], S. MAHON[1,2], S. CHARPIER[1,2]
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**Abstract:** Epileptic seizures result from aberrant cellular and/or synaptic properties that may alter the capacity of neurons to process ongoing information. For instance, during absence seizures spike-and-wave discharges (SWDs) likely interfere with incoming sensory inputs, participating in the looseness of conscious experience. However, the mechanisms by which SWDs alter conscious sensory perception remain unclear. Using the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), a validated animal model of absence epilepsy, we identified the cellular correlates subtending the negative interactions between epileptic discharges and sensory processing. By combining *in vivo* electrocorticographic and intracellular recordings from the somatosensory cortex of GAERS, we found that the intrinsic excitability of cortical neurons was dynamically altered during seizures, alternating periods of increased and decreased cell responsiveness. To investigate how this time-dependent change in cortical excitability affected sensory processing during absence seizures, we examined the whisker-evoked sensory responses in the related thalamo-cortical system in the course of SWDs. Consistent with the fluctuating neuronal excitability, sensory stimuli were still processed in primary sensory cortices and

thalamic nuclei during SWDs but with a severe time-to-time instability. This lack of consistent sensory responses in the somatosensory thalamo-cortical network could, at least partly, explain the interruption of conscious perception during absence seizures.

**Disclosures: M. Williams:** None. **S. Lecas:** None. **S. Mahon:** None. **S. Charpier:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 562.03/D16

**Topic:** B.11. Epilepsy

**Title:** Autonomic dysfunction and increased arrhythmogenic potential in mice following status epilepticus

**Authors: *A. LEVINE**[1], A. DAO[2], A. E. ANDERSON[3]
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**Abstract:** Status epilepticus (SE) is a prevalent disorder, which is associated with significant morbidity, including the development of epilepsy and mortality. Studies indicate that lethal cardiac arrhythmias contribute to death following SE as well as sudden unexpected death in epilepsy (SUDEP). A range of potentially lethal cardiac arrhythmias (i.e. tachycardia, bradycardia, and asystole) are observed in epilepsy and are indicative of underlying autonomic nervous system (ANS) dysfunction. Tachycardia is the most commonly reported seizure-related to arrhythmia but asystole and bradycardia have been observed and may occur ictally in people with temporal lobe epilepsy (TLE). Studies have described ANS imbalance during ictal and postictal periods but less is understood about ANS function during interictal periods. We sought to understand ANS changes following SE by monitoring cardiac electrical activity in a chemoconvulsant mouse model of TLE. To simultaneously investigate alterations in electrocardiography (EKG) and video synchronized electroencephalographic (vEEG) signals following SE. Recordings showed ictal bradycardia and post-ictal tachycardia, which had been described in other mouse models of SE, as well as humans. Interictally, no changes were seen in heart rate, RR interval, QTc interval, and PR interval between saline and kainate treated animals. However, SE animals exhibited decreased interictal beat-to-beat variability of the QTc and decreased PR intervals for the two weeks of monitoring after SE or until a death event. Sinus pause with a junctional escape beat, premature ventricular contractions, and accelerated ventricular rhythm were observed interictally following SE during sleep. Additionally, death events were captured and showed seizure-related increases in beat-to-beat variability in the RR, QTc, and PR intervals preceding death. Our mouse model recapitulates changes that are observed in human TLE. Although average values for heart rate, R-R interval, QTc interval, and

PR interval showed no difference interictally between saline and kainate treated animals, the potentially lethal arrhythmias observed and beat-to-beat variations indicate ANS dysfunction. Interestingly, the arrhythmias were observed commonly during sleep and more than half of all cases of SUDEP are reported to occur during sleep. Further research into the mechanisms of ANS dysfunction following SE may be fruitful in providing greater understanding as well as treatments to prevent future cases of SUDEP.

**Disclosures:** **A. Levine:** None. **A. Dao:** None. **A.E. Anderson:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** B.11. Epilepsy

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Institute fund from Huntington Medical Research Institutes

**Title:** Faster flux of neurotransmitter glutamate during seizure --- *In vivo* evidence from $^{13}C$-enrichment of extracellular glutamate in the hippocampus of the kainate rat model of temporal lobe epilepsy

**Authors: *K. KANAMORI**[1,2]
[1]Neurosci. Res., Lab. Launch, Monrovia, CA; [2]Epilepsy Dept., Huntington Med. Res. Inst., Pasadena, CA

**Abstract: Aim**: Examine how the flux of neurotransmitter glutamate from neurons to the extracellular fluid, as measured by the rate of $^{13}C$ enrichment of extracellular glutamate ($GLU_{ECF}$), changes in response to seizures in the kainate (KA)-induced rat model of temporal-lobe epilepsy.
**Method:** Following intrahippocampal KA injection, $GLU_{ECF}$ was collected by microdialysis from the CA1/CA3 region of awake rats, in combination with EEG recording of chronic-phase recurrent seizures and intravenous infusion of [2,5-$^{13}C$]glucose. Progressive $^{13}C$ enrichment of $GLU_{ECF}$ C5 at ~ 10 picomol level was analyzed by gas-chromatography mass-spectrometry (GCMS), by measuring the increase in the peak area of m/z 331 vs m/z 330 ion (Fig. 1).
**Results**: The $^{13}C$ enrichment of $GLU_{ECF}$ C5 increased much faster in a rat with frequent seizures (shown by arrows) than in controls (Fig. 2A). The mean enrichment rate (the increase in fractional enrichment/min for $t$ = 0-120 min) was 0.0029 ± 0.0001 in frequently-seizing (10 ± 0.7 seizures/3 h) rats ($n$ = 4), vs controls, 0.00167 ± 0.0001/min ($n$ = 6) ($p < 0.01$) (Fig. 2B).
**Significance**: The *flux* of the excitatory neurotransmitter GLU, as measured for the first time in

vivo by $^{13}C$ enrichment analyses, is significantly enhanced by seizures, in addition to the previously-reported rise in $GLU_{ECF}$ *concentration.*

**Clinical potential**: To examine seizure-induced change in GLU flux, the time-resolution can be improved >10-fold in the human brain, where a 70-mm (instead of 2-mm) probe can sample more GLU per unit time.

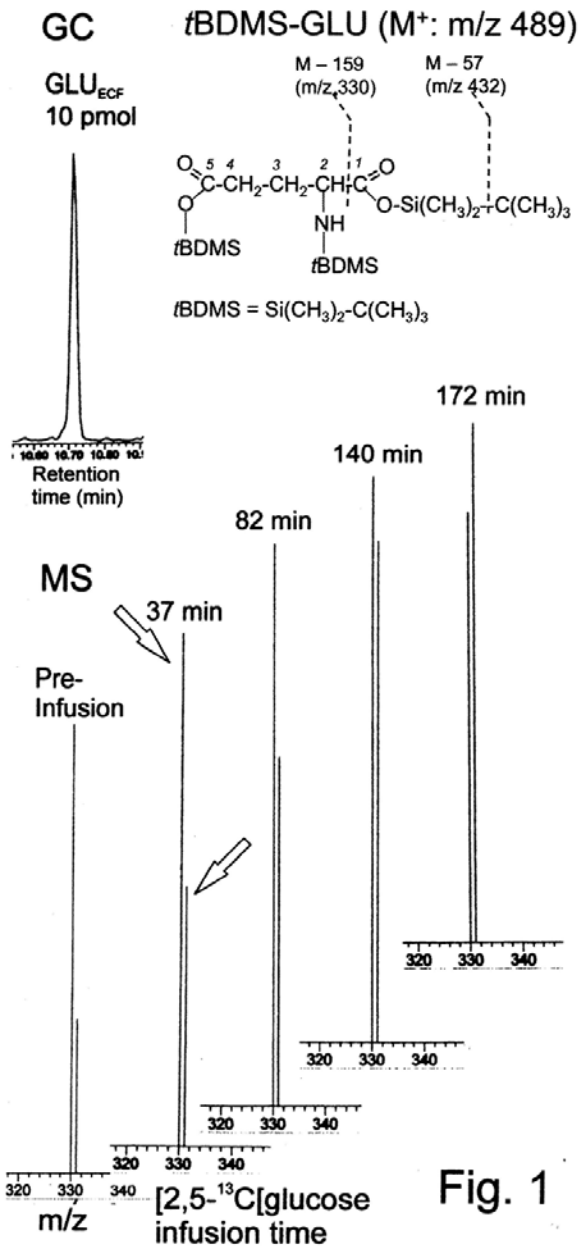
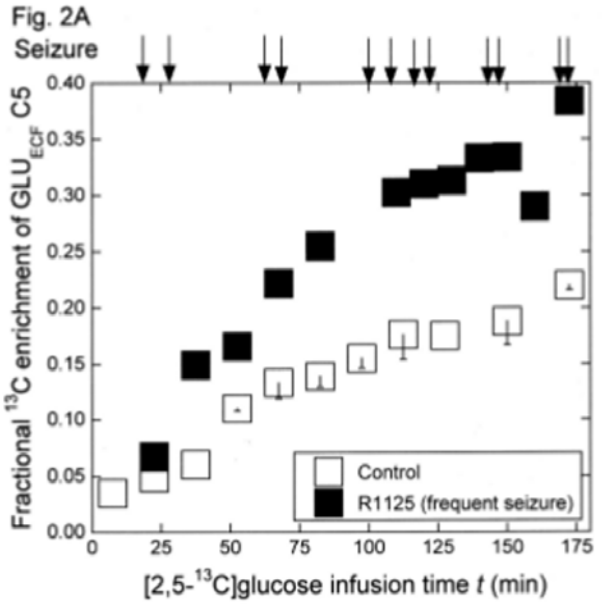
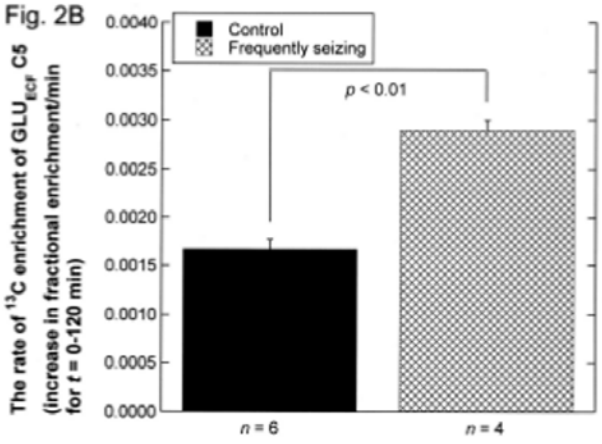



Fig. 1




**Disclosures: K. Kanamori:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

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**Topic:** B.11. Epilepsy

**Support:** Swedish Research Council

ALF

Royal Physiographic Sociaty of Lund

**Title:** Running as a modulator of epileptogenesis in a genetic mouse model of epilepsy

**Authors: *M. AHL**[1], U. AVDIC[2,3], T. DEIERBORG[4], C. T. EKDAHL[5]
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**Abstract: Background**: Exercise is a known for its modulatory and beneficial effect on brain function. The development of epilepsy often includes a primary insult i.e genetic changes or a head trauma that initiates an epileptogenic phase that will lead to epilepsy and spontaneous seizures. We therefore set out to study if exercise by running could be a modulator of the epileptogenesis in a genetic mouse model of epilepsy notably the synapsin 2 knockout (KO), which will develop handling induced seizures from 2.5-3 months of age.
**Method**: Seizures were induced with handling by lifting the mice into another cage. Synapsin 2 KO mice (n=98) were housed with or without free access to a running wheel. All the handling and provocations of animals were done systematically for 8 weeks; first 3 times a week for 5 weeks and then once a week for additionally 3 weeks. Wheels were introduced at different time points in different groups, both before and after seizure onset. All seizures were recorded, and seizure onset, frequency and seizure severity was compared between running and control animals. Corticosterone levels and social ability of animals were also investigated before and after wheel inset.
**Results:** Animals that had wheels introduced before the development of seizures had a significantly later seizure onset compared to controls. The majority of these running animals did remarkably not develop any seizures. Running before seizure onset had a huge effect on seizure development even when the mice were not running during the provocation period. However, when wheels were introduced after seizure onset the effect of supressing seizures failed. In the groups that had a strong positive correlation between exercise and suppression of seizure onset, no change in seizure severity could be found. In addition, there was a decrease of corticosterone levels from 1 month to 2 months of age in both experimental groups. Furthermore, only running animals increased their social interaction time compared to non-runners.
**Conclusion**: These are the first results showing that exercise modulates epileptogenesis and seizure development in mice. These results may be of significance for the prevention and disease modifying strategies in the treatment of epilepsy.

**Disclosures:** **M. Ahl:** None. **U. Avdic:** None. **T. Deierborg:** None. **C.T. Ekdahl:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 562.06/D19

**Topic:** B.11. Epilepsy

**Support:** NIH Grant R15S088776

**Title:** Effect of early life seizures on development of autistic-like behavior in two mouse strains: 129SvEvTac and C57BL/6 mice

**Authors: *S. L. HODGES**[1], S. O. NOLAN[2], A. J. HOLLEY[2], M. S. BINDER[2], J. T. OKOH[2], K. J. ACKERMAN[2], J. N. LUGO[1]
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**Abstract:** Epilepsy and Autism spectrum disorder (ASD) have a high comorbidity rate, with the co-occurrence of having both disorders being approximately 30% in children with either disorder. Researchers have begun to investigate this relationship by examining how early life seizures can lead to the development of autistic-like behavior in rodent epilepsy models. However, utilization of different seizure induction methods and background strain of mouse used has shown variable results, and thus it is critical we continue to investigate this relationship to determine the most optimal model to study the comorbidity. In this study, we used the inhalant flurothyl to induce seizures in male and female 129SvEvTac and C57BL/6 mice on postnatal days 7 to 11. Each mouse received 3 seizures per day, each approximately 2 hours apart (15 seizures total). All mice went through behavioral testing 3 months later in adulthood to examine how flurothyl seizures in early developmental periods effected the behavioral phenotype. We examined activity levels, social behavior, anxiety, repetitive behavior, learning/memory, and sensory gating abilities in all mice. The 129SvEvTac mice did not exhibit the typical ASD-like behavior we would have expected. Rather, the 129SvEvTac seizure mice exhibited significantly decreased repetitive behavior in the nose poke task compared to controls ($p < .05$). Results from 129SvEvTac mice will be compared to the behavioral phenotype of a more active mouse strain, C57BL/6 mice. Understanding mechanistically how early life seizures can contribute to the development of autistic-like behavior will be critical for elucidation of new therapeutic approaches for both disorders.

**Disclosures: S.L. Hodges:** None. **S.O. Nolan:** None. **A.J. Holley:** None. **M.S. Binder:** None. **J.T. Okoh:** None. **K.J. Ackerman:** None. **J.N. Lugo:** None.

**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 562.07/D20

**Topic:** B.11. Epilepsy

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**Title:** The impact of early-life seizures on ultrasonic vocalization behavior in 129 SvEvTac mice: A seizure model comparison

**Authors: *S. O. NOLAN**[1], S. L. HODGES[2], C. D. REYNOLDS[3], A. J. HOLLEY[1], M. S. BINDER[1], G. D. SMITH[2], J. N. LUGO, JR[1]
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**Abstract:** Epilepsy is a neurodevelopmental disorder that is highly prevalent during the first year of life. Early life seizures are known to cause deficits in a variety of behavioral domains and have been shown to contribute to the development of autistic-like behaviors. Previous data from our lab using Ultravox equipment has demonstrated that a single acute early life seizure results in male-specific suppression of 50kHz ultrasonic vocalizations (USVs) in 129 SvEvTac mice. However, the impact of other types of seizure inductions have not been studied. For our studies, we evaluated the impact of seizures on vocalization development in 129 SvEvTac male and female mice across two different seizure paradigms, a single bout of continuous seizures on postnatal day (PD) 10 and multiple flurothyl seizures across PD 7-11. For the first paradigm, we administered kainic acid to mice on PD 10 to induce status epilepticus. On PD 12, pups were separated from their mothers and quantitative and spectrographic analysis was conducted to analyze USVs using Avisoft equipment and software. Results indicated that a single acute seizure suppressed USV quantity and duration, $p < 0.05$, as well as altered call-type utilization, $p < 0.05$. For the second paradigm, we administered flurothyl to mice on PD 7 to PD 11. The animals received 3 flurothyl-induced seizures per day, with 2 hours between each seizure, through PD 11. Similar to the first paradigm, on PD 12, pups were separated from dams and USV production was recorded for 2 minutes. These results indicate that call-type utilization was significantly impacted by seizures, $p < 0.05$, with an increased utilization of complex calls and a decreased use of chevron and upward call types. This study provides evidence that the suppression of USVs by seizures is not limited to a particular type of seizure induction, as call-type utilization was significantly impacted in both models. These are also the first results to examine the impact of a multiple seizure paradigm using flurothyl on vocalization development in mice.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

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Fondazione Ente Cassa di Risparmio di Firenze

**Title:** Optical mapping of neuronal activity in a zebrafish model of epilepsy

**Authors: *L. TURRINI**[1], C. FORNETTO[2], G. MARCHETTO[2], M. C. MÜLLENBROICH[1,5], N. TISO[6], A. VETTORI[6], F. RESTA[1], A. MASI[3], G. MANNAIONI[3], F. S. PAVONE[1,5,4], F. VANZI[1,2]
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**Abstract:** Conventionally, epileptic seizures are detected and characterized in zebrafish either as behavioral alterations or by means of direct electrographic recordings. To overcome the limitations of these conventional approaches (namely limited sensitivity of behavioural assays and lack of spatial resolution of electrographic recordings), we implemented the real time optical mapping of neuronal activity during the onset and propagation of epileptic seizures. We employed a transgenic zebrafish line with pan-neuronal expression of the genetically encoded calcium indicator GCaMP6s to measure neuronal activity in zebrafish larvae during seizures induced by pentylenetetrazole (PTZ). With this approach, we simultaneously measured neuronal activity in different regions of the larva brain together with tail locomotor activity, showing the high sensitivity of this method to detect different levels of alteration, as induced by increasing PTZ concentrations and treatments with various drugs. Moreover, we applied this optical method to the development of a high-throughput drug-screening assay able to simultaneously record brain and locomotor activity, demonstrating that GCaMP measurements can be more sensitive than behavioral assays for the detection of subclinical epileptic seizures. The methodological approach described here could provide a deeper comprehension of the mechanisms underlying epilepsy, enabling investigations on hypomorphic human mutations, and could improve the development of new anticonvulsant drugs. Furthermore, this method can also be easily applied to the study of other human neuropathologies modelled in zebrafish, allowing a simple and yet detailed investigation of brain activity alterations associated with the pathological phenotype.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

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**Program#/Poster#:** 562.09/D22

**Topic:** B.11. Epilepsy

**Support:** NIH

PERF

**Title:** Models, mechanisms and therapeutic development of focal cortical dysplasia

**Authors: *Y. WANG**[1], S. HU[3], T. JI, 48105[1], K. GLANOWSKA[1], G. G. MURPHY[2], J. M. PARENT[1]
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**Abstract:** Focal cortical dysplasia (FCD) is a major cause of intractable focal epilepsies and has been clearly linked to abnormal MTOR signaling pathway. Recently, genetic mutations in this pathway have been increasingly identified in familial and sporadic focal epilepsies. However, there is no animal model that concordantly recapitulates pathological and electrophysiological changes observed in resected human tissues. Therefore, it has been challenging to develop new effective medical or surgical therapies. Here, we use CRISPR genomic editing tools to establish a novel FCD animal model that shows cortical dyslamination, ctyomegaly and increased intrinsic excitability. Everolimus is able to reverse the increased soma size and ameliorate the seizure burden. We also use genetically engineered human stem cells for in vitro study with hopes to develop a cell-based transplant therapy and a medium-throughput drug-screen platform.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

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**Title:** Changes in spatial encoding specificity of CA1 pyramidal cells in models of heightened dentate gyrus excitability

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**Abstract:** The dentate gyrus plays a central role in regulating activity in the extended hippocampal network and is thus critical for its normal functioning regarding cognition but also with regard to pathological conditions such as epilepsy, in which dentate granule cells (DGCs) are hyperactive. In order to better understand how altered dentate function affects downstream processing we decided to look at how cellular activity in areas such as the CA1 hippocampal region is affected when normal dentate function is perturbed, either through injury or through pharmacogenetic manipulations. For this purpose, we implemented an in vivo approach and performed two-photon calcium imaging in the CA1 of awake, behaving, mice traversing a virtual reality environment. CA1 is characterized by the presence of cells that encode spatial information (“place cells”). Our experimental setup allowed us to monitor the activity of CA1 pyramidal cells and assess their spatial tuning with regard to the animal's position within the virtual environment. Our preliminary data suggests that, compared to control animals, CA1 cells in animals that had experienced pilocarpine-induced status epilepticus (SE), which is devastating to DGCs, had a lower spatial specificity. Following SE, DGCs exhibit hyperactivity which persists permanently, so the reduction of spatial tuning specificity in CA1 cells could be attributed to a downstream flood of propagated activity.
Interestingly enough however, when animals expressing CaMKIIa.hM3D in their dentate were treated with clozapine-N-oxide (CNO), thus hyperexciting DGCs (mimicking the conditions seen in SE animals), there was no disruption of spatial tuning specificity in CA1 cells, nor was there any effect on their overall activity levels. In fact, CNO administration tended to increase spatial specificity in these cells, but not in a statistically significant manner. These diametrically opposite effects on CA1 cells following dentate hyperexcitability suggest that a complex pattern of interactions between the two structures may be at play.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

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AES/EFA Predoctoral Award

**Title:** Neuron Restrictive Silencing Factor mediates long term learning and memory deficits provoked by developmental long febrile seizures

**Authors: *M. M. CURRAN**[1], K. P. PATTERSON[2], J. M. BARRY[3], A. SINGH-TAYLOR[4], G. P. BRENNAN[5], N. RISMANCHI[6], M. PAGE[3], Y. NOAM[7], G. M. HOLMES[3], T. Z. BARAM[1]
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**Abstract: Rationale**: In a subset of children experiencing prolonged febrile seizures (FSs), the most common type of childhood seizures, cognitive outcomes are compromised. Similarly, our lab has identified significant, enduring spatial memory problems in rats following experimental prolonged FS (febrile status epilepticus; eFSE). The underlying mechanisms of these cognitive changes are unknown. Our lab has discovered Neuron Restrictive Silencing Factor (NRSF, also known as REST), a transcriptional repressor, as one of the mechanisms underlying the development of spontaneous seizures following status epilepticus in adult rats[1]. NRSF is known to contribute to neuronal differentiation during development and to programmed gene expression in mature neurons. Thus, we aim to determine if dysregulation of NRSF contributes to long term learning and memory deficits following eFSE.
**Methods:** Experimental FSE is induced in 10-11 day old Sprague Dawley rats as previously described.[2] To determine the role of NRSF following eFSE, we administer decoy oligodeoxynucleotides (ODNs) to acutely block NRSF function after eFSE. The first cohort consists of all male rats. At adulthood, we test their learning and memory function via the active avoidance spatial memory task, and then use golgi staining to analyze structural changes within

the hippocampus. To better understand how exposure to eFSE affects the developing brain, we use the novel object location task in a second cohort of male and female rats.
**Results:** Spatial memory, measured as the avoidance of the shock zone in the active avoidance task, is reduced after eFSE. Remarkably, these deficits are abolished by transient, post hoc interference with the chromatin binding of NRSF. Additionally, eFSE provokes region-specific dendritic loss in the hippocampus and aberrant generation of excitatory synapses in dentate gyrus granule cells. NRSF prevents the granule cell changes, but does not prevent dendritic loss in the CA1. The novel object location tasks in both males and females are ongoing.
**Conclusions:** Together, these studies provide novel insights into developing networks that underlie memory, the mechanisms by which early-life seizures influence them, and the means to abrogate the ensuing cognitive problems. We show that NRSF-based transcriptional repression is required for the development of long term memory deficits as tested in the active avoidance task and we will determine if that extends to other tests and to females as well.
1. McClelland, S. *et al. Ann. Neurol.* **70,** 454-64 (2011).
2. Dubé, C. *et al. Brain* **129,** 911-22 (2006).

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

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**Title:** Online classification of behavior using miniature motion sensors during electrical recordings in freely moving rodents

**Authors: *H. POLDER**[1], N. ZIEGENSPECK[2], P. ZHAO[2], B. KLEINER[2], J. PLANCK[1], M. WESKAMP[1], A. DRAGUHN[3], M. BOTH[3], J. BRANKAčK[3]
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**Abstract:** Correlation of neuronal activity and behavior is a substantial goal of modern neuroscience research. For the investigation of pathological states (e.g. epilepsy, sleep disorders, drug addiction) the examination of the interconnection between behavioral patterns and electrophysiological data is of great importance. However, synchronous acquisition and

combined analysis of electrophysiological and quantitative behavioral data is still a challenging task. Currently, video tracking systems are the standard for behavioral monitoring. However these systems require a considerable amount of hardware, computing power and manual work for offline analysis.
We developed a method for automatic classification of behavioral states based on tracking and analyzing the motion of rodents using a set of miniature motion tracking sensors. Our approach is based on synchronization of multiple motion sensor data with local field potentials (EEG, LFP) in freely moving rodents for automatic classification of behavioral states. Classification of behavior is based on a learning algorithm working on a hierarchy of behavior states.
The headstage on the rodent consists of a miniature inertial measurement unit (IMU) with nine degrees of freedom (DOF), multiple channel recording of electric signal patterns (EEG, LFP) and a LED marker set for motion capturing. A stereo camera system placed above the recording cage tracks the LED signals and synchronizes them with the IMU data for estimation of the movement trajectory with 6 DOF and an accuracy of 2 cm. The camera system can be used to define behavioral states in the algorithm for automatic classification and is not used by the algorithm any more once the behavioral states are defined.
Compared to previously used approaches, this method yields lower false positive rates and a better association of motion features with behavioral state. For every layer of hierarchy, we identified the most informative feature components. For example, exploratory behavior was identified by the correlation between location, head orientation and acceleration signals. Among the classifiers tested, Neuronal Network, Gradient Boosted Trees and Random Forest achieved the best results. With these methods, first results show a classification accuracy of 99 % for immobility vs. movement and 96 % for detection of REM-sleep. Further behavioral states like classification of locomotion, exploration, or other behaviors can be flexibly added using appropriate training data.
The method is suited to correlate multi-dimensional physiological data (e.g., EEG) and behavioral states in a semi-automatic, highly reliable and reproducible manner.

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**Poster**

**562. Epilepsy: *In Vivo* and Behavior - *In vivo* Imaging, Seizure Mapping, and Mechanisms**

**Location:** Halls A-C

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**Title:** Does atomoxetine, a norepinephrine reuptake inhibitor, reduce seizure-induced respiratory arrest by modulating cardiorespiratory function?

**Authors: *H.-J. FENG**[1], H. ZHAO[1,2], J. COTTEN[1], X. LONG[2]
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**Abstract: Background:** Sudden unexpected death in epilepsy (SUDEP) is a significant public health burden. The mechanisms of SUDEP are elusive, although cardiorespiratory dysfunction is a likely contributor. Clinical and animal studies indicate that seizure-induced respiratory arrest (S-IRA) is the primary event leading to death in many SUDEP cases. Our prior studies demonstrated that intraperitoneal (IP) injection of atomoxetine, a norepinephrine reuptake inhibitor (NRI), suppresses S-IRA in DBA/1 mice. In the current study, we injected atomoxetine intracerebroventricularly (ICV) and measured its effect on S-IRA in DBA/1 mice to determine its central effects. Additionally, we tested our hypothesis that atomoxetine reduces S-IRA via altering cardiorespiratory function.
**Methods:** DBA/1 mice were primed by daily subjecting to acoustic stimulation for 3-4 days to establish consistent susceptibility to audiogenic seizures (AGSz) and S-IRA. Atomoxetine was applied ICV using microinjection in behaving DBA/1 mice. We examined the effect of atomoxetine on respiratory and cardiac function using non-invasive plethysmography and ECG in anesthetized DBA/1 mice. Blood pressure and heart rate were measured using a tail-cuff system in conscious DBA/1 mice.
**Results:** The incidence of S-IRA was significantly reduced 2 hr after ICV administration of atomoxetine at 200 (40%, $n = 10$; $p < 0.01$) and 250 nmol (0%, $n = 6$; $p < 0.001$) as compared with vehicle control (100%, $n = 12$). Although atomoxetine suppressed tonic AGSz in 33.3% of mice in both dosages, it did not block susceptibility of DBA/1 mice to AGSz. IP atomoxetine administration at a dosage (15 mg/kg) that reduces S-IRA slightly increased basal ventilation (116.4% of vehicle control, $n = 7$; $p < 0.01$) as compared with vehicle control ($n = 7$), but exerted no effect on heart rate in anesthetized DBA/1 mice. IP injection of atomoxetine produced no effect on the heart rate and blood pressures in conscious mice.
**Conclusions:** Our data indicate that atomoxetine reduces S-IRA through direct effects on the CNS and potentially through modest effect on lung ventilation in DBA/1 mice. Atomoxetine, at a dosage that inhibits S-IRA, produced no detectable effects on the cardiovascular system. As a medication widely used for treatment of attention deficit hyperactivity disorder, atomoxetine is potentially useful to prevent SUDEP.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

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Cure AHC

**Title:** Knock-in mutation in Na/K-ATPase α3 increases hippocampal excitability with underlying GABAergic dysfunction

**Authors: *A. S. HUNANYAN**[1], A. R. HELSETH[1], E. ABDELNOUR[1], M. SACHDEV[1], M. SZABO[1], L. CHUNG[2], M. MASOUD[1], J. RICHARDSON[1], Q. LI[4,6], J. V. NADLER[5], S. D. MOORE[4,6], D. W. HOCHMAN[3], M. A. MIKATI[1,2]
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**Abstract:** Na/K-ATPase dysfunction, primary (mutation) or secondary (energy crisis, neurodegenerative disease) increases neuronal excitability in the brain. The mechanisms underlying this increased excitability remain to be fully understood. To evaluate increased excitability we performed extracellular as well as whole cell patch clamp recordings from the brain slices. We also performed immunohistochemistry to count the hippocampal cells. Here we demonstrate in the CA1 region of knock-in mice carrying the D801N $\alpha_3$ subunit mutation the following as compared to wild type. 1) Increased polyspiking evoked by electrical stimulation of Schaffer collaterals. 2) Equalization by bicuculline of the number of polyspikes induced by Schaffer collaterals stimulation. 3) Reduced miniature, spontaneous, and evoked, inhibitory postsynaptic currents, but no change in excitatory postsynaptic currents. 4) Robust action potential frequency adaptation in response to depolarizing current injection in stratum oriens fast-spiking/parvalbumin and quasi fast-spiking/somatostatin/neuropeptide Y interneurons. 5) No change in number of neuropeptide Y, somatostatin or pyramidal cells, but reduced number of parvalbumin positive interneurons. Our data indicate that, in our model, parvalbumin and somatostatin GABAergic interneuron dysfunction is a major contributor to Na/K-ATPase related increased excitability.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

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**Topic:** B.11. Epilepsy

**Support:** NSF CAREER Award 1149446

**Title:** Capnotaxis in the African naked mole-rat regulates GABA efficacy

**Authors: *M. ZIONS**[1], D. P. MCCLOSKEY[2]
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**Abstract:** Rationale: The African naked mole-rat (H. glaber), a species with a broad tolerance for low oxygen and high carbon dioxide, presents a unique opportunity to study how the mammalian brain accommodates environmental conditions. We have demonstrated that this species displays spontaneous seizure behavior and activity in vivo and in vitro under normal environmental conditions, which are exacerbated under conditions which lower inhaled carbon dioxide (hyperthermia, hyperventilation, hypocapnia).
Here we explored a mechanism that may explain both the environmental tolerance and the vulnerability to seizure: carbon dioxide regulation of GABAergic tone.
Methods: Adult naked mole-rats were injected with diazepam (5 mg/kg ip) and recorded with video and/or EEG. All animals exhibited seizures within 10 minutes. Treatment with 5% carbon dioxide allowed a second dose of diazepam to stop the seizure. Behavioral analysis using RFID tracking in the housing colony showed a strong preference of all colony members for the nest chamber, which contained the highest levels of carbon dioxide. When exogenous carbon dioxide (11%) was infused into the colony, animals spent more time away from the nest chamber and near the gas source.Conclusions: Diazepam typically suppresses seizure by promoting binding of GABA at its receptors, increasing chloride influx down its gradient. In naked mole-rats diazepam induces seizures in normal environmental conditions, but blocks seizures in hypercapnic conditions, indicating a chloride gradient which is tightly coupled to availability of carbon dioxide. Naked mole-rats demonstrate capnotaxis toward carbon dioxide sources, indicating not just a tolerance but a preference for hypercapnic environments. We therefore propose that naked mole-rats experience an anxiolytic effect through the enhanced efficacy of GABA when in the presence of their carbon dioxide exhaled by colony mates, a physiological adaptation which may be linked to the emergence of eusociality in this species.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

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**Topic:** B.11. Epilepsy

**Title:** Altered functional efficacy of hippocampal interneuron during epileptogenesis following febrile seizures

**Authors:** ***Y. YU**[1], D.-S. KIM[2]
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**Abstract:** Febrile seizure (FS) is the most common seizure type in infants and young children. FS may induce functional changes in the hippocampal circuitries. Abnormality of excitatory and inhibitory neurotransmissions was previously related to wide-spread seizure attack in the hippocampus following recurrent seizure onset. To clarify the involvement of expressional changes and functional alterations of hippocampal interneurons with epileptogenesis following FS, we investigated long-term effects following recurrent seizure in a hyperthermia-induced seizure animal model. At 12 weeks following FS, the recurrent seizure time period, local field potentials (LFP) revealed high amplitude potential and a sharp wave characteristic of epilepsy. Mossy fiber reorganization in the hippocampus was also detected as abnormal synaptic connection at 8 weeks. Calretinin (CR) -positive interneurons were transiently enhanced during epileptogenic period at 7-9 weeks after FS in the CA1 and DG region and it is double labeled with VGLUT-1. However, although GABAA-α1 immunoreactivities were un-changed as similar to control hippocampus at 7-9 weeks after seizure onset, its expression was significantly enhanced at 4 weeks and 12 weeks and it is colocalized with GABA. Furthermore, the field excitatory postsynaptic potential (fEPSP) and the paired-pulse responses including population spike (PS) latency, excitability ratio and PS2/PS1 ratio were markedly altered in the CA1 and DG region at 12 weeks after FS. Therefore, our findings in present study indicate that these time-dependent changes may be based on the persistent alterations of hippocampal neuronal circuits in balance between excitatory and inhibitory responses, and may lead to the epileptogenesis and spread of seizure activity following FS.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

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**Title:** Synaptic balance of adult-generated dentate granule cells in the rat pilocarpine temporal lobe epilepsy model

**Authors: *K. M. GLANOWSKA**, G. G. MURPHY, J. M. PARENT
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**Abstract:** Excessive excitatory or altered inhibitory signaling is proposed to underlie the abnormal synchrony of hippocampal neuronal networks which are thought to contribute to seizures in human and experimental temporal lobe epilepsy (TLE). Increased dentate granule cell (DGC) neurogenesis is implicated in these epileptogenic mechanisms, as DGCs born around or shortly after the time of epileptogenic insult, especially hilar-ectopic DGCs, show abnormal integration into pre-existing circuits. However, specific alterations in both glutamatergic and GABAergic innervation of individual, birthdated DGCs during epileptogenesis has not been studied before. We investigated changes in excitatory and inhibitory synaptic inputs onto individual DGCs born during epileptogenesis using retroviral DGC labelling and whole cell voltage clamp techniques in the rat pilocarpine model of TLE. We compared neurons born shortly after induction of status epilepticus (SE) in adult male rats with DGCs born neonatally in age-matched controls after SHAM treatment. Additionally, ongoing studies are investigating synaptic balance in adult-born DGCs from SHAM animals. Birthdating of DGCs was achieved using retrovirus expressing GFP or mCherry driven by a synapsin promoter. All electrophysiological recordings were performed 8 to 10 weeks after SE or SHAM. We recorded both glutamatergic and GABAergic spontaneous synaptic activity in normal mature DGCs and those born during epileptogenesis. We calculated ratios of frequencies and amplitudes of excitatory to inhibitory postsynaptic currents (PSCs) to compare the synaptic balance index for individual neurons. This allowed us to identify candidate hub neurons exhibiting a disproportionately high contribution of one type of synaptic inputs over another. The vast majority of adult-born DGCs in the epileptic brain did not differ from normal controls; however, a small fraction, approximately one in 15 cells, showed a substantial increase in the ratio of glumatergic to GABAergic PSCs frequencies. We speculate that those cells serve as network hubs facilitating seizure development after an epileptogenic insult. An understanding of DGC integration into pre-existing networks may be crucial for the development of antiepileptogenic therapies.

**Disclosures:** **K.M. Glanowska:** None. **G.G. Murphy:** None. **J.M. Parent:** None.

**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 563.05/D31

**Topic:** B.11. Epilepsy

**Support:** NIH Grant NS18201

NIH Grant NS083932

**Title:** Immediate hippocampal granule cell epileptogenesis in experimental temporal lobe epilepsy

**Authors: A. V. BUMANGLAG**, *R. S. SLOVITER
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**Abstract:** Acquired temporal lobe epilepsy with hippocampal sclerosis is a common neurological disorder defined, in part, by hippocampal formation neuron loss. It has been hypothesized that normally quiescent dentate granule cells generate spontaneous epileptiform discharges, either immediately, as a possible consequence of neuron loss, or after a seizure-free “latent period” when a secondary epileptogenic process gradually develops. Here we sought to determine whether and when granule cell epileptogenesis occurs after hippocampal injury, and whether the “latent period” is a clinically subtle epileptic state or a seizure-free “pre-epileptic” state. Perforant path stimulation-induced nonconvulsive status epilepticus for 24 hrs in urethane-sedated rats (n=16) caused selective injury to the hippocampus and entorhinal cortex. Continuous granule cell layer recording at 10 KHz in awake rats (n=16) revealed immediate and spontaneous granule cell population spikes and epileptiform discharges in all animals. These high-amplitude granule cell layer events were not identified by parallel surface EEG recording. Video data revealed spontaneous granule cell layer discharges lasting <10 sec had no detectable behavioral correlate, whereas discharges lasting ~15-40 sec reliably triggered subtle focal seizures (behavioral arrest, facial automatisms, and “wet-dog” shakes). Only granule cell layer discharges lasting ~40-130 sec caused clinically obvious seizures (behavioral arrest, facial automatisms, and forepaw clonus, with or without rearing), which ended the "latent period" 2-4 wks post-injury. Prolonged hippocampal excitation at 2 Hz for 24 hrs caused no obvious neuron loss and no spontaneous epileptiform discharges during the 1-month observation period (n=4). These results indicate that post-injury granule cell "epileptogenesis" occurs without delay, and that focal hippocampal discharges associated with non-convulsive seizures are shorter than those associated with convulsive seizures. The "latent period" is therefore a subtle epileptic state in gradual transition to a clinically obvious epileptic state, and not a seizure-free “gestational" state

when an unidentified epileptogenic mechanism gradually develops. Surface EEG recording appears to be an insensitive method that fails to detect focal hippocampal seizures readily detected by granule cell layer recording. Thus, even continuous EEG recording has a high probability of yielding the false negative conclusion that seizures do not occur during the latent period, which probably explains why the latent period has long been incorrectly assumed to be a seizure-free, pre-epileptic state.

**Disclosures: A.V. Bumanglag:** None. **R.S. Sloviter:** None.

**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 563.06/D32

**Topic:** B.11. Epilepsy

**Support:** NC123240.1

**Title:** Electrical stimulation of the reticular nucleus of the thalamus protected against the epileptic status induced by pentylenetetrazole

**Authors: *G. CONTRERAS-MURILLO**[1], D. M. AGUASCALIENTES[2], A. VALDÉS-CRUZ[3], D. MARTÍNEZ-VARGAS[4], J. ESCOTTO-RAMÍREZ[5], A. MARTÍNEZ[5], V. MAGDALENO-MADRIGAL[5]
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**Abstract:** The use of electrical stimulation therapy for epilepsy is currently being studied in preclinical and clinical protocols. We have reported that the high-frequency stimulation (HFS) in the thalamic reticular nucleus (TRN) induced an anti-epileptogenic effect. The goal of the present study was to evaluate the effects of TRN electrical stimulation on the expression of pentylenetetrazole-induced (PTZ) seizures. Experiments were performed using Wistar male rats, with electrodes stereotaxically implanted in the left TRN (AP -1.4mm, L 1.6mm, H 6.2mm). Epidural EEG recording screws were implanted in the prefrontal cortex for EEG recording. The rats were classified as follows: Control, received only PTZ injection; HFS 10 min, treated with HFS for 10 min; LFS 10 min, subjected to LFS 10 min; HFS 60 min, HFS application for 60 min; LFS 60 min, received LFS for 60 min. EEG recordings were obtained from the cortex and were evaluated to assess ictal behavior over 30 min. The electrical stimulation of TRN induced a

significant decrease in seizure severity. DBS in the TRN induced a significant decrease in seizure severity and duration of TCGCs. Additionally, increased in latency of TCGCs and protected against death as a consequence of *status epilepticus*. These data support the benefical effects of TRN in mediating the expression of experimental seizures. In the future, the thalamic reticular nucleus may be a hopeful target for electrical stimulation to treat intractable seizures in humans.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

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**Topic:** B.11. Epilepsy

**Support:** NSF

NIH (NINDS & OD)

**Title:** Loss of parvalbumin-immunoreactive interneurons in epileptic California sea lions

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**Abstract:** Temporal lobe epilepsy is common in humans. Seizures typically originate in the hippocampus, but the cause is unknown. The hippocampal dentate gyrus of human patients displays neuropathological abnormalities, including the loss of parvalbumin-immunoreactive interneurons. Normally, parvalbumin interneurons strongly inhibit excitatory neurons. Loss of parvalbumin interneurons in temporal lobe epilepsy might cause seizures. Rodent models of temporal lobe epilepsy do not perfectly replicate the parvalbumin interneuron loss found in human patients, so better animal models are needed. California sea lions (*Zalophus californianus*) develop temporal lobe epilepsy after exposure to the excitatory neurotoxin domoic acid, which enters the marine food chain during harmful algal blooms. We hypothesized that epileptic sea lions would display significant loss of parvalbumin interneurons in the dentate gyrus. To test this hypothesis, sea lions were intracardially perfused with formaldehyde immediately upon euthanasia because of failed response to treatment and poor prognosis. Brains were sectioned (40 μm) coronally. Nissl staining revealed obvious hippocampal neuron loss

(sclerosis) unilaterally in 22 sea lions and bilaterally in 15. Control sea lions (n=14), that were euthanized because of other conditions (e.g., septicemia and severe malnutrition) with a poor prognosis and failure to respond to treatment, displayed no obvious loss of hippocampal neurons. Stereology and a Neurolucida system are being used to estimate the number of parvalbumin-positive interneurons per dentate gyrus. Preliminary data suggest control sea lions have 14,200 ± 1600 parvalbumin interneurons per dentate gyrus. The average number of parvalbumin interneurons per dentate gyrus is reduced to only 6% of controls in sclerotic hippocampi of epileptic sea lions, similar to reports for human patients with temporal lobe epilepsy. If confirmed, these findings would suggest that epileptic sea lions can be used as a large animal model of human temporal lobe epilepsy and as candidates to test novel anti-epileptogenic treatments before human clinical trials.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

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**Program#/Poster#:** 563.08/D34

**Topic:** B.11. Epilepsy

**Support:** NIH R01 NS08056501A1

**Title:** Early-life seizures disrupt parvalbumin circuit maturation and spread of thalamocortical activity in auditory cortex

**Authors: *Y. J. SONG**[1], E. E. DIEL[2], A. E. TAKESIAN[2,3], L. T. MASSARO[1], E. L. HONIG[1], J. J. LIPPMAN-BELL[4], T. K. HENSCH[2,3], F. E. JENSEN[1]
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**Abstract:** Neonatal seizures are associated with long-term cognitive and behavioral deficits, including autism and intellectual disability. We have found that pentylenetetrazol (PTZ)-induced seizures early in life prematurely unsilence thalamocortical (TC) synapses to disrupt tonotopic plasticity in the primary auditory cortex (A1; Sun et al, in review). Here, we examine whether early-life PTZ seizures may further disrupt E-I balance by altering the postnatal maturational profile of parvalbumin (PV+) cells across A1. Notably, this particular inhibitory circuitry is underdeveloped at birth and matures postnatally, signaling both the onset of critical period plasticity and its closure as they become tightly enwrapped by perineuronal nets (PNN).
We induced acute seizures in male C57BL6 mice by PTZ injection (60mg/kg daily i.p.) from P9-

11. Both mRNA and protein expression for PV and PNN were evaluated across the A1 critical period (P12-15) and beyond (P20, P40). By *in situ* hybridization, PTZ-mice at P13 had reduced PV transcripts strongly correlated with PNN gene expression. Immunohistochemistry revealed no differences in the number of PV+ cells with PNN in A1 across development. However, a significant decrease in cells only positive for PV+ (without PNNs) emerged in P20 PTZ mice (n=10) relative to that of saline-treated controls (n=8) within layer IV (respectively, 71.11±7.9% vs 100±10.45%; p=0.039) and layer V (78.88±7.32% vs 100±4.95%; p=0.038).
Across the tonotopic axis, TC responses in A1 mature at differential rates with earlier strengthening in rostral, high frequency regions (Barkat et al., 2011), as confirmed by vGluT2 labeling before and after the critical period. Because PV+ cells receive stronger TC innervation (Cruikshank et al., 2007), and impact the horizontal spread of TC driven activity, we further examined functional sensitivity to PTZ treatment across the tonotopic axis using voltage-sensitive dye imaging in an acute TC slice preparation. We compared naive (no tone-rearing) mice that were injected either with PTZ or saline from P9-11 and imaged at P15 upon stimulating the central thalamic (MGBv) sites for alignment and plotting the ΔF/F signal decay across layers. Surprisingly, the PTZ-injected mice displayed greater spread of activity only in the caudal direction and restricted to thalamo-recipient layers I and IV.
Taken together, our results suggest that in addition to unsilencing auditory synapses, PTZ-induced seizures may also impact PV circuit maturation which may contribute to biased spread of excitability toward late maturing portions of the caudal tonotopic map.

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**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

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**Topic:** B.11. Epilepsy

**Support:** NIH/NINDS grant #R37 NS071785-07 (to S.C.B.)

**Title:** Long-term seizure suppression and rescue of behavioral comorbidities in epileptic mice with hippocampal transplantation of GABA progenitors from the medial ganglionic eminence

**Authors: *M. L. CASALIA**[1], M. A. HOWARD[2], S. C. BARABAN[3]
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**Abstract:** Epilepsy is a neurological disorder characterized by unprovoked seizures, behavioral and cognitive comorbidities. We recently showed that adult intra-hippocampal transplantation of

interneuron progenitors from the embryonic medial ganglionic eminence (MGE) dramatically reduce spontaneous electrographic seizures and rescue comorbidities observed in a mouse model of acquired epilepsy (Hunt et al. 2013). Whether the therapeutic benefits of transplanted interneuron progenitors are long-lasting or whether other types of ganglionic eminence derived interneurons are also therapeutic is not known. Here adult male CD1 mice (n = 134) were administered pilocarpine to elicit status epilepticus (SE). Only mice that survived acute SE and subsequently exhibit at least two unprovoked tonic-clonic seizures were designated as “epileptic” (n = 63; 90%) and became candidates for intra-hippocampal progenitor cell transplantation. In the open field (OF) test, epileptic mice showed progressive impairments compared to controls that were rescued 60 days after bilateral MGE transplantation (n = 26). In ‘epileptic+MGE’ mice continuous 14-day video-electroencephalographic (vEEG) monitoring at 180 days after transplant (DAT) showed an 84% reduction in mean seizure frequency and an 88% reduction in the total number of seizures. *Post hoc* immunohistochemistry at 210 and 360 DAT showed that MGE cells co-express neuronal (Tuj1 and GAD67) and interneuron markers (PV, SOM and nNOS), as expected. Slice electrophysiology studies at 270 DAT showed that inhibitory postsynaptic current frequency was reduced in epileptic mice (n = 7) but restored to naïve levels in ‘epileptic+MGE’ mice (n = 6). In a separate group of naïve mice, intra-hippocampal transplantation of CGE progenitors resulted in mice that exhibited “interictal” bursts at 30 DAT (n = 3) and seizure-like events with high-frequency, high-voltage, rhythmic activity with clear onset, progression and termination at 90 DAT (n = 3). Although CGE progenitors integrated as vasoactive intestinal protein- and reelin-positive interneurons in ‘epileptic+CGE’ mice, the pattern and frequency of electrographic seizure activity did not diminish during the 14 days of continuous vEEG monitoring (n = 5). Taken together, we demonstrate that MGE (but not CGE) progenitors enhance functional GABA-mediated inhibition, dramatically reduce spontaneous seizure frequency and rescue behavioral deficits in epileptic mice more than six months after treatment. We conclude that MGE progenitor cell transplantation exerts a long-term therapeutic effect in pre-clinical epilepsy models.

**Disclosures:** **M.L. Casalia:** None. **M.A. Howard:** None. **S.C. Baraban:** None.

**Poster**

**563. Epilepsy: Interneurons and Animal models**

**Location:** Halls A-C

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**Topic:** B.11. Epilepsy

**Support:** NIH/NIGMS T32 training grant 5T32GM008328-24

NIH grant 1R01NS099586-01

**Title:** Slow $Cl^-$ extrusion promotes the spread of activity among reticular thalamic neurons

**Authors: *P. M. KLEIN**[1], A. LU[2], M. P. BEENHAKKER[3]
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**Abstract:** Inhibitory GABAergic signaling among reticular thalamic (RT) neurons is thought to form an important choke point that prevents the occurrence of seizures. We have previously demonstrated that despite diminished expression of the $Cl^-$ cotransporter KCC2 in the RT nucleus, RT neurons still maintain a sufficiently hyperpolarized reversal potential for GABA-induced currents ($E_{GABA}$) to promote inhibition. However, our previous $E_{GABA}$ measurements only reflect the ability of RT neurons to maintain low intracellular $Cl^-$ concentrations ($[Cl^-]_i$) during short periods of mild GABAergic signaling. We predict that low KCC2 expression leaves RT neurons susceptible to activity-dependent increases in $[Cl^-]_i$, allowing prolonged stimulation to render GABAergic signaling among RT neurons excitatory and to then promote spreading activation within the RT nucleus. Using a paradigm of repeated GABAergic activation in neurons voltage-clamped at depolarized potentials, we determined that $Cl^-$ accumulates more rapidly in RT neurons than in neurons of the ventrobasal (VB) thalamic nuclei in P10-20 Sprague Dawley rats. The time constant for recovery from $Cl^-$ loading ($\tau_{rec}$) in RT neurons (30.3±3.7 sec, n=11) was also much slower than in VB neurons (18.3±2.3 sec, n=15, p=0.008). The KCC2 antagonist VU0463271 slowed $\tau_{rec}$ in VB neurons (baseline: 16.4±3.6 sec; 10 minutes post-VU: 28.2±4.0 sec, n=8, p=0.045), but had no impact on $\tau_{rec}$ in RT neurons (baseline: 25.5±3.3 sec; 10 minutes post-VU: 27.2±3.1 sec, n=6, p=0.47). VU0463271 may not alter the $\tau_{rec}$ of RT neurons because KCC2 expression is already sufficiently low, such that further reductions have little impact in this experimental paradigm. Based on our experimental findings, we produced a computational model of the RT nucleus, with 100 RT neurons arranged in a linear array and each cell projecting GABAergic synapses to the eight nearest neurons. When RT neurons were assigned a slow $\tau_{rec}$ reflecting our experimental data (~30 sec), stimulation of neurons within our network provoked GABA-mediated action potentials more rapidly, and in a greater number of neurons, than with faster $\tau_{rec}$ values. Less frequent stimulation also produced a greater spread of activation within our network when slower $\tau_{rec}$ values were modeled. Our results indicate that the reduced $Cl^-$ extrusion capability of RT neurons leaves the RT nucleus more susceptible to undergoing an activity-dependent switch to excitatory GABAergic signaling. The loss of inhibitory GABAergic signaling among RT neurons breaks open this important seizure choke point and implicates upregulating $Cl^-$ extrusion in RT neurons as a therapeutic strategy for treating absence epilepsy.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.01/D37

**Topic:** B.12. Glial Mechanisms

**Support:** Brain and Behavior Foundation

Quinnipiac University

**Title:** Impact of maternal separation on dorsal and ventral hippocampal expression of protein markers for inflammation, deacetylation, and glutamatergic function

**Authors: *K. S. JONES**[1], L. TELISKA[1], I. SCHIANO[2], R. ROTOLO[3], C. LITTLE[1], T. STRANGE[1], C. FLYNN[3], C. QUAILEY[3], C. ROSE[3], T. MEDWID[3], M. SZAHAJ[1], J. DEMURO[2], T. FRAZIER[3], M. MIRRIONE[4], A. J. BETZ[1]
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**Abstract:** There is a great demand for therapeutic advancements with respect to mood disorders and a significant link between stress and major depressive disorder (MDD) exists. Maternal separation in rodents is a widely used animal model used to induce early-life stress. This model has reliably demonstrated an increased risk of depressive-like behavior later in life. Existing research also supports that individuals with MDD have learning and memory deficits. Two regions of the hippocampus, the dorsal and the ventral regions, are suggested to be functionally different while the dorsal hippocampus is involved in cognitive function and the ventral hippocampus is involved in emotion and stress. Additionally, microglia activity is associated with causing the atrophy and pathology of these brain regions and may be implicated in MDD. The proliferation and stability of microglia across the development into adulthood differs. We aimed to examine the expression of dorsal and ventral hippocampal microglia in adolescent rats that experience postnatal maternal separation during early development. In the present study, Sprague Dawley male pups were separated from the dam from PND 2 to PND 14 for three hours a day. A control condition of non-separated pups was maintained. First, we examined behavioral tasks during adolescence and found separated offspring spent more time in closed arms of an elevated plus maze. Second, we found epigenetic markers correlating with anxious behavior in the elevated plus maze. Also, we found decreased Iba1 protein expression and increased Rel A protein expression in both hippocampal compartments. Further, we found increased CD11b protein in only the ventral portion of the hippocampus. Decreased levels of histone deacetylase 4 were found in the ventral hippocampus but not dorsal hippocampus. There were no changes in glutamatergic transporters in neurons or astrocytes. To understand the functional connectivity of our findings, we used immunohistochemistry to examine the alterations in dorsal and ventral hippocampal input and output regions. Microglia were quantified in the nucleus accumbens, prelimbic and orbital cortex and based on morphology of Iba1 expressing cells. Our data may provide insight as to the molecular mechanisms responsible for MDD vulnerability, pathogenesis and possible therapeutic remedies.

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**Poster**

**564. Microglia in Disease**

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**Program#/Poster#:** 564.02/D38

**Topic:** B.12. Glial Mechanisms

**Title:** Molecular evolution of adeno-associated virus for targeting of microglia

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**Abstract:** Adeno-associated virus (AAV) is a small virus that causes very mild immune response and no disease. Lack of pathogenicity makes AAV an attractive and powerful gene therapy tool. The AAV genome contains two open reading frames named rep and cap. The cap gene encodes the proteins that form the capsid. A plethora of various AAV serotypes have been identified, all differing in the capsid gene, which dictates cellular receptor binding and thus the tropism of that particular serotype. However, although AAV transduces a variety of cell types with high efficiency, microglia remains remarkably refractory to transduction. The purpose with this project was to utilize molecular evolution to engineer novel AAV capsid mutants that efficiently transduce microglia, thereby creating a novel tool to aid investigations in to the role of microglia in disease. We chose microglia because ongoing research by us and others demonstrate that neuroinflammation plays an important role in the pathophysiology of Parkinson's disease (PD) and other neurodegenerative disorders. Neuroinflammation in PD involves an activated microglia response along with an increase in inflammatory markers. Targeting microglia by AAV opens the door for modulating neuroinflammation, potentially interfering with the disease process in PD, and providing a new tool to study PD etiology.
We utilized 2 different methods to generate diverse libraries of AAV cap mutants 1) Random DNA shuffling of naturally occurring mutant capsids in combination with rationally engineered mutant capsids. With this approach various cap genes were digested and randomly reassembled to generate chimeric capsids. 2) Insertion of peptide libraries in to the AAV cap. Introduction of short peptides in to certain motifs of the AAV capsids can alter the tropism of the virus by mediating capsid attachment to non-canonical receptors that are specifically expressed on the surface of the target cell, in our case the microglia. In our study, we utilized a random peptide library as well as a library based on CD11b, an integrin family member expressed on surface of the microglia. AAV was thereafter generated and injected in to the CNS (striatum) of adult rats. Ongoing work is aimed at 1) Determining the diversity of our viral library, and 2) Isolate and sequence chimeric cap genes contained within microglia. Identified capsids will thereafter be subject to additional rounds of evolution in order to further improve the efficacy of microglial transduction. Although this work is focused on microglia per se, it is important to note the power

of this approach, as it can be applied to a variety of cell types to facilitate research in to a variety of pathologies.

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**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

**Support:** Norwegian Research Council Grant 240844

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**Title:** Selective loss of glutamine synthetase in the cerebral cortex initiates a sequence of neuropathological events that culminate in epilepsy and neurodegeneration

**Authors: *N. C. DANBOLT**[1], Y. ZHOU[2], R. DHAHER[4], M. PARENT[5], Q.-X. HU[2], B. HASSEL[3], S.-P. YEE[6], F. HYDER[5], S. E. GRUENBAUM[4], T. EID[4]
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**Abstract:** The enzyme glutamine synthetase (a.k.a. glutamate ammonia ligase, Glul) is enriched in astrocytes and serves as the primary pathway for synaptic glutamate clearance and brain ammonia detoxification. Loss of astrocytic Glul has been implicated in several CNS disorders, such as epilepsy; however, the mechanism by which Glul deficiency might cause disease is not understood. Here we selectively deleted Glul in the hippocampus and neocortex of mice to study the consequences of Glul deficiency. This resulted in a viable mouse model which allows detailed studies of the pathological progress leading to epilepsy and neurodegeneration. At two weeks of age, the brain cytoarchitecture and behavior of Glul deficient mice were largely unremarkable; however, the brain chemistry, microglial cells and blood vessels were altered. At four weeks of age, other changes became apparent, such as slowed brain growth, altered functional connectivity, reduced cerebrovascular reactivity, behavioral abnormalities, epileptic seizures and progressive neuron loss. Conclusions: Lack of Glul is in itself sufficient to cause epilepsy and neurodegeneration. However, Glul deficiency does not immediately cause seizures,

but rather triggers a pathological process involving early changes to microglia and blood vessels, as well as astrocytes, before culminating in epileptic seizures and progressive neuron loss.

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**Poster**

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**Topic:** B.12. Glial Mechanisms

**Support:** NYU Challenge Grant 2016-17

**Title:** Phagocytic profile of microglia in postmortem autism spectrum disorder temporal cortex

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**Abstract:** In typically developing individuals, dendritic spine density peaks between 12 and 36 months after birth and is then pruned during maturation. Autism spectrum disorder (ASD) appears to lack this normal pruning process and spine density remains high up to adulthood. Microglia are resident immune cells of the brain that are involved in synaptic pruning during development. It is possible that there is a change in phagocytic microglia in ASD that leads to a decrease in synaptic pruning. To address this, we used an unbiased stereological approach to quantify phagocytic cups and phagocytic microglia in postmortem human temporal cortex immunocytochemically labeled with Iba1. We observed and report for the first time, phagocytic cups and phagocytic microglia in the tissue sample we analyzed. The total density of all microglia phenotypes and phagocytic microglia did not differ between ASD donors (n=10, 14.6 yrs, range 2.8-29 yrs) and typically developing individual donors (controls, n=9, 14.9 yrs, 1.8-32 yrs). However, the phagocytic capacity of microglia was significantly higher in ASD compared to controls, which suggests increased phagocytosis of either apoptotic or proliferating cells. There was a significant negative correlation with the density of ramified microglia and a significant positive correlation with the density of amoeboid/rod/dystrophic microglia in ASD. It may be that these phagocytic microglia are not associated with synaptic pruning, but with increased inflammatory responses or cell death. Further investigations of phagocytosis by microglia using different functional markers (e.g., CD68 or CD11b, PSD-95) may be a step forward in understanding its significance in synaptic pruning and neuroinflammation in ASD.

**Disclosures:** **A.S. Lee:** None. **M. Perez-Pouchoulen:** None. **P.M. Whitaker-Azmitia:** None. **E.C. Azmitia:** None.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.05/D41

**Topic:** B.12. Glial Mechanisms

**Support:** JSPS KAKENHI Grant number JP26860921

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**Title:** Increased microglial activation in anorexia nervosa: A [$^{11}$]DPA713 PET study

**Authors: *M. YOKOKURA**[1], T. TERADA[2], T. BUNAI[2], K. NAKAIZUMI[1], Y. KATO[1], M. FUTATSUBASHI[3], E. YOSHIKAWA[3], H. YAMASUE[1], Y. OUCHI[2]
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**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 studies showed significant increases in inflammatory cytokines (i.e. TNF-α, IL-1β, IL-6) and immunoreactive protein (i.e. corticotropin release hormone) in AN patients. Although it is reported that changes of these cytokines in the peripheral blood are linked with activation of microglia in various diseases, no study exists on microglial activation in the AN brain. Here, we aim to clarify that microglial activation is present in the active state of AN and that the level of microglial activation is associated with clinical scores of the AN severity using PET with [$^{11}$C]DPA713.
***Methods;*** Twenty female AN patients (mean age±SD, 25.0±6.2 years old, mean BMI±SD, 14.1±1.3) and 20 healthy female subjects (22.9±3.7years old, BMI 20.6±2.4) underwent [$^{11}$C]DPA713 PET measurements and neurocognitive tasks. $BP_{ND}$ of [$^{11}$C]DPA713 was estimated with a simplified reference tissue model. We examined the whole brain using a voxel-wise analysis, SPM8 (Wellcome Department of Cognitive Neurology, London, UK).
***Results;*** The levels of [$^{11}$C]DPA $BP_{ND}$ in AN patients were significantly higher in the broad brain regions except for the parietal cortex compared to healthy subjects. General cognitive abilities evaluated with Raven colored progressive matrices (visual and spatial cognition), Stroop test (frontal lobe function), and Iowa Gambling Task (decision making) did not differ between AN patients and healthy subjects. No significant correlation was found between [$^{11}$C]DPA $BP_{ND}$

values and these clinical parameters.
***Discussion;*** Our results showed a significant increase in microglial activation in the extensive brain areas but the parietal cortex in the active state of AN. The heterogeneous activation of microglia in the brain and no correlation between microglial activation and clinical scores unlike evidence reported in mild cognitive impairment or dementias suggest that the microglial activation depicted during the aggravated period may be reversible in the AN brain.

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**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

**Support:** NSFC Grant No. 81571174

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Key Technologies R&D Program of Sichuan Province (2015SZ0058-5)

Fundamental Research Funds for the Central Universities

**Title:** Microglial activation regulates cognitive deficit in young offspring of maternal immune activation

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**Abstract: Introduction:** Maternal immune activation (MIA) during pregnancy increases the risk of psychiatric disorders such as schizophrenia, autism-spectrum disorder and depression in offspring. But the underlying mechanism of MIA-induced neurodevelopmental and behavioral abnormality in prepuberty offspring has not yet elaborated. The present study was intended to investigate the effects of MIA on cognitive behavior in young offspring and its potential neuroinflammatory pathway. **Methods:** Animal model of MIA was induced by injection of pregnant Wistar rats with polyinosinic-polycytidylic acid (Poly(I:C)), a synthetic analogue of the viral double-stranded RNA. The male prepuberty offspring (21 days old) were used in this experiment. Cognitive behavior was estimated by the Morris Water Maze. Inflammatory

cytokines and microglial activated phenotype (classical activated microglia: M1 phenotype; alternative activated microglia: M2 phenotype) were confirmed by Real-Time PCR, ELISA and IHC. The offspring were injected with different dose of pioglitazone by intraperitoneal administration for 7 days. **Results:** The young offspring with MIA showed impaired spatial learning and memory in Morris Water Maze task, increased expression of pro-inflammatory cytokines (IL-1β, IL-6, TNFα, iNOS), and decreased expression of anti-inflammatory mediators (IL-4, IL-10). Microglial M1 activation was observed in MIA offspring. Pioglitazone administration inhibited M1 activation, promoted M2 polarization, improved spatial learning and memory and the balance of pro- and anti-inflammatory cytokines in offspring rats. **Conclusion:** MIA-induced cognitive deficit was modulated by microglia-dependent inflammatory response in the prepuberty offspring rats.

**Keywords:** Microglial activation; neuroinflammation; cognitive behavior; offspring; maternal immune activation

**Support:** This work was supported by the National Natural Science Foundation of China (No. 81571174, 81603503), Key Technologies R & D Program of Sichuan Province (2015SZ0058-5), and Fundamental Research Funds for the Central Universities.

**Disclosures: Q. Zhao:** None. **Z. You:** None.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.07/D43

**Topic:** B.12. Glial Mechanisms

**Title:** Microglia are more active in schizophrenia as evidenced by gene expression signatures

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**Abstract:** The pathological mechanisms of schizophrenia (SCZ) are still unknown. This has left drug treatments essentially the same as they were four decades ago despite that these drugs have considerable side effects and do not address all the symptoms. Recent findings show genetic and environmental risk for SCZ is associated with a number of immune response factors. Microglia, the principal immune effectors in the brain, play a critical role in synaptic pruning and increase their rate of pruning in response to inflammation. This suggests that microglia could be responsible for the synaptic loss observed postmortem in SCZ. We hypothesize that a type of microglial activation similar to their inflammatory responses play a key role in the synaptic loss in SCZ. Using Affymetrix microarray data from Martinez et al (*J. Immunol.*, 2006), we identified a gene expression signature indicative of microglial activation. From the initial 19,530 genes in the dataset, the bottom 10% least abundant probes were eliminated from the analysis. MANOVA

with Hotelling-Lawley statistic identified 1723 genes that were differentially expressed in response to LPS and IFN-γ treatment (to induce what is known traditionally as an 'M1' state) using FDR of 0.05, and subsequent fold change cutoff of 1.2. Of these 426 genes, 305 were eliminated because they were expressed more highly in other brain cell types than in microglia, based on Zhang et al data (*J. Neurosci.*, 2014). These 121 genes were used to investigate microglial activity within SCZ RNA-Seq dataset from Zhao et al (*Mol. Psychiatry*, 2015). Using principal components analysis as a dimension reduction technique, overall expression scores for genes indicative of microglial activation and overall expression scores for genes indicative of synaptic density were obtained for both SCZ and controls samples. SCZ samples showed overall higher levels of microglial activation gene expression ($t= -4.10$, $p<0.001$) and lower synaptic gene expression ($t= 4.9446$, $p<0.001$). Overall, synaptic density and microglia activation estimated in this manner were negatively correlated to each other ($R= -0.52$, $p<0.001$). Overall, these data show the plausibility of our microglia-centered disease model of SCZ.

**Disclosures: C.L. Savonen:** None. **M.A. Reimers:** None.

**Poster**

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**Location:** Halls A-C

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**Topic:** B.12. Glial Mechanisms

**Support:** NIH R01AT007429

NIH R21NS095166

**Title:** The agonists of $PGE_2$ EP1 receptor potentiate microglial CD36 recycling without affecting migration

**Authors: *B. MA**[1,2], K. GOODWIN[1], S. DORÉ[3,2]
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**Abstract:** We previously documented that the deletion of the prostaglandin E2 (PGE2) EP1 receptor undermined microglial phagocytosis in vivo. However, the related mechanism still remains unknown. CD36 is a type II scavenger receptor that mediates microglial phagocytosis, which contributes to hematoma clearance after intracerebral hemorrhage (ICH). In this study, we sought to identify the effects of two EP1 receptor agonists on CD36 receptor recycling and cell migration using primary microglial cultures.
The EP1 agonists 17-pt-PGE2 and ONO-DI-004 were used in our study as the treatment.

Fluorescent latex beads and CFSE-labeled red blood cells (CFSE-RBCs) were added to microglial cells independently at a concentration of 10 beads or CFSE-RBCs per cell for live-cell imaging. Immunocytochemistry was performed with the antibody against ionized calcium-binding adapter molecule 1 (Iba1, a microglial marker). We analyzed microglial CD36 receptor recycling by using an established receptor recycling assay. As migration also affects microglial phagocytosis, we used the DUNN chamber to monitor microglial chemotaxis.
We found that 10μM 17-pt-PGE2 significantly increased the number of attached beads to microglial cells from 40 to 120min of incubation ($p<0.01$). Furthermore, we analyzed CD36 receptor recycling using an established receptor recycling assay, and the quantitative results showed that a 10μM 17-pt-PGE2 treatment markedly increased the positive staining area of recycled CD36 receptor in microglial cells ($p<0.05$). We then treated primary microglial cells with another EP1 agonist, ONO-DI-004, for 120min at concentrations of 5 and 10μM. The fluorescent area of CFSE-RBCs after ice-cold PBS washing was markedly increased with the 10μM ONO-DI-004 treatment ($p<0.05$). In the live-cell imaging experiment, a 10μM ONO-DI-004 treatment also significantly increased the number of attached CFSE-RBCs to microglial cells from 25 to 60min of incubation ($p<0.01$). However, the DUNN chamber results indicated that neither EP1 agonist had an effect on microglial travelling distance or velocity.
This study demonstrates that the PGE2 EP1 agonists 17-pt-PGE2 and ONO-DI-004 can potentiate microglial phagocytosis in vitro through enhanced CD36 receptor recycling.
**Key Words:** G-protein-coupled receptors, hemorrhage, live imaging, DUNN chamber, prostanoids, stroke

**Disclosures: B. Ma:** None. **K. Goodwin:** None. **S. Doré:** None.

**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

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**Title:** Electroconvulsive shock enhances microglial responsive motility in the mouse hippocampus

**Authors: *A. SEPULVEDA RODRIGUEZ**[1,2], C. A. CARLONE[1], S. VICINI[1,2,3], P. A. FORCELLI[1,2,3]

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**Abstract:** Microglia, the resident macrophages of the CNS, play a central role in the neuroinflammatory response that characteristic of many brain disorders. A specific pattern of changes in microglial physiology, motility and morphology have been described in the mouse hippocampus after severe seizures (Sz) known as *Status Epilepticus* (SE) (Avignone et al. J Neuro 28(37):9133 2008). SE creates two potent and distinct activators of microglial activation (neuronal hyperactivity and neurodegeneration) and results in the development of spontaneous Sz reminiscent of Temporal Lobe Epilepsy (TLE).

This study aims to further characterize the microglial response to abnormal neuronal hyperactivity using Sz induced by a range of *in vivo* transcorneal Electroconvulsive Shock (ECS) regimens on transgenic reporter mice. Unlike chemoconvulsants, ECS yields reproducible Sz of varying severity without neuronal damage or disruption of endogenous neurotransmission. We employ a well-established in-slice functional assay of microglial responsive motility to monitor the physiological state of the microglia. P28-P35 female and male CX3CR1$^{eGFP/+}$ mice (with and without P2rx4-tdTomato) were randomized to treatment and control groups, then subjected to one of the following ECS regimens: 1 minimal (clonic) Sz, 3 minimal Sz in 1d, 3d with 3 minimal Sz each, or 1 maximal (tonic-clonic) Sz. Horizontal slices of hippocampus were prepared 24h after the last Sz, following standard protocols. Confocal fluorescence z-stacks were taken every 30s to capture the response of microglial processes in CA1 towards a patch pipette with 3mM ATP. Extent and velocity of responsive motility was quantified using Manual Tracking (ImageJ). Microglial expression of P2RX4 was evaluated by tdTomato fluorescence intensity.

None of our minimal ECS regimens affected microglial responsive motility, while a single exposure to maximal ECS significantly enhanced the velocity of the response. The correlation of these changes with microglial P2RX4 expression will be reported.

Our data suggest that unlike minimal Sz, maximal Sz recruit microglial activation pathways reminiscent of the response to SE. In sharp contrast to SE, such Sz are not thought to cause hippocampal degeneration nor result in TLE-like spontaneous Sz. In conclusion, our data suggest that at least part of the SE-induced microglial response can be recapitulated with much less severe Sz. These results could reveal novel biomarkers and therapeutic targets for hippocampal sclerosis, neuroinflammation and epileptogenesis.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.10/D46

**Topic:** B.12. Glial Mechanisms

**Title:** Activation of microglia and loss of synapse in the developing hippocampus of mice prenatally treated by valproate

**Authors: *T. HONDA**[1], Y. ISHIHARA[1,2], A. ISHIDA[1], T. YAMAZAKI[1]
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**Abstract:** Valproate (VPA) is the first choice drug for treatment of generalized epilepsy, but taking VPA in pregnant increases the risk of autism spectrum disorder (ASD). Recently, it is reported that the anatomical and morphological characteristics as well as the number of microglia are abnormal in ASD patients, suggesting that microglia are involved in ASD pathology. The purpose of this study is to unravel the mechanism of abnormal behavior induced by VPA, focusing on microglial activation. Pregnant ICR mice were orally administered with pure water or VPA at 800mg/kg dosage on gestational day 11 (GD11). Behaviors in male pups were examined using open field test in 4-week old to evaluate locomotor activity and Y-maze test, social interaction test and marble burying test in 5-week old to evaluate cognitive function, social preference and repetitive behavior, respectively. On postnatal day 10 (P10), neuroinflammation was investigated by proinflammatory cytokine expression using real-time PCR. Microglial activity was evaluated by ionized calcium-binding adopter molecule 1 (Iba1) and cluster of differentiation 68 (CD68) staining, and synaptic formation was assessed by postsynaptic density protein 95 (PSD95) staining on P10 and in 6 week-old. There was no change in locomotor activity between VPA-treated group and vehicle-treated group, but VPA-treated mice showed deficit in alternation, decreases in contact with a novel mouse and increment of burying behavior as compared to vehicle-treated mice, suggesting that VPA induces impairment of working memory, abnormal social behavior and symptom of repetitive behavior, respectively. VPA up-regulated expression of interleukin 1β (IL-1β) in the hippocampus. There was no change in the number of microglia between VPA-treated murine brain and vehicle-treated murine brain, but VPA enlarged microglial cell body and up-regulated CD68 expression in hippocampal CA1 region on P10 but not in 6 week-old. These results clearly show that exposure to VPA during fetal stage activates microglia accompanied with increased phagocytic activity in the hippocampus. VPA reduced the number of PSD95 puncta in the CA1 hippocampus, but the alteration of PSD95 expression disappeared in 6-week old mice. Therefore, VPA could transiently induce synaptic loss in developing brain. Taken together, the present study has demonstrated that fetal exposure to VPA elevates microglial activity and decreases the number of synapse in hippocampus during developmental period. Impairment of developmental synapse pruning by activated microglia might elicit abnormal behaviors accompanied with prenatal VPA exposure.

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**Poster**

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**Topic:** B.12. Glial Mechanisms

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National Natural Science Foundation of China 81603503

Key Technologies R & D Program of Sichuan Province 2015SZ0058-5

**Title:** Effects of microglial phenotypic on hippocampal neurogenesis and depressive-like behavior in CMS-exposed mice

**Authors: *J. ZHANG**, J. ZHANG, L. ZHANG, H. HE, L. MO, M. WANG, Y. HAN, Z. YOU
Sch. of Life Sci. and Technology, Ctr. for Informational Biol., Univ. of Electronic Sci. and Technol. of, sichuan, China

**Abstract:** Major depressive disorder (MDD) is a mood disorder of multifactorial origin and a significant contributor to the global burden of disease. The effective antidepressant treatments are insufficient as result of the considerable heterogeneity of depression and a lack of defined etiology. Growing body evidence from human postmortem and animal researches suggests that the change in structure and function of microglia contributes to the physiopathology of major depression disorders and associated deficits in neuroplasticity and neurogenesis. Recent studies reported that groups of individuals with depression demonstrated significantly changes in the levels of a variety of inflammatory biomarkers in central nervous system. However, the relationship between microglia-mediated neuroinflammation and hippocampal neurogenesis remain poorly understood. In this study, we provided an underlying mechanism by which microglial phenotype transformed by stress from resting state to M1 state suppresses hippocampal neurogenesis and results in depressive-like behavior. The animal model of depression was established by chronic mild stress. Base on the data from behavioral test, the mice were divided in two group, including high-susceptible to stress (HS) and low-susceptible to stress (LS). The neurogenesis and microglial morphology was examined by immunohistochemistry. And the microglial phenotype was assessed by qRT-PCR. The results showed the microglia polarized to M1 state in HS group. And microglial M1 polarization and depressive-like behavior were positively correlated. Hippocampal neurogenesis was decreased in HS group, and associated with depressive-like behavior. In a separate in vitro experiment, IFN-γ-induced M1 primary microglia suppressed neural stem cell proliferation, differentiation, and survival. These results suggested that the phenotypic polarization of microglia contribute to neuroinflammation, hippocampal neurogenesis and stress-induced depressive symptomatology.

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**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

**Support:** Aresty Research Center, Rutgers University

Graduate School of Biological Sciences, Rutgers University

NIH RO1 grant #NS089578

**Title:** A potential role for class IIa HDACs in mediating neuroinflammation following traumatic brain injury

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**Abstract:** Each year millions of Americans will suffer either a traumatic injury to the brain or the spinal cord. At present, there is no effective cure. One major factor preventing recovery within the central nervous system is ongoing neuroinflammation triggered by the injury. Prolonged inflammation can lead to continued loss of neurons and consequently loss of function. A major player in the inflammatory response is microglia, the brain's resident immune cells. Microglia are highly ramified at "rest" but change into rounded phagocytic cells following injury. The mechanism associated with this activation and morphological change is poorly understood.
To better understand the molecular mechanisms that might be responsible for control of microglial activation, we have focused our attention on class IIa zinc dependent histone deacetylases (HDACs). Many members of this class (e.g., HDAC4, 5 and 7) are expressed in macrophages and microglia and seem to play an important role in innate immune responses. Classically HDACs were recognized as enzymes that deacetylated the lysine residues of histones within the chromatin, regulating gene expression. They are balanced by other enzymes termed histone acetyl transferases. The class IIa HDACs are unique in that they possess minimal enzymatic activity and their actions depend on forming complexes with class I HDACs and transcription factors. Further, their function is dependent on their cellular location which is determined by their phosphorylation state. When phosphorylated they are sequestered in the

cytoplasm. When dephosphorylated they enter the nucleus to regulate gene expression. In mice subjected to traumatic brain injury (TBI), we observed intense immunoreactivity for HDAC7 within activated microglia and reactive astrocytes. Sham-operates and naive controls showed minimal staining for HDAC7 in microglia and astrocytes. In-vitro studies using activated, immortalized microglia (BV2 cells) and primary cultures of astrocytes confirmed cellular expression of HDAC7. Our results indicate that increased HDAC7 expression may mediate the inflammatory response after neural injuries.

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**Poster**

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**Program#/Poster#:** 564.13/D49

**Topic:** B.12. Glial Mechanisms

**Support:** NIH Grant NS078247

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**Title:** Cathepsin B contributes to NLRP3 inflammasome activation via lysosomal membrane permeabilization in mouse microglial cells exposed to Rotenone and LPS

**Authors: V. LAWANA**[1], N. SINGH[1], H. JIN[1], V. ANANTHARAM[2], A. G. KANTHASAMY[2], *A. KANTHASAMY[1]
[1]Biomed. Sci., [2]Biomed Sci, Iowa Ctr. for Advanced Neurotoxicology, Iowa State Univ., Ames, IA

**Abstract:** Parkinson’s Disease (PD) is a progressive neurodegenerative disease characterized by the pronounced loss of dopaminergic neurons. In recent years, mitochondria dependent oxidative stress and inflammation have been implicated in the mechanism of dopaminergic neurodegeneration. In this context, the activation of innate immune response is becoming a pivotal component of neurodegeneration. Here we investigated the mechanisms by which minimally toxic concentration of LPS and rotenone (ROT) elicited microglial activation response via NOD-like receptor containing a pyrin domain 3 (NLRP3) inflammasome using mouse microglial BV2 cells and primary microglia. Upon sequential treatment of BV2 cells with ROT and LPS, a marked time-dependent increase in ROS generation, mitochondrial membrane potential (MMP) collapse was found to accompany an impaired autophagolysosomal system (ALS). Moreover, a concomitant increase in the activation of cathepsin B (cath B), a lysosomal protease, and NLRP3 inflammasome as evidenced by a marked increase in NLRP3 expression

and secretion of IL-1β and caspase-1 were also evidenced in these cells. Additionally, ROT/LPS exposure also lead to the extracellular release of high-mobility group box 1 (HMGB1) protein, further enhancing microglial activation. Intriguingly, blocking cath B activation by RNAi or pharmacological blockade using CA074 attenuated NLRP3 inflammasome activation, ROS generation and mitochondrial dysfunction, as was well as accumulation of autophagic vacuoles (AVs) and lysosomal destabilization in ROT/LPS treated microglial cells. Conversely, cath B overexpression accentuated the levels of NLRP3 inflammasome markers and p62, an autophagy adaptor protein in ROT and LPS treated cells indicative of impaired autophagic flux mechanisms. Our results indicate that low non-cytotoxic concentrations of ROT and LPS, amplifies NLRP3-dependent inflammatory response via enhanced lysosomal membrane permeabilization (LMP) and subsequent release of cath B into the cytosol by controlling the activation of the autophagy mechanism.

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**Poster**

**564. Microglia in Disease**

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**Program#/Poster#:** 564.14/D50

**Topic:** B.12. Glial Mechanisms

**Support:** NIH

**Title:** Microglia priming through maternal immune stress influences brain function and behavior

**Authors: *L. N. HAYES**[1], K. AN[2], M. KIM[2], R. NARODU[3], A. J. CHANG[3], G. DOLEN[3], A. SAWA[2,3]
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**Abstract:** Genomic and molecular studies suggest a role for immune dysfunction in psychiatric conditions. Furthermore, prenatal infections in mothers lead to an increased risk for psychiatric disorders in the offspring. However, the mechanisms in the pathological trajectory are unclear. We hypothesize that early immune activation permanently alters microglia (MG) function, which effects neural circuit development, immune responsiveness, and adult behavior. We can test this by using the maternal immune activation (MIA) model that delivers polyinosinic:polycytidylic acid to pregnant dams at embryonic day 9.5. In the MIA offspring, we observed several behavioral alterations relevant to psychiatric conditions, including psychostimulant hypersensitivity and dysfunctional social behaviors. We measured MG activation in response to lipopolysaccharide (LPS) *in vitro* and found that both embryonic and adult MG, especially from

the striatum, showed reduced TNFα and IL-6 suggesting a blunted MG responsiveness. Furthermore, MIA animals showed increased MG activation and survival factors at baseline as measured by CD68, IL-34, and TNFα in the striatum. We stimulated the mice with LPS *in vivo* and observed induction of IL-6 in the striatum and IL-4 in the cortex only in MIA mice. However, surprisingly, we found increased variability in the expression of major proinflammatory cytokines, TNFα and IL1β in MIA animals. The correlation of TNFα and IL-1β expressions in both cortex and striatum, only in MIA, suggests the program of transcriptional response maybe dysregulated after MIA. We now evaluate MG specific RNA sequencing results to discover other transcriptional programs that are desynchronized by the early immune stress. We evaluated the MG maturation because MG play a critical role in neural circuit development. We found an increase in MG density in the dorsal striatum at postnatal day (P)3 that was unsustained resulting in a decreased MG density and activation in the dorsal and ventral striatum by P30. We quantified the spine density of medium spiny neurons in the striatum to determine if the decreased MG density influenced spine formation, but we observed a normal spine density. Now we extend our studies to evaluate other physiosiological properties. Altogether, we argue that early maternal immune stress constitutes a priming stimulus for microglia in the offspring. These primed microglia show a disrupted developmental trajectory, immune responsiveness, and gene expression program. We are now investigating how dysfunctional microglia impact neural circuitry and whether together these are causal to the observed behavioral deficits.

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**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

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Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning(NRF-2017R1A2B4002922)

**Title:** Morphological and ultrastructural characterization of NG2 glia in the striatum of rats subjected to the mitochondrial toxin 3-nitropropionic acid

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**Abstract:** NG2 glia, which are characterized by expression of chondroitin sulfate proteoglycan, have been recognized as a distinct class of glial cells in the mammalian central nervous system (CNS). Recently, it has been reported that NG2 glia can be activated in response to a variety of CNS insults, and their activation is closely related to astroglial and microglial reactions. In this study, we examined the spatiotemporal distribution profiles and detailed morphological characteristics of resting and reactive NG2 glia in the striatum of rats treated with a mitochondrial toxin 3-nitropropionic acid (3-NP), which induces pan-necrosis of all cell types in the lesion core and reactive astrogliosis in the perilesional area. In the striatum of saline-treated control rats, weak NG2 immunoreactivity was restricted to small stellate-shaped cell, typical NG2 glia. However, prominent NG2 expression was also noted on vasculature-associated cells including pericytes and smooth muscle cells and activated microglia/macrophages in the lesion core after 3-NP injection. The lesion core was initially characterized by the absence of microglia and astrocytes due to cell death, but was infiltrated with activated microglia/macrophages, most of which revealed NG2 immunoreactivity, over a 7-day period post-injection. In parallel with microglial infiltration, resting NG2 glia began to change into the reactive phenotype including larger cell bodies and thicker processes. The activation and proliferation of NG2 glia were first observed at the edge of the lesion core and were gradually found within the epicenter, eventually in all areas of the lesion core at 7 days post-lesion, which was both spatially and temporally correlated with the infiltrating microglia/macrophages. Reactive NG2 glia revealed progressively more complicated processes-bearing morphology during the experimental period until 28 days, when they formed the structures surrounding the cystic cavities by linking to each other, being devoid of glial fibrillary acidic protein-positive astrocytes. Immunoelectron microscopic findings demonstrated that NG2 glia were characterized by large euchromatic nuclei with prominent nucleoli and well-developed Golgi-complex. Thus, our data provide the evidence that the activation of NG2 glia could be attributed to the possible interaction with infiltrating microglia/macrophages. In addition, the distribution pattern of reactive NG2 glia forming the complex network in the lesion core and particularly the walls of the cystic cavities suggest that NG2 glia participate in fibrotic scar formation and tissue remodeling after brain insults.

**Disclosures: X. Jin:** None. **T. Riew:** None. **H. Kim:** None. **J. Choi:** None. **M. Lee:** None.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.16/D52

**Topic:** B.12. Glial Mechanisms

**Support:** Intramural Research Program of the NIH

**Title:** Norepinephrine deficiency accelerates ascending sequential neurodegeneration and progression of non-motor/motor symptoms in an inflammatory Parkinson's Diseases mouse model

**Authors: *S. SONG**[1], S.-H. CHEN[1], S. S. MOY[2], Q. WANG[3], J.-S. HONG[4]
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**Abstract:** We have previously shown that mice injected with a single dose of LPS (5 mg/kg; ip) displayed a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and PD-like motor dysfunction within 10 months. Since the well-known anti-inflammatory property of norepinephrine (NE) and its important role in regulating non-motor behaviors, we hypothesized that prior damage of locus coeruleus (LC) noradrenergic neurons in this inflammatory mouse model may render neurons in upper brain regions more vulnerable to inflammation-mediated degeneration and accelerate the appearance of non-motor and motor symptoms. For this purpose, male mice received a single systemic injection of DSP-4, a selective noradrenergic neurotoxin one week before LPS dosing. We found that DSP-4 intoxication potentiated LPS-induced sequential neurodegeneration in SNpc, hippocampus, and cortex. Mechanistic study revealed that DSP-4 enhanced LPS-induced microglial activation and subsequently elevated neuronal oxidative stress in different brain regions through a sequential ascending pattern. To further demonstrate the effects of DSP-4 on non-motor and motor symptoms in LPS model, the related functional behavior tests were performed at different time points after treatment. Consistent with the enhanced neurodegeneration, DSP-4 intoxication accelerated the progressive deficits of non-motor symptoms including hyposmia, constipation, anxiety, sociability, exaggerated startle response and memory loss, and motor symptoms including decreased rotarod activity, grip strength, and gait disturbance in LPS-injected mice. In summary, our studies not only provided an excellent model that recapitulates the features of sequential neuron loss and the progression of motor/non-motor symptoms of PD, but also revealed the critical role of early LC noradrenergic neuron damage in the pathogenesis of PD.

**Disclosures: S. Song:** None. **S. Chen:** None. **S.S. Moy:** None. **Q. Wang:** None. **J. Hong:** None.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

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**Program#/Poster#:** 564.17/D53

**Topic:** B.12. Glial Mechanisms

**Support:** T32 NS 082145

1F31 MH102070-01A

R01 MH090127

VA I01BX003195

**Title:** Brain-derived neurotrophic factor-TrkB receptor signaling negatively regulates the pro-inflammatory response of microglia to lipopolysaccharide

**Authors: *A. M. GARRISON**[1], J. M. PARROTT[2], L. REDUS[3], J. C. O'CONNOR[4]
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**Abstract:** Peripheral inflammation concurrently increases microglial pro-inflammatory cytokine expression and reduces expression of brain-derived neurotrophic factor (BDNF) in the brain. Both neuroinflammation and reduced BDNF have been implicated as contributing factors in the pathogenesis of anxiety and depression, but the interaction between these factors has not been thoroughly investigated. We have previously observed that BDNF deficient ($BDNF^{+/-}$) mice exhibit an exaggerated neuroinflammatory and depressive-like behavioral response to peripheral lipopolysaccharide (LPS), suggesting that endogenous BDNF could have an important negative regulatory role in the neuroinflammatory process. Therefore, we utilized the BV-2 mouse microglial cell line to investigate directly whether BDNF regulates the pro-inflammatory response to LPS challenge. BV-2 microglia were treated with vehicle or LPS (100 ng/ml) in the presence or absence of BDNF (0, 1, 10, 100 ng/ml). After 6h, tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), IL-10, and ionized calcium-binding adapter molecule-1 (IBA1) mRNA transcripts were increased in response to LPS along with extracellular nitrite. Interestingly, co-treatment with BDNF significantly reduced the induction of pro-inflammatory factors, while significantly increasing expression of the anti-inflammatory cytokine IL-10. To confirm whether the effect of BDNF is mediated via activation of the high affinity TrkB receptor, BV-2 cells were challenged with LPS in the presence or absence of 7,8-dihydroxyflavone (0.1, 1, 10, 100 μM). Activation of TrkB receptor using the selective agonist completely replicated the modulatory effect of BDNF at 10 and 100 μM. These data suggest that BDNF can modulate the inflammatory responses of microglia, which may be involved in conferring vulnerability/resilience to stress- or inflammation-induced psychiatric disorders.

**Disclosures:** **A.M. Garrison:** None. **J.M. Parrott:** None. **L. Redus:** None. **J.C. O'Connor:** None.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

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**Program#/Poster#:** 564.18/D54

**Topic:** B.12. Glial Mechanisms

**Support:** NIH Grant R01NS084298

CHDI

HDSA

**Title:** Microglia mediate early loss of specific synaptic connections in Huntington's disease

**Authors: *D. WILTON**[1], M. HELLER[1], A. DAGGETT[2], A. KIM[1], A. FROUIN[1], M. ESZES[3], R. L. FAULL[3], X. W. YANG[2], B. A. STEVENS[1]
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**Abstract:** Synaptic dysfunction is becoming increasingly recognized as one of the earliest pathological events in many neurodegenerative diseases, including Huntington's disease (HD). Despite HD being a monogentic disease with a well characterized pathology, the molecular mechanisms underlying synaptic dysfunction remain poorly understood. In this study, we show that loss of specific synaptic connections can be observed as early as 3 months in multiple slow progressive Huntington's disease mouse models prior to the onset of motor or cognitive impairment and that microglia contribute to this via a complement-dependent mechanism. During development, synapse loss as a result of pruning is a normal and highly regulated process required for the correct wiring of the brain. Microglia together with complement proteins, a key component of the innate immune system, play a key role in regulating this process (Stevens et al, 2007; Schafer et al., 2012; Paolicelli et al., 2011). Here we provide evidence that aspects of this developmental mechanism are aberrantly reactivated in Huntington's disease mouse models leading to the loss of specific synaptic inputs. Using super resolution imaging, genetic ablation and viral-mediated labeling, we demonstrate that complement proteins selectively target cortico-striatal connections for engulfment by microglia and that this process requires mutant huntingtin expression in both striatal and cortical neurons. Moreover, blocking this process at multiple levels through both genetic and antibody mediated approaches is able to prevent synaptic pathology. Notably, we find that aspects of this mechanism may also be conserved in human disease, as post-mortem tissue from Huntington's disease patients reveals evidence of synapse loss, complement deposition at synapses and phagocytic microglia. Together these findings demonstrate that elements of the innate immune system previously only thought to be involved

in late stage inflammation mediate the loss of specific synaptic connections early in Huntington's disease pathology.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.19/D55

**Topic:** B.12. Glial Mechanisms

**Support:** Ministry of Education (MOE) Academic Research Fund (AcRF) Tier-1 Grant (WBS No: R-181-000-153-112)

**Title:** MiR-146a mediates tumorigenic gene expression by targeting SMAD4 in glioma-associated microglia

**Authors:** ***A. KARTHIKEYAN**[1], N. GUPTA[1], L. LEI[3], C. TANG[4], B. T. ANG[4], K. MALLILANKARAMAN[2], E. A. LING[1], S. T. DHEEN[1]
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**Abstract:** Gliomas are malignant brain tumors that show complex heterogeneity at the cellular and molecular level. In addition to the neoplastic cells in a glioma, microglia form a significant portion of the tumor mass and secrete soluble factors that aid in the tumorigenesis. The Transforming Growth Factor β (TGFβ) is a pleiotropic cytokine that exerts both tumor suppressive and tumor promoting effects, depending on a cellular and gene specific context. TGFβ is known to be enriched in higher-grade glioma tumors sand glioma-associated microglia; however, the role of its signaling pathway in glioma progression is poorly understood. This study investigates the role of SMAD4, a key mediator of the TGFβ signaling pathway, found in glioma associated microglia on tumorigenesis. *In vitro* analysis showed that SMAD4 expression levels were increased in microglia upon glioma conditioned medium (GCM) treatment. On the other hand, miR-146a which is predicted to target SMAD4 was found to be downregulated in microglia upon GCM treatment. Using loss- and gain-of function studies and luciferase assay, SMAD4 was confirmed to be a direct target of miR-146a in microglia. Overexpression of miR-146a in microglia suppressed the expression of tumor supportive factors, VEGFa and MMP9 in microglia, and conversely, miR-146a inhibition, resulted in an increase in the expression of these factors. Stable knockdown of SMAD4 in microglia revealed a decrease in the expression level of MMP9 as compared to control microglia, indicating a crucial role for SMAD4 in regulating tumor supportive genes in microglia. Taken together, this study shows that miR-146a targets

SMAD4, which mediates the regulation of tumor supportive genes in glioma-associated microglia. Further studies are needed to evaluate therapeutic potential of miR-146a in suppressing microglia specific tumor supportive gene expression in gliomas.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.20/D56

**Topic:** B.12. Glial Mechanisms

**Support:** NIH FG17801

**Title:** Nicotine increases limbic microglial expression and cocaine self-administration in the adolescent rat by a D2 receptor mechanism

**Authors: *K. LINKER**[1], M. A. WOOD[3], P. TAWADROUS[2], F. M. LESLIE[4]
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**Abstract:** Epidemiological studies indicate that the vast majority of smokers begin tobacco use during adolescence, and rodent studies demonstrate that adolescent nicotine exposure has unique effects on brain and behavior. It is well established that adolescent nicotine exposure increases subsequent cocaine-associated behaviors. Specifically, our lab has demonstrated that brief (4-day), low dose (60μg/kg/day) nicotine pretreatment during early adolescence, but not adulthood, increases subsequent cocaine-self administration in rats. This nicotine pretreatment also induces age-specific changes in D2 receptor sensitivity. To assess if D2 receptors are necessary for the nicotine-induced increase in cocaine reinforcement, the D2 antagonist, raclopride (.5mg/kg/day), was administered during nicotine pretreatment. We have shown that this D2 receptor antagonism blocks adolescent nicotine-induced increases in cocaine self-administration. This finding aligns with literature demonstrating that adolescent nicotine exposure uniquely alters dopaminergic and limbic function. While multiple studies have elucidated age-specific changes in neurons after nicotine exposure, few have looked at microglia. However, microglia are in constant communication with neurons, are important during brain development and express D2 receptors. Whereas many drugs of abuse activate microglia, we have confirmed that nicotine suppresses microglia in the adult brain. We now show, however, that brief low dose early adolescent nicotine exposure increases microglia marker (IBA1) expression and microglia cell number. These increases in microglia are specific to the nucleus accumbens (NAc) and basolateral amygdala (BLA), two regions that are associated with reward and are actively maturing during

adolescence. Both these regions also have dopamine innervation and express D2 receptors. In alignment with our behavioral data, administering the D2 antagonist, raclopride, during the nicotine pretreatment blocks increased microglial expression in the NAc and BLA. Together, these studies demonstrate that D2 receptors are necessary for nicotine-induced increases in microglia and cocaine self-administration in early adolescent rats.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.21/D57

**Topic:** B.12. Glial Mechanisms

**Title:** Acetaminophen rescues microglial defects and cognitive impairment in the DP16 murine model of Down syndrome

**Authors: *B. PINTO**[1,2], A. PETRETTO[3], M. BARTOLUCCI[3], L. PERLINI[1], L. CANCEDDA[1]
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**Abstract:** Down syndrome (DS) is caused by the presence of a supernumerary chromosome 21 (Chr21), and it represents the most frequent cause of genetic intellectual disability. Interestingly, some of the genes located on Chr21 are essential for the correct function of the immune system, and DS individuals often suffer from immunological disorders. Microglia are the main immune cells of the brain and play important roles in its development and mechanisms essential for cognitive functions. However, it is not known whether microglial defects are involved in the cognitive deficits associated with DS. Here, we investigated the presence of microglial alterations and their possible implication in cognitive impairment in the DP16 murine model of DS. At postnatal day (P22), we found microglial morphological defects both in the hippocampus and somatosensory cortex of DP16 mice. The trisomic microglia showed enlarged cell body and decreased ramifications, morphological alterations typically associated with increased microglial phagocytic activity. Interestingly, administration of acetaminophen, a commonly used non-steroidal anti-inflammatory drug, restored the cell-body size to the WT level, with no significant effect on ramifications. Remarkably, this treatment had a paradoxical effect on WT microglia: it caused an enlargement of the cell body and a significant decrease of the ramification. The morphological alterations of microglia correlated with cognitive performance of the mice in the novel object recognition test: DP16 mice showed cognitive impairment, and the acetaminophen treatment improved their cognitive performance to untreated WT littermate levels; WT mice showed cognitive impairment upon acetaminophen treatment. JAK1/2 are important signaling kinases downstream to many pro-inflammatory cytokines including Inferon Gamma (IFNγ),

which is dysregulated in DS and strongly expressed in microglia. Remarkably, the administration of Ruxolitinib (a JAK1/2 inhibitor) recovered microglial morphology in the DP16 mice, without causing a cognitive impairment in the WT littermates. Altogether, our data suggest a possible involvement of microglia in the cognitive defects observed in adult DS animals. Thus, microglia may be a potential target for interventional therapies for the treatment of cognitive impairment in DS individuals and acetaminophen, a widely used, safe and over-the-counter drug, could be a potential treatment for cognitive defects in DS.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

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**Topic:** B.12. Glial Mechanisms

**Support:** NIH T32MH073124-06

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Autism Speaks Foundation (#7567)

Jane Botsford Johnson Foundation

**Title:** Microglia isolated from offspring of dams with allergic asthma exhibit methylation and transcriptional alterations to genes dysregulated in autism

**Authors: *A. VOGEL CIERNIA**, M. CAREAGA, J. LASALLE, P. ASHWOOD
Univ. of California Davis, Davis, CA

**Abstract:** Dysregulation in immune responses during pregnancy increase the risk of a having a child with an autism spectrum disorder (ASD). Asthma is one of the most common chronic diseases among pregnant women, and symptoms often worsen during pregnancy. We recently developed a mouse model of maternal allergic asthma (MAA) that induces alterations in behavioral outcomes of the offspring including changes in sociability, repetitive and perseverative behaviors. Since epigenetic changes help a static genome adapt to the maternal

environment, activation of the immune system may epigenetically alter fetal microglia, which are the brain's resident immune cells. We therefore sought to test the hypothesis that transcriptional and DNA methylation alterations to microglia may be involved in the long-lived behavioral alterations in offspring exposed to gestational MAA. Here we used the genome-wide approaches of whole genome bisulfite sequencing to examine DNA methylation combined with RNA sequencing to examine gene expression in microglia from juvenile MAA offspring. Differential expression analysis revealed significant alterations in the epigenome of microglia from MAA compared to PBS treated control offspring and identified genes involved in controlling microglial sensitivity to the environment and shaping both synaptic and long-range neuronal connections in the developing brain. Differentially methylated regions (DMRs) in MAA microglia were enriched for immune signaling pathways and important microglial developmental transcription factor binding motifs. Differentially expressed and DMR associated genes significantly overlapped genes with altered expression in human ASD cortex, supporting a role for microglia in the pathogenesis of ASD.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.23/D59

**Topic:** B.12. Glial Mechanisms

**Support:** Intra-Cellular Therapies, Inc.

Intramural funds from the Centers for Disease Control and Prevention-National Institute for Occupational Safety and Health

**Title:** ITI-214, a novel and selective phosphodiesterase inhibitor, reverses LPS-induced inflammatory responses in BV2 cells and in mice

**Authors:** ***J. J. O'BRIEN**[1], L. P. WENNOGLE[2], J. P. O'CALLAGHAN[4], D. B. MILLER[4], S. DUTHEIL[3], G. L. SNYDER[3], R. E. DAVIS[2], J. P. HENDRICK[1]
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**Abstract:** ITI-214 is a a potent and specific inhibitor of PDE1 that has important beneficial effects in mice, rats, and dogs (Li, et al., 2016, J. Med. Chem. 59:1149; Snyder et al., 2016, Psychopharm. 233:3113). In humans, this compound has been well-tolerated across a broad range of doses in multiple Phase I studies. It is currently in development for the treatment of

CNS and non-CNS diseases. Enzymes of the PDE1 family hydrolyze both cAMP and cGMP and are activated by calcium. PDE1 is expressed in microglia, and isofoms of PDE1 may be involved in the regulation of inflammatory responses by modulating microglial intracellular cyclic nucleotide pools. Inhibition of PDE1 activity in microglia, therefore, may provide a promising avenue for therapeutic intervention in CNS disorders in which inflammation is a prominent feature. Increasing intracellular cGMP by either stimulating production or inhibiting hydrolysis has been shown to attenuate lipopolysaccharide (LPS)-induced responses in microglia (Baltrons et al., 2008, Neurochem Res 33:2427; Pifarre et al., 2010, J Neurochem 112:807; Zhao et al, 2011, Intl Immunopharm 11:468). Additionally, cGMP has been shown to play a role in LPS-induced motility of microglia (Scheiblich et al., 2014, Brain Res 1564:9). Cyclic adenosine monophosphate (cAMP) is also a key regulator of inflammatory responses. We tested the effects of ITI-214 in LPS-stimulated immortalized murine microglial cells, BV2 cells, by measuring changes in cytokine release using ELISA and in gene expression using RT-qPCR. PDE1 inhibition with ITI-214 prevented LPS-induced increases in TNFalpha release from BV2 cells. Similarly, LPS-induced increases in TNFalpha, IL-1beta, and Ccl2 mRNA expression were reduced by greater than 50 percent both in BV2 cells and in mice (p is less than 0.01) upon PDE1 inhibition. Transcriptome analysis has shown that ITI-214 triggers changes in a unique, signature pattern of genes under LPS-induced and basal conditions in BV2 cells. Our data support the importance of ITI-214 in regulating microglial responses and its use in treating neuroinflammation associated with neurodegenerative and neuropsychiatric disease.

**Disclosures:** **J.J. O'Brien:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **L.P. Wennogle:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **J.P. O'Callaghan:** 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 funds from the Centers for Disease Control and Prevention-National Institute for Occupational Safety and Health. **D.B. Miller:** 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 funds from the Centers for Disease Control and Prevention-National Institute for Occupational Safety and Health. **S. Dutheil:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **G.L. Snyder:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **R.E. Davis:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc. **J.P. Hendrick:** A. Employment/Salary (full or part-time):; Intra-Cellular Therapies, Inc.

**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.24/D60

**Topic:** B.12. Glial Mechanisms

**Support:** DA043138

DA035203

DA036157

**Title:** $CX_3CR_1$ deficiency elevates neuroinflammation and potentiates cocaine-mediated psychomotor activity

**Authors: *M. GUO**[1], Y. KOOK[2], E. CHIVERO[2], S. CALLEN[2], S. BUCH[3]
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**Abstract:** Emerging evidence demonstrates a close link between neuroinflammation and drug addiction. The CX3CL1/CX3CR1 axis plays critical roles in both neuron- microglia communication and in maintaining microglial quiescence. Previous studies have reported increased inflammatory responses, as well as increased LPS-mediated neurotoxicity in the CX3CR1-GFP mice (CX3CR1 deficiency). The detailed signaling mechanism(s) underlying such exaggerated immune responses and what if any, effect CX3CR1 deficiency has on other CNS cells types such as the astrocytes and neurons, however, remains unexplored. In the current study, we demonstrate increased basal glial (microglia and astrocytes) activation in CX3CR1-GFP mice compared to the wild type controls. CX3CR1-GFP mice exhibited increased expression of the inflammasome marker NLRP3 in microglia, astrocytes and neurons. Intriguingly, in response to phsychotimulant such as cocaine, there was an even more increased inflammatory response. These findings were complemented with behavioral assays, wherein the CX3CR1-GFP mice showed increased cocaine-mediated locomotor activity and condition place preference compared with the controls. Taken together our findings suggest a link between the NLRP3 inflammasome signaling, neuroinflammation the development of drug addiction in the context of CX3CR1 deficiency. Modulation of the NLRP3 pathway could thus be targeted and developed as an alternative approach for the treatment of drug addiction.

**Disclosures:** **M. Guo:** None. **Y. Kook:** None. **E. Chivero:** None. **S. Callen:** None. **S. Buch:** None.

**Poster**

**564. Microglia in Disease**

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**Topic:** B.12. Glial Mechanisms

**Support:** NIH Grant R01NS097195-01 (JZ)

NIH Grant R03 NS094071-01 (YH)

**Title:** A novel mechanism of action for carbonoxolone via the inhibition of mitochondrial glutaminase

**Authors: Y. LI**[1], J. ZHENG[2], *Y. HUANG[3]
[1]Shanghai Tenth People's Hosp. affiliated to Tongji Univ. Sch. of Med., Shanghai, China; [2]Dept Pharmacol. & Exptl. Neurosci., Omaha, NE; [3]Univ. Nebraska Med. Ctr., Omaha, NE

**Abstract:** Carbenoxolone (CBX) is a glycyrrhetinic acid derivative that is well known to inhibit endogenous glucocorticoids by potently inhibiting 11b-hydroxysteroid dehydrogenase, and it is also known in cellular physiology as an effective and water-soluble gap junction blocker. There are many reports of the neural modulating effects of CBX. However, given the drug's multiple sites of action, it has been difficult to pinpoint the exact mechanism of action. Here we found that CBX reduced intracellular glutamate levels, indicating it may directly affect glutamate metabolism, and more specifically on glutaminolysis, a metabolic process that converts glutamine to glutamate. Glutaminase is the mitochondrial enzyme that catalyzes the first step of glutaminolysis. Two genes encode at least four isoforms of glutaminase in humans. GLS1 gene encodes isoforms kidney-type glutaminase (KGA) and glutaminase C (GAC) through alternative splicing, whereas GLS2 gene encodes liver-type glutaminase isoforms. Using our established glutaminase activity assay based on rat brain mitochondrial lysates, we demonstrated that CBX inhibited glutaminase activity in a dose dependent manner. To eliminate any irrelevant components of the enzyme reaction, we generated recombinant KGA and GAC proteins and CBX inhibited glutaminase activity in these proteins in a dose dependent manner. Pharmacological analyses of CBX-mediated inhibition of glutaminase suggest it is more potent and efficient than the commercially available 6-diazo-5-oxo-L-norleucine. Based on two recently published models of the structure basis for GLS1 inhibition, we applied the Glide docking of CBX to the GLS1 and identified that CBX binds to the interface of a tetramer of the GLS1. After confirming that CBX is an effective glutaminase inhibitor, we applied it to a macrophage-tropic HIV-1 infected primary microglia culture system that is known to have excess glutamate production and neurotoxicity. As expected, CBX potently reduced intracellular and extracellular glutamate levels and completely abolished neurotoxicity in HIV-1-infected microglia. While CBX is known as a hemichannel blocker that blocks glutamate release, we found that hemichannel blocking only modestly reduced glutamate levels, suggesting that CBX blocked the neurotoxicity of HIV-1-infected microglia through inhibition of glutaminase. This novel mechanism of action for CBX via the inhibition of mitochondrial glutaminase will have broad implications for neuroscience studies that apply CBX.

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**Poster**

**564. Microglia in Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 564.26/D62

**Topic:** B.12. Glial Mechanisms

**Support:** NIH

**Title:** Microglial activation of the nuclear GAPDH cascade mediates cognitive inflexibility in an inflammatory mouse model

**Authors: *A. RAMOS**[1], F. E. DOMINGUEZ[2], N. J. ELKINS[3], K. MAULDING[4], K. ISHIZUKA[5], A. SAWA[6]
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**Abstract:** Behavioral flexibility is one of the most important behavioral constructs that is required for adaptability. This behavior is affected under stress conditions, and its disturbance is involved in many neuropsychiatric disorders, in particular schizophrenia. Nonetheless, molecular and cellular mediators for this behavior are unclear. Also, it has been proposed that neuroinflammation and excess oxidative stress may play a role in the pathology of neuropsychiatric disorders. Therefore we wanted to study how these stressors can mediate deficits in behavioral flexibility. For that, we have specifically focused on the nuclear GAPDH (Glyceraldehyde-3-phospate Dehydrogenase) cascade since GAPDH can act as a sensor of oxidative stress: our group has reported that under stress condition, a small fraction of GAPDH is posttranslationally modified and translocated to the nuclei to mediate stress signaling (which we call the “nuclear GAPDH cascade”).
In this study, we used LPS (Lipopolysaccharide)-treated mice. We found these mice displayed behavioral inflexibility in a paradigm using rule shifting. Interestingly, the behavioral deficits were ameliorated by a compound, (1R,3R)-1,3-dymethyl-2-propargyl-1,2,3,4tetrahidrosiquinoline (called RR) that is a blocker of the nuclear GAPDH cascade (Hara *et al*. Nat Cell Biol. 2005). Therefore, we next validated whether the nuclear GAPDH cascade was indeed activated in the LPS-treated mice. We found that this cascade was selectively activated in microglia in the cortex, but not in the striatum. This region specificity is consistent with preclinical and clinical findings that the prefrontal cortex is involved in controlling behavioral flexibility. To understand how the activation of nuclear GAPDH cascade in microglia leads to neural circuitry dysfunction, we conducted molecular profiling of *in vivo* microglia by CHIP-Seq and RNA-Seq. Finally, we have introduced the conditional K225A-GAPDH$^{f/f}$ mouse model in

which lysine (K) 225 of GAPDH is replaced by alanine (A), preventing GAPDH from nuclear translocation. Using this model we genetically target microglia and corroborate the activation of the nuclear GAPDH cascade in these cells is sufficient to cause behavioral inflexibility. Taken together, we now propose that the transcriptional regulation mediated by nuclear GAPDH cascade in cortical microglia is a key molecular and cellular mediator for behavioral flexibility. Note, the potential utility of the nuclear GAPDH cascade as a clinically-available high throughput biomarker for cognitive deficits in major mental disorders is demonstrated by Dominguez *et al* in this meeting.

**Disclosures: A. Ramos:** None. **F.E. Dominguez:** None. **N.J. Elkins:** None. **K. Maulding:** None. **K. ishizuka:** None. **A. Sawa:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.01/D63

**Topic:** B.12. Glial Mechanisms

**Support:** The New Jersey Commission on Brain Injury Research (CBIR11PTJT0102)

**Title:** Mechanisms of myelin loss after mild traumatic brain injury

**Authors: *A. A. ADAMS**[1], B. J. PFISTER[2], H. A. KIM[1]
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**Abstract:** Traumatic brain injury (TBI) is one of the leading causes of hospitalization and death in the United States. The effects of TBI on myelin structures in the brain have not been characterized until fairly recently, given the assumption that myelin loss occurs as a secondary effect after axonal degeneration. Recent animal studies indicate that mild TBI (mTBI) induces primary demyelination. Importantly, myelin loss in the brain has been shown to leave previously myelinated axons more vulnerable to degeneration after a second mild injury. Appropriate axo-glial signaling is essential for the maintenance of myelin homeostasis. Axons are known to release aberrant levels of glutamate following injury induced increases in excitatory neurotransmission. Our recent study demonstrates that neurons distal to the site of injury remain hyperexcitable for up to one hour after *in vitro* injury. Previous studies have suggested that NMDAR mediated calcium entry in oligodendrocytes (OL) may play a role in myelin damage after CNS injury. We hypothesize that glutamate release from hyperexcited axons activates glutamate mediated calcium dependent signaling in OLs that disrupts myelin homeostasis. Using an *in vitro* stretch-injury device combined with a neuron-OL co-culture system, we show that injury to neurons results in intracellular calcium increases in non-injured neighboring OLs, suggesting the occurrence of an injury induced neuron to OL signaling event. Further, NMDA

treatment in mature OL monoculture induces downregulation of myelin basic protein expression in the absence of cell death. The effect of NMDA was attenuated upon inhibition of the ERK pathway. I*n vivo*, we show that mild fluid percussion type injury in rodents increases ERK activity in CC1+ cells within white matter tracts. Our findings suggest that neuronal hyperexcitability and aberrant glutamate release may initiate calcium mediated ERK activation in the OL that results in loss of myelin.

**Disclosures: A.A. Adams:** None. **B.J. Pfister:** None. **H.A. Kim:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.02/E1

**Topic:** B.12. Glial Mechanisms

**Title:** Chd7 collaborates with Sox2 to regulate activation of oligodendrocyte precursor cells after spinal cord injury

**Authors: *T. DOI**[1,2], T. OGATA[1], Y. SAWADA[1], S. TANAKA[2], M. NAGAO[1]
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**Abstract:** Oligodendrocyte precursor cells (OPCs) act as a reservoir of new oligodendrocytes (OLs) in homeostatic and pathological conditions. OPCs are activated in response to traumatic neural injury such as spinal cord injury (SCI), to generate myelinating OLs. This OPC activation is the first step in the process of remyelination, and proper control of OPC activation is important for tissue repair following SCI. However, the molecular mechanisms underlying OPC activation, especially its epigenetic regulation, remain mostly unknown. Here we demonstrate that Chromodomain helicase DNA binding protein 7 (Chd7), which is a member of the Chd family of chromatin remodelers, regulates the proliferation and identity maintenance of OPCs following SCI. Chd7 was expressed in OPCs in the intact adult spinal cord, and its expression level was upregulated with a concomitant increase in Sox2 expression at 3 days post injury (dpi) in a mouse model of SCI. OPC-specific deletion of *Chd7* (*PDGFRα-CreER*;*Chd7*$^{flox/flox}$;*CAG-CAT-EGFP* mice, *Chd7* cKO) in the injured spinal cord lead to reduced OPC proliferation and the loss of OPC identity, but did not affect the survival of OPCs at 3 dpi. Furthermore, *Chd7* cKO attenuated OPC differentiation and remyelination at 42 dpi. Behavioral assessment using the Basso Mouse Scale showed that *Chd7* cKO mice performed significantly worse than control mice at 28, 35, and 42 dpi. Knockdown of Chd7 or Sox2 in cultured OPCs showed similar phenotypes to those observed in *Chd7* cKO mice. Co-immunoprecipitation experiments revealed that Chd7 and Sox2 formed a complex in cultured OPCs. We performed microarray analysis of OPCs following Chd7 knockdown and found that regulator of cell cycle (Rgcc) and protein

kinase Cθ (PKCθ) are novel target genes of Chd7 for OPC activation. Knockdown of Sox2 also reduced the expression of Rgcc and PKCθ. Chromatin immunoprecipitation analysis showed that Chd7 and Sox2 bound to the regulatory regions of Rgcc and PKCθ genes to induce their expression. Knockdown of either Rgcc or PKCθ decreased OPC proliferation and identity maintenance. Conversely, overexpression of Rgcc or PKCθ promoted OPC proliferation. Furthermore, the Chd7 knockdown phenotypes were fully restored by overexpression of both Rgcc and PKCθ. In conclusion, Chd7 is a key regulator of OPC activation, in which it cooperates with Sox2, and acts through direct induction of Rgcc and PKCθ expression after SCI.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.03/E2

**Topic:** B.12. Glial Mechanisms

**Support:** JSPS KAKENHI 25461794

**Title:** A novel system for functional analysis of adult oligodendrocyte progenitor cells

**Authors: *N. KIKUCHI(NIHONMATSU)**[1], Y. XIUJUN[4], Y. MATSUDA[2], M. WATANABE[2], K. AOKI[2], H. KONDO[3], Y. TATEBAYASHI[2]
[2]Affective disorders reserach project, [3]Technol. research, [1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [4]Second Hosp. of Hebei Med. Univ., Hebei Province, China

**Abstract:** Oligodendrocyte (OL) progenitor cells (OPCs) are widely distributed throughout the brain, both in gray and white matter. Whereas BrdU labeling studies have revealed that the cell cycle time of OPCs increased from early postnatal to adult, the production of mature OLs decreases steadily during adulthood, suggesting that adult OPCs might play a more important role in the maintenance of as-yet-unknown physical conditions than in the production of mature OLs. The lack of technique to purify and culture OPCs from adult brain is one major disadvantage of studying of their functions. We have recently established a novel method to isolate and culture OPCs from adult rat brains. In the present study, we report a progress of our OPC culture system in more detail with a finding of a novel OPC subtype. To isolate OPCs from other CSF cells, we used a step gradient composed of the 4 different buoyant densities with Optiprep™. After centrifugation, a major thick layer was apparently formed on the top of the layers containing microglia and neuronal stem cells. After this thick layer was put on the culture dishes more than for 30min, the supernatants including non-adherent cells and the debris were removed. After these purification procedures, adherent cells on the culture dishes were our

culture OPCs from adult rat brains (aOPCs-culture). aOPCs-culture effectively continued to proliferate in response to basic fibroblast growth factor (FGF2) instead of platelet-derived growth factor (PDGF). FGF2 withdrawal from aOPCs-culture increased the proportion of plexin-B3-expressing cells. Immunohistochemical studies revealed that plexin-B3 is expressed in olig2$^{+}$ aOPC-like glia cells distributed throughout the adult rat brain (plexin-B3$^{+}$ aOPCs). They could be clearly distinguished from neurons, astrocytes, microglia, OLs and strongly NG-expressing OPCs. Furthermore, cortical spreading depression (CSD), a self-propagating wave of cellular depolarization that has been implicated as a fundamental common mechanism of progressive cortical injury seen in stroke and head trauma, significantly increased densities of plexin-B3$^{+}$ aOPCs in the remote ipsilateral in compared with contralateral cortex. These data suggest that brain injuries induce unique delayed cortical oligodendro(gliosis) of plexin-B3$^{+}$ aOPCs. Taken together, our data is highly suggestive that aOPC-culture is a novel system for investigating the physiological mechanisms of OPCs in the adult brain.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.04/E3

**Topic:** B.12. Glial Mechanisms

**Title:** RyR3-mediated ER calcium release is dynamically regulated and crucial for oligodendroglial differentiation RyR3-mediated ER calcium release is dynamically regulated and crucial for oligodendroglial differentiation

**Authors: *L. XIAO**, T. LI
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**Abstract:** It has been known that dynamical intercellular calcium ($Ca^{2+}$) increase in oligodendrocyte progenitor cells (OPCs) is important to initiate differentiation,while the intracellular calcium-release channel involved in this process remains unclear. As one of the intracellular calcium channels which mediate ER calcium-release, the role of Ryanodine receptors (RyRs) in oligodendroglial development is understudied. In this study, we demonstrated that: (1) Among RyRs, RyR3 is specifically expressed in oligodendroglial lineage cells but down-regulated following OPC differentiation. The strong RyR3 positive reactions were distributed all over the cytoplasm and processes in OPCs and/or immature oligodendrocytes (imOLs), while it gradually decreased and located around the perinuclear region in mature OLs. (2) RyR3 mediated intercellular $Ca^{2+}$ waves following caffeine stimulation were correlated with their expression pattern, in which high flat $Ca^{2+}$ fluctuations and spontaneous oscillatory $Ca^{2+}$

waves were more frequently recorded in OPCs and/or imOLs. (3) RyR antagonist ryanodine pretreatment can neutralize the increase of intracellular $Ca^{2+}$ induced by OPC differentiation, and reduced the number of mature OLs. Finally (4) Knockdown RyR3 in OPCs resulted in inhibition of OPC differentiation. Taking together, our results indicated that RyR3-mediated ER $Ca^{2+}$ release is dynamically regulated and crucial for oligodendroglial differentiation.
This work is supported by CNSF (31171046) and CSTCKJCXLJRC07.

**Disclosures: L. Xiao:** None. **T. Li:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

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**Program#/Poster#:** 565.05/E4

**Topic:** B.12. Glial Mechanisms

**Support:** National Institutes of Health (R01NS089586 to S.H.K, R21NS092009 to D.E.B.)

Shriners Hospitals for Children (85500-PHI-14 to S.H. K., 84298-PHI to H.J.)

**Title:** Role of ionotropic glutamate receptor-mediated calcium signaling in oligodendrocyte regeneration after neonatal white matter injury

**Authors: *R. R. KHAWAJA**[1,2], A. AGARWAL[3], H. JEONG[2], M. MISHINA[4], D. E. BERGLES[3], S. H. KANG[5]
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**Abstract:** Oligodendrocytes (OLs) form myelin sheaths that are critical for rapid axonal conduction and metabolic support of axons in the CNS. These cells are highly vulnerable to injury, and OL loss and hypomyelination are frequently observed in numerous brain injuries. Earlier studies have suggested that NMDA receptor (NMDAR) or AMPA receptor (AMPAR)-mediated glutamate excitotoxicity is the major mechanism leading to OL damage in response to CNS insults, such as hypoxic ischemia (H/I). However, supporting *in vivo* evidence has been limited to outcomes of systemic administrations of NMDAR or AMPAR-targeted reagents, which are not cell-specific and likely impact neurons and other glia, including oligodendrocyte progenitor cells (OPCs) that express glutamate receptors and form synapses with neurons. To define the impact of glutamate receptor-dependent $Ca^{2+}$ signaling within oligodendroglia, we employed cell-specific, *in vivo* genetic approaches to selectively block NMDAR- (by deleting *Grin1,* an essential NMDAR subunit) or AMPAR-mediated $Ca^{2+}$ influx (by

overexpressing EGFP-fused *Gria2*, a $Ca^{2+}$-impermeable AMPAR subunit) into OL lineage cells in a mouse model of neonatal H/I brain injury. Removal of *Grin1* from OPCs or all oligodendroglia did not significantly change OL development, in accordance with previous results, or attenuate OL loss in response to the H/I injury. Although abolishment of AMPAR $Ca^{2+}$ signaling also did not affect the rate of normal OL development or OL loss following H/I injury, this manipulation accelerated OL lineage transition in the injured brain, promoting the formation of new OLs. These results suggest that neither NMDAR- nor AMPAR-mediated $Ca^{2+}$ influx is a major cytotoxic contributor to H/I-induced OL loss, but that AMPAR-mediated $Ca^{2+}$ signaling in OPCs or immature OLs inhibits post-injury regeneration of myelin.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.06/E5

**Topic:** B.12. Glial Mechanisms

**Support:** NHMRC Project Grant #APP1058647

**Title:** Brain-derived neurotrophic factor haploinsufficiency results in region-specific, differential oligodendroglial development in the central nervous system

**Authors: *M. NICHOLSON**[1], R. J. WOOD[1], J. FLETCHER[1], S. S. MURRAY[1,2], J. XIAO[1,2]
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**Abstract:** Myelination is a complex biological process and the extracellular factors responsible for inducing myelination in the central nervous system (CNS) remain incompletely understood. We have previously identified brain-derived neurotrophic factor (BDNF) as a key player in promoting CNS myelination during development, as BDNF heterozygous mice exhibit a severe hypomyelinating phenotype during early postnatal weeks. However, it remains controversial whether BDNF exerts an influence upon oligodendroglial cell numbers and lineage progression during CNS development *in vivo.*
To investigate this, BDNF heterozygous (HET) and BDNF wild type (WT) littermate control mice were sacrificed at P6, P9, P15 and P30. Both white and grey matter of the brain and lumbar spinal cord were immunohistochemically analysed for total density of oligodendroglial (Olig2+) cells. Further analysis of oligodendroglial development identified sub-populations of these cells at early and late stages of lineage progression; immature oligodendrocyte precursor cells (PDGFRa+) and mature oligodendrocytes (CC1+).

We found significantly fewer oligodendroglial lineage cells (Olig2+) in the dorsal white matter tracts and grey matter of the spinal cord of HET mice compared to WT controls. Whilst overall there were lower numbers of both immature and mature oligodendroglial sub-populations, proportions of these cells relative to the total oligodendroglial density remained not significantly different, indicating BDNF is likely influencing oligodendroglial proliferation and/or survival, but not differentiation and lineage progression during CNS development *in vivo*.
Interestingly, we found a significantly higher proportion of mature oligodendrocytes in both spinal cord and cortical grey matter of HET mice compared to WT controls at P6, indicating BDNF may temporally influence oligodendroglial differentiation during early postnatal development.
Together, this study reveals an important role of BDNF signalling in regulating oligodendroglial populations in different CNS regions during early postnatal development, providing a new insight into understanding the cellular and molecular signalling pathways that govern CNS myelination.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.07/E6

**Topic:** B.12. Glial Mechanisms

**Title:** Motor neuron sonic hedgehog specifies the first wave of oligodendrogenesis in the vertebrate spinal cord

**Authors: *L. STARIKOV**[1,2,3], A. SAJAN[1], A. H. KOTTMANN[1,2,3]
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**Abstract:** Oligodendrocytes are a heterogeneous group of myelin forming cells which ensheath axons to increase electrical conductance of action potentials and provide trophic support to neurons. In the spinal cord, oligodendrocytes are produced from two domains that are spatially and temporally distinct and give rise to oligodendrocyte populations that react differently to injury. The first born lineage produces ventral oligodendrocyte precursors (vOPs) from the Olig2 precursor domain in the ventricular zone. This lineage provides 80% of all mature oligodendrocytes, and is developmentally dependent on the signaling protein Sonic hedgehog (Shh). A second group of oligodendrocyte precursors, dOPs, arises two days later from the dorsally located Msx3 domain in a Shh independent manner. The mechanisms of cell lineage

specification in these two precursor domains and whether oligodendrocytes derived from them can functionally compensate for each other is not fully investigated. It is widely assumed that the relevant source of the Shh signal needed for specification of vOPs emanates from the midline, i.e. notochord and floorplate. However, due to the lack of selectivity of currently available Cre expression alleles, extra midline sources of Shh cannot be excluded. Curiously, post mitotic motor neurons (MNs) begin to express Shh when they settle in their lateral positions in the spinal cord, coinciding with a switch from MN to vOP production by the ventricular Olig2 precursor domain. By analyzing an allelic series of selective ablation of Shh expression by Olig2cre in MNs, we observe a Shh gene dose-dependent reduction in the specification and migration of vOPs from the Olig2 precursor domain without alteration in the qualitative or quantitative outcome of neurogenesis from either more ventral or more dorsal located precursor domains of the ventricular zone. The ablation of Shh from MNs mediated by ChATcre, which is expressed around the same time as Shh in MNs has no effect on vOP production. Together our results define a narrow temporal window during which Shh expression specifically from nascent MNs is essential for the production of vOPs. Our data attributes a so far unappreciated morphogenic signaling function to MNs and points to a neuronal based mechanism by which morphogenic activity can impact select precursor domains once the advanced size of the germinal niche prohibits the formation of instructive gradients.

**Disclosures:** **L. Starikov:** None. **A. Sajan:** None. **A.H. Kottmann:** None.

**Poster**

**565. Oligodendrocytes**

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**Program#/Poster#:** 565.08/E7

**Topic:** B.12. Glial Mechanisms

**Support:** Rutgers IMRT grant

NJCBIR grant CBIR11PJT012

**Title:** Calcium-dependent Erk1/2 MAPK activation following mechanical stretch injury induces myelin loss in oligodendrocytes

**Authors: *J. KIM**[1], P. GOKINA[1], M. T. LONG[2], B. J. PFISTER[2], H. A. KIM[1]
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**Abstract:** Traumatic brain injury (TBI) is caused by mechanical force to the brain, and it is associated with the disability of cognition or neuronal behavior. It has been shown that oligodendrocyte (OL) pathology following TBI that is associated with demyelination and OL

death. Since myelin is crucial for neuronal function and survival, loss of myelin is likely to impair axonal integrity, resulting in axonal degeneration. Balances in cellular signaling pathways are important for maintenance of myelin homeostasis. Disruption of calcium homeostasis or activation of MAPK following stretch injury has been shown in previous research in neurons and glial cells, leading to cell death. While axon pathology associated with TBI has been actively studied, the molecular mechanisms how myelin and OL respond to stretch injury are not fully understood.
In this study, we tested whether TBI induces changes in calcium homeostasis and Erk1/2 MAPK activity in mature OLs that may contribute to TBI-induced myelin damage. TBI-induced Erk1/2 activation and loss of myelin basic protein (MBP) were observed in mature OL within white matter tracts, such as the corpus callosum and external capsules, of the brain. To elucidate OL autonomous response to TBI, we have developed OL monocultures on deformable silicone membranes, which are later rapidly stretched to induce a mechanical stretch injury to OLs *in vitro*. The stretch injury induced efflux of calcium in mature OLs. Also, stretch injury-induced Erk1/2 activation and MBP loss were detected in mature OLs without cell death, and inhibition of Erk1/2 activation restored the MBP loss following injury. In addition, increasing intracellular calcium with a calcium ionophore was sufficient to activate Erk1/2 that results in MBP loss, while chelating intracellular calcium during the injury reduced Erk1/2 activation following injury. Altogether, we found a molecular mechanism of OL pathology that is associated with TBI: the stretch injury induces calcium-dependent Erk1/2 activation in mature OL, resulting in MBP loss.

**Disclosures:** **J. Kim:** None. **P. Gokina:** None. **M.T. Long:** None. **B.J. Pfister:** None. **H.A. Kim:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.09/E8

**Topic:** B.12. Glial Mechanisms

**Support:** A Grant-in-Aid for Scientific Research (C) of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) in Japan (25860701)

**Title:** Teneurin-4 is a transmembrane protein regulating cell adhesion and cytoskeleton organisation in oligodendrocytes

**Authors:** ***N. SUZUKI**[1], C. HAYASHI[1], N. KIKURA[1], M. HYODO[1], Y. MABUCHI[1], S. DE VEGA[2], Y. YAMADA[3], C. AKAZAWA[1]
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**Abstract:** Myelination by oligodendrocytes is essential for the rapid propagation of action potential and the maintenance of axons in the central nervous system (CNS). Cell-cell interactions between oligodendrocytes and neuronal axons or other glia cells play a role in the myelin formation. Therefore, defects in the cell-cell adhesions impair oligodendrocyte differentiation/maturation and myelination, which results in neuological disorders, such as tremors. Previously, we reported that Teneurin-4 (Ten-4), a type II tramsmembrane protein, is required for oligodendrocyte differentiation and myelination of small diameter axons in the CNS and Ten-4-deficient mice develop tremors. However, cellular and molecular functions of Ten-4 in oligodendrocytes and myelination have not been fully elucidated yet. In this study, we first found that the defects in Ten-4-deficient mice appear at the early postnatal stage, when oligodendrocyte precursor cells (OPCs) attach axons and start to differentiate into oligodendrocytes. We then investigated whether Ten-4 possesses cell adhesion activity and found that Ten-4-overexpressing cells formed larger cell aggregations, compared with control cells. Furthermore, the recombinant extracellular domain of Ten-4 (rTen-4ECD) exerted OPC attachment activity. Oligodendrocytes formed lamellar-type morphology on the rTen-4ECD-coated culture slides and myelin-like structure surrounding the rTen-4ECD-coated nanofibers. In addition, we screened Ten-4 binding proteins and found that they formed a molecular complex with a couple of intracellular proteins that contain SH3 domains and are critically involved in cell-cell adhesion and cytoskeletal organization. These results revealed that Ten-4 is a key regulator of cell adhesion and cytoskeletal organization in oligodendrocytes for CNS myelination.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.10/E9

**Topic:** B.12. Glial Mechanisms

**Support:** NIH NS080223

**Title:** Expression of Rictor in oligodendrocyte precursor cells is required for proper maturation and myelination

**Authors: *H. A. HATHAWAY**, W. B. MACKLIN
Cell and Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

**Abstract:** Mammalian target of rapamycin (mTOR) is a protein kinase that regulates a variety of cellular processes, from protein translation to cytoskeletal organization. One such process is the

differentiation of oligodendrocyte precursor cells (OPCs) into mature, myelinating oligodendrocytes. The substrate specificity of mTOR is determined through the formation of complexes with adaptor proteins. Many studies have investigated the role of Raptor-containing mTOR complex 1 (mTORC1) in oligodendrocyte development, and suggest that mTORC1 plays a role in myelination within the CNS. However, few studies support a similar role for Rictor-containing mTORC2. Here, we present the first report of Rictor playing an important role in CNS myelination. Rictor was deleted specifically from platelet-derived growth factor receptor α (PDGFRα)-expressing OPCs. These Rictor knockout mice exhibited a decrease in myelinated axons, reduced expression of myelin proteins, and fewer mature oligodendrocytes relative to wildtype littermates. Additionally, Rictor knockout mice had reduced expression of proteins involved in cytoskeletal regulation. Together, these data suggest that Rictor is required for proper oligodendrocyte maturation and myelination and that this pathway may involve cytoskeletal reorganization. These studies supported by NIH NS080223.

**Disclosures: H.A. Hathaway:** None. **W.B. Macklin:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.11/E10

**Topic:** B.12. Glial Mechanisms

**Support:** National Multiple Sclerosis Society

**Title:** Apoptosis and proliferation in mixed glial culture from connexin knockout mice

**Authors: *S. KEIL**[1], M. FREIDIN[2], C. K. ABRAMS[3]
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**Abstract:** Glial cells of the central nervous system (CNS) express a number of different connexins. These connexins are integral membrane proteins that form gap junction channels and provide a low resistance pathway for the diffusion of small molecules and ions between coupled cells. Diseases like X-linked Charcot-Marie-Tooth disease (CMT1X) and Pelizaeus-Merzbacher-Like disease 1 (PMLD1), caused by mutations in connexin 32 (Cx32), and connexin 47 (Cx47) respectively, provide evidence that oligodendrocytes rely on connexins to effectively function in the CNS. Recent data suggests that connexins may also have a role in the regulation of cell growth and resistance to apoptotic and necrotic cell death. In this experiment, we investigated the effect disrupting Cx32 and Cx47 has on the oligodendrocytes' overall capacity for proliferation and survival. Utilizing immunolabeling we examined cell proliferation, and apoptotic cell death in the different cell populations from the mixed glial cultures of Cx32KO, Cx47KO, T55I mice

when compared to wild-type controls. Total $O4^+$and $GFAP^+$ cell counts were performed. The results continue to support and highlight the critical role connexins play in oligodendrocyte and CNS myelin, and implicates further investigation into therapeutic targets.

**Disclosures: S. Keil:** None. **M. Freidin:** None. **C.K. Abrams:** None.

**Poster**

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**Topic:** B.12. Glial Mechanisms

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Rachleff Family Endowment

**Title:** Selective estrogen receptor modulators enhance remyelination in an estrogen receptor-independent manner

**Authors: K. A. RANKIN**[1], F. MEI[2], Y.-A. A. SHEN[3], S. R. MAYORAL[4], C. DESPONTS[5], D. S. LORRAIN[5], A. J. GREEN[3], R. BOVE[3], *J. R. CHAN[3]
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**Abstract:** Multiple sclerosis (MS) is a neurologic disease characterized by widespread demyelination with subsequent degeneration of axons. Remyelination of these axons has been widely proposed as a key therapeutic target to reverse clinical disability associated with disease progression. However, there is a large unmet need for FDA-approved remyelinating therapeutics for patients with MS. Utilizing a high-throughput screening platform, we identified a cluster of small molecules that enhance oligodendrocyte precursor cell (OPC) differentiation: Selective Estrogen Receptor Modulators (SERMs). While several SERMs have previously been implicated, most are not well-tolerated in patients. However, we have identified one attractive candidate FDA-approved SERM that has previously proven very tolerable in clinical trials for menopausal symptoms: Bazedoxifene (BZA). Using mouse as well as human OPCs *in vitro*, we

validated BZA's potent, cell-autonomous effects on OPC differentiation and myelination. When tested in an *in vivo* focal demyelination model, BZA showed significantly enhanced and accelerated remyelination. Lastly, we sought to validate BZA's mechanism of action through Estrogen Receptor using OPCs isolated from total ERα, ERβ and double ER knockout animals. Instead, our results indicate that BZA elicits its robust OPC differentiation and remyelination results in an ER-independent manner, in contrast to previous reports for other SERMs. Further bioinformatics profiling has led to the implication of several attractive target candidates for a variety of FDA-approved SERMs that present exciting opportunities for future drug development of remyelinating therapeutics. Our results implicate bazedoxifene as a potent regulator of remyelination that could be available at an accelerated timeline to patients, and its off-target effect has indicated a previously unknown mechanism of action through a novel receptor hit by a plethora of SERMs.

**Disclosures: K.A. Rankin:** None. **F. Mei:** None. **Y.A. Shen:** None. **S.R. Mayoral:** None. **C. Desponts:** None. **D.S. Lorrain:** None. **A.J. Green:** None. **R. Bove:** None. **J.R. Chan:** None.

**Poster**

**565. Oligodendrocytes**

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**Program#/Poster#:** 565.13/E12

**Topic:** B.12. Glial Mechanisms

**Support:** National Multiple Sclerosis Society

**Title:** Gli1 fate-labeling during postnatal development identifies sonic hedgehog (Shh) responsive cells that give rise to oligodendrocyte lineage cells during myelination and in adulthood contribute to remyelination

**Authors: *M. A. SANCHEZ**, R. C. ARMSTRONG
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**Abstract:** The forebrain oligodendrocyte population undergoes dramatic amplification to accomplish extensive myelination during postnatal development. This increased generation of oligodendrocytes correlates with sonic hedgehog (Shh) signaling in the dorsal subventricular zone (SVZ) beneath the corpus callosum (CC) (CK Tong et al., 2015, Stem Cell Reports). We examined Shh signaling relative to this wave of forebrain oligodendrogenesis to determine whether postnatal Shh-responsive cells contribute to myelination in development and to remyelination in adults. Transgenic mouse lines were generated for induced genetic fate-labeling of cells actively transcribing *Shh* or *Gli1*. *Gli1* transcription is an effective readout for canonical Shh signaling. $Shh^{CreERT2}$ mice and $Gli1^{CreERT2}$ mice were each crossed to reporter lines, either $R26^{tdTomato}$ mice to label cells with red fluorescence, or, $R26^{IAP}$ mice to label cell membranes with

alkaline phosphatase. When tamoxifen (TMX) was given i.p. on postnatal days 6-9 (P6-9), Shh ligand synthesis was prevalent in neurons of *Shh*$^{CreERT2}$ mice. In *Gli1*$^{CreERT2}$ mice, TMX at P6-9 detected Gli1 expression in cells that populated the CC, with the vast majority localized laterally. This lateral distribution was maintained through adulthood, which contrasted with the pattern of myelination that progressed from lateral to medial in the CC between P14-56. Delaying TMX to P14-17, after the peak of oligodendrogenesis, significantly reduced labeling of Shh synthesizing neurons and Gli1 expressing cells in the CC. Subsequent studies used TMX from P6-9 in *Gli1*$^{CreERT2}$ mice to label Gli1 expressing cells and examine their fate in the adult CC. Gli1 fate-labeled cells gave rise to astrocytes and oligodendrocyte lineage cells, including cycling oligodendrocyte progenitor cells identified by EdU incorporation and NG2 immunolabeling. The SVZ also contained Gli1 fate-labeled cells, including neural stem cells immunolabeled for GFAP. Finally, the cuprizone model of demyelination was used to examine the potential for Gli1 fate-labeled cells to contribute to oligodendrocyte repopulation during remyelination. Gli1 fate-labeled cells incorporated EdU in response to chronic demyelination. In addition, Gli1 fate-labeled cells were immunolabeled by NG2 early during remyelination and formed myelin-like membranes after longer periods for remyelination to progress. These studies reveal a population of cells with active Shh signaling during postnatal development that contributes to the generation of oligodendrocytes during CC myelination and gives rise to cells that continue to proliferate in adulthood and contribute to CC remyelination.

**Disclosures: M.A. Sanchez:** None. **R.C. Armstrong:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.14/F1

**Topic:** B.12. Glial Mechanisms

**Support:** PRIN 2015 (2015E8EMCM_002)

**Title:** Selective activation of adenosine $A_{2B}$ receptors reduces outward $K^+$ currents and maturation of oligodendrocyte precursor cells *In vitro* by interacting with sphingosine kinase/sphingosine 1-phosphate signaling axis

**Authors: I. FUSCO**[1], I. DETTORI[1], F. CENCETTI[2], L. GAVIANO[1], *R. CORRADETTI[1], F. PEDATA[1], E. COPPI[1], A. M. PUGLIESE[1]
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**Abstract:** The ability to regenerate oligodendrocytes depends on the availability of neural progenitors called oligodendrocyte precursor cells (OPCs). They are present throughout the adult

brain and spinal cord and can replace oligodendrocytes lost due to injury, aging or disease such as Multiple Sclerosis (MS). OPCs are massively recruited to the site of injury in damaged myelinate axons, but in MS patients remyelination is often ineffective. The factors that promote the initiation of OPC differentiation and overcome the block for successful remyelination in demyelinating diseases are poorly defined. Different pathways may contribute to ameliorate/impair remyelination in MS lesions, including adenosine and sphingosine kinase/sphingosine 1-phosphate (SphK/S1P) signaling axis. At all maturational stages OPCs express all four adenosine receptor subtypes ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$). S1P, produced by the action of SphK (two isoforms: SphK1 and SphK2), is a bioactive lipid that regulates remyelination and cell injury. An unexpected finding is that FTY720 (Fingolimod), approved as orally active drug for relapsing MS, modulates S1P receptors. Here, by electrophysiological recordings combined with Real-time PCR and Western Blot analysis, we studied the effects of adenosine $A_{2B}$ receptors and SphK/S1P signaling on membrane currents and differentiation of purified primary OPCs isolated from the rat cortex.
Selective stimulation of adenosine $A_{2B}$ receptors by 2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide (BAY60-6583, 0.1-30 µM, n=43), an adenosine $A_{2B}$ receptor agonist, inhibited $K^+$ outward currents elicited by a voltage ramp protocol. This effect was prevented by the selective adenosine $A_{2B}$ receptor antagonist *N*-(4-Acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)phenoxy]acetamide (MRS 1706, 10 µM, n=5) and was reduced by VPC96047 (500 nM, n= 4), a pan-SphK inhibitor. Similarly, FTY720 phosphate (1 µM, n=4), the active metabolite of FTY720, mimicked and partially occluded the effect of 10 µM BAY60-6583 on ramp-evoked current. In cultured OPCs, SphK1 phosphorylation was enhanced after acute (10 minutes) application of 10 µM BAY60-6583 in the medium cultures, demonstrating an interaction between SphK/S1P pathway and $A_{2B}$ activation. Finally, chronic $A_{2B}$ stimulation (by 10 µM BAY60-6583 for 6 days in the medium cultures) reduced the expression of mature oligodendrocyte markers, as determined by Real-time PCR analysis.
Our findings reveal that activation of adenosine $A_{2B}$ receptors and the SphK/S1P pathway are involved in the maturation of OPCs. Support: PRIN 2015 (2015E8EMCM_002).

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.15/F2

**Topic:** B.12. Glial Mechanisms

**Support:** National Multiple Sclerosis Society Agency(PP-1603-08106)

**Title:** RNA methylation plays a critical role in CNS myelination

**Authors: *H. XU**[1], R. B. KUNJAMMA[1], X. ZHUANG[2], Q. FEI[3], C. HE[3], B. J. POPKO[1]
[1]Neurol., Univ. of Chicago, Chicago, IL; [2]Dept Neurobiol, Univ. of Chicago Dept. of Neurobio., Chicago, IL; [3]Dept. of Chem. and Inst. for Biophysical Dynamics, Chicago, IL

**Abstract:** Recent evidence has shown that RNA modification is a dynamic and reversible process that adds a new layer of epigenetic regulation in biological processes. N6-methyladenosine ($m^6A$), the most abundant internal modification site in eukaryotic messenger RNA (mRNA), is the first example of reversible RNA methylation. The conservative enrichment of $m^6A$ in stop codons, 3'-untranslated regions and long internal exons in mouse and human suggests its fundamental importance in RNA biology. Discoveries of $m^6A$ methyltransferase protein "writers", demethylase protein "erasers" and functional recognizer "reader" proteins suggest their dynamic regulatory roles in reversible RNA modification. A number of in vitro and in vivo studies have shown that reversible methylation of $m^6A$ is essential to cell survival and development. Nevertheless, the role of reversible RNA epigenetic modification in specialized cells, including myelinating cells, remains unknown. In our study, we used a Cre-loxP genetic approach to examine the role of RNA methylation in different developmental stages of myelinating cells. Methyltransferase like (METTL) 3 and 14 are active enzymatic components of the $m^6A$ "writer", and the knockdown of either component leads to substantially decreased $m^6A$-containing mRNA levels in the cells. We used mice containing a floxed *Mettl14* allele, which were crossed with mice that express Cre in oligodendrocytes in the CNS, to examine the role of $m^6A$ RNA methylation in CNS myelination. The Cre lines used were 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP)-Cre mice and oligodendrocyte transcription factor 2(Olig2)-Cre mice, which expresses Cre in mature oligodendrocytes and oligodendrocyte lineage cells respectively. *Mettl14* floxed mice lacking the Cre-expressing loci were used as controls. Molecular, biochemical, histological, and behavioral approaches were used to examine the role of RNA methylation in oligodendrocyte lineage cells. The CNP-Cre $Mettl14^{fl/fl}$ mice and the Olig2-Cre $Mettl14^{fl/fl}$ mice appear phenotypically normal until four to six months of age when they begin to display clinical symptoms that include tremor and ataxia, which are typical symptoms for CNS myelin abnormalities. The clinical symptoms displayed by the mutant mice correlate with reduced oligodendrocyte numbers and severe myelin abnormalities. These results indicate that $m^6A$ RNA methylation plays an essential role in oligodendrocyte function and myelin maintenance.

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**Poster**

**565. Oligodendrocytes**

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**Program#/Poster#:** 565.16/F3

**Topic:** B.12. Glial Mechanisms

**Support:** MOST 103-2314-B-006-007-MY3

MOST 105-2811-B-006-010

**Title:** BRCA1 BRCA2-containing complex subunit 3 mediates oligodendrocyte maturation

**Authors: C.-Y. WANG**[1], C.-H. HO[1], *S.-F. TZENG[2]
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**Abstract:** Oligodendrocytes (OLGs) produce myelin to wrap axons and promote action potential propagation in the central nervous system (CNS). BRCA1/BRCA2-containing complex subunit 3 (BRCC3), a lys63-specific deubiquitinase, was originally discovered to participate in DNA repair complex against double strand break. In addition, BRCC3 has been reported to regulate the activation of inflammasome by deubiquitinating NLRP3. Our previous study has shown that BRCC3 gene knockdown (KD) can increase the sensitization of glioma cells to alkylated agents. Recently, we also found that the reduction of BRCC3 expression in OLG precursor cells (OPCs) by lentivirus-mediated delivery of shRNA against BRCC3 (BRCC3-KD) suppressed OPC cell growth and sphere formation of OPCs. Moreover, suppression of BRCC3 led to the downregulation of DNA repair genes, such as BRCA1 and Rad51. Through the measurement of the OLG process complexity and myelin basic protein (MBP) production, BRCC3-KD increased OLG maturation when OPCs were maintained in the differentiation medium. The expression of genes (e.g. Sox10, Myrf, and MBP) that are responsible for OLG maturation was upregulated in BRCC3-KD cultures compared to that detected in the scrambled control cultures. The observations from neuron-OLG co-culture also showed that BRCC3-KD increased the maturation of OLGs derived from OPCs. The molecular mechanism underlying BRCC3-mediated OLG maturation is under investigation.

**Disclosures:** **C. Wang:** None. **C. Ho:** None. **S. Tzeng:** None.

**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

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**Topic:** B.12. Glial Mechanisms

**Support:** NSF-GRFP DGE-1553798

NIH Grant NS095679

**Title:** Oligodendrocyte contact induces presynaptic specialization in axons

**Authors: *A. N. HUGHES**[1], B. APPEL[2]
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**Abstract:** Many neural circuits depend on the coincident arrival of neural impulses at common targets. However, action potentials travel along axons of unequal lengths and conduction velocities. One mechanism by which the timing of impulses can be tuned is by regulating the amount of myelin membrane that ensheathes axons. To craft the temporal precision demanded by circuits, oligodendrocytes (OLs), the myelinating cell type of the CNS, must determine which axons should be myelinated and how much myelin to deliver to selected axons. What informs an OL of which axons should be myelinated? Our group and others found that preventing vesicular release in neurons reduces the number of stable myelin sheaths that form on axons. However, we know little about how neuronal vesicular release serves myelination. Previous work from our lab indicates that a major synaptic vesicle protein, synaptophysin, clusters under myelin sheaths that form on *phox2b+* reticulospinal axons, raising the possibility that vesicles are released directly onto apposing myelin sheath. Other groups have performed proteomic analysis of purified myelin to reveal several factors, including neuroligin1, CaMKII, and synCAM3 and -4, known to function at postsynaptic terminals. Drawing on these data, we hypothesized that OL contact induces the formation of a synapse-like structure by inducing presynaptic specialization in the axon. To test this, we use zebrafish as a model system to combine genetic and pharmacological tests of gene function with direct observation of axon ensheathment using time-lapse imaging. Our preliminary data indicate that neurons respond to OL contact with local calcium transients, reminiscent of those that occur in motor neurons upon contact with target muscle cells during neuromuscular junction formation. Additionally, we show that the presynaptic terminal protein, synaptophysin, is recruited to nascent sheaths. Together, these data support a role for OL contact in inducing presynaptic differentiation in neurons.

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**Poster**

**565. Oligodendrocytes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 565.18/F5

**Topic:** B.12. Glial Mechanisms

**Title:** Differential targeting of sodium channels in axons with intermittent myelination

**Authors: C. M. BACMEISTER**[1,2], S. R. LEVINSON[3], *E. G. HUGHES[1]
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[3]Dept. of Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO

**Abstract:** Myelination and the localization of voltage-gated ion channels to nodes of Ranvier enables axons to conduct signals in an efficient and timely manner. While the traditional model of myelination assumes a uniform distribution of myelin sheaths along the length of the axon, the recent discovery of diverse myelin profiles in the cortex—termed intermittent myelination—reveals the necessity for a greater understanding of the structure and function of these diverse myelination profiles in the adult brain. Here, we evaluated the localization of ion channels on cortical axons with intermittent myelination using immunohistochemical analysis to characterize the distribution of sodium channels at paranodes in superficial layers of cortex from 1.5 to 10 months of age. A differential distribution of sodium channels was found adjacent to nodal and heminodal paranodes and a large proportion of heminodes showed no sodium channel immunoreactivity. Furthermore, paranodes of terminal myelin sheaths showed a greater propensity for clustering sodium channels than paranodes of isolated myelin sheaths. Nav1.6, the predominant sodium channel isoform at nodes of Ranvier, was absent from the majority of heminodes even in 10 month old mice. Together, these data indicate that the differential targeting and clustering of sodium channel isoforms to nodes and heminodes in the superficial cortex is related to the distribution of myelin. This varied distribution of voltage-gated ion channels may suggest additional roles for myelin beyond regulating conduction velocity in axons with intermittent myelination.

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**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

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**Program#/Poster#:** 566.01/F6

**Topic:** B.14. Neuro-Oncology

**Support:** NIH Grant P01CA069246

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**Title:** Decoding of single extracellular vesicles

**Authors: *K. B. FRASER**, K. LEE, B. GHADDAR, K. YANG, L. BALAJ, E. A. CHIOCCA, X. O. BREAKEFIELD, H. LEE, R. WEISSLEDER
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**Abstract:** Extracellular vesicles (EVs) describe a broad family of cell-secreted, membrane-containing nanoparticles of varying cellular origins capable of diverse biological function, diagnostic potential and therapeutic applications. The relatively small size of these EVs (~50nm - 1μm) has mainly required the use of bulk analyses, making more nuanced biological investigations into cell of origin or population heterogeneity difficult. Here we describe a new technique for single EV analysis (SEA) that provides a multiplexable and sensitive method to probe proteomic information with single vesicle resolution. We used this method to profile glioblastoma EVs and found high variation in both pan-EV and tumor cell markers. This initial investigation provides some of the first evidence of heterogeneity among biomarker profiles across tumor EVs. The data demonstrates the capacity of the SEA technology to be used in an array of clinical applications and to address key questions in EV biology moving forward.

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**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.02/F7

**Topic:** B.14. Neuro-Oncology

**Title:** Determining the elastic moduli of normal and cancerous brain to develop a 3D culture mimic

**Authors: *T. RIGGINS**[1], H. MCNALLY[2], R. T. BENTLEY[3], J. RICKUS, 47907[4]
[1]Biomed. Engin., Purdue Univ., Lafayette, IN; [2]Dept. of Electrical Engin. Technol., [3]Dept. of Vet. Clin. Sci., [4]Dept. of Agr. and Biol. Engin., Purdue Univ., West Lafayette, IN

**Abstract:** Glioblastoma (GBM) is the most common and deadly primary brain tumor of the adult CNS. GBM cells remodel their local extracellular matrix (ECM) to create a tumor microenvironment, which in turn supports the tumor progression. Collagen I, which is not usually expressed in the normal adult brain, is over-expressed within the tumor core relative to the peritumor region in patients with short survival times. Collagen-based 3D culture models show that altering the collagen I density and in turn the elastic modulus of the matrix, alters the migration mode and rate of GBM cells. Little is known, however, regarding the elastic modulus within the native GBM tumor microenvironment and whether there is a potentially instructive mechanical gradient from tumor to the native brain. Literature provides a wide range of estimated elastic moduli of normal brain as result of varying tissue preparation methods, temperature conditions, testing methods, and post mortem time. To address these issues, we aim to measure the mechanical properties in the tumor and peritumor tissue from canine patients with spontaneous glioma and attempt to match these properties in a 3D brain mimic culture model. We measure elastic moduli by Atomic Force Microscopy (AFM), using a Bruker Bioscope Catalyst with Nanoscope V Controller mounted on an Olympus inverted microscope, using a cantilever with a nominal spring constant (k) of 0.12 N/m. To delineate the effects of preservation methods, brain tumor and brain samples will be flash frozen within 3 hrs post mortem, while other brain tumor and brain samples will be incubated ($37^o$, 5% $CO_2$) within 3 hrs post mortem. These results will determine how tissue preservation affects the mechanical properties, and will be the baseline to adjust the mechanical properties of the 3D tunable culture. We expect that matching the brain environment in culture will encourage a typical genetic tumor expression profile and help create a more representative pathophysiological model to study GBMs.

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**Poster**

**566. Brain Tumor Biology**

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**Program#/Poster#:** 566.03/F8

**Topic:** B.14. Neuro-Oncology

**Support:** QNRF NPRP No.:6-089-3-021

**Title:** Pharmacological modulation of calcium signaling to overcome acquired cisplatin resistance in neuroblastoma cells

**Authors: *A. M. FLOREA**[1], J. MCCALLUM[2], E. VARGHESE[2], G. REIFENBERGER[1], D. BÜSSELBERG[2]
[1]Heinrich Heine Univ. Düsseldorf, Uniklinikum, Düsseldorf, Germany; [2]Weill Cornell Med. in Qatar, Qatar Foundation-Education City, Doha, Qatar

**Abstract:** High-risk neuroblastoma (NB) patients are treated with chemotherapy protocols including e.g. cisplatin (CDDP) and topotecan (TOPO) that is successful in only 30-40% of cases due to acquired chemotherapy resistance, thus improved therapies are urgent. Relapsed or metastatic NB tumors are difficult to treat as the molecular mechanisms leading to drug resistance are not understood. Since alterations of intracellular calcium homeostasis induced by anticancer drugs have been demonstrated in NB before it is suggested that manipulation of key modulators of calcium signaling associated with apoptosis may provide novel treatment strategies in NB. In this study, we assessed whether calcium signaling modulators enhanced the effects of CDDP and TOPO in parental SH-SY5Y and cisplatin (CDDP) resistant SH-SY5Y_R cells. In vitro cellular cytotoxicity was utilized as an endpoint. Treatment schemes included untreated control cells (72h), cells treated with a calcium modulator alone (72h), cells treated with CDDP or TOPO (72h), and cells treated with a combination of a calcium modulator and CDDP or TOPO. In the combinatory treatment, the cells were first exposed to the calcium modulator for 30min. followed by CDDP or TOPO for 72h. We tested the following compounds: thapsigargin (THAPS), 2-aminoethoxydiphenylborane (2-APB), cylopiazonic acid (CPZ), dantrolene (DANTR), ryanodine (RYA), verapamil (VERA), nifedipine (NIFE), cyclosporine A (CYCLA), 1,2-Bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid known as BAPTA/BAPTA-AM, the $Na^+/Ca^{2+}$ exchange inhibitor YM58483, reversan (REV), as well as the calmodulin (CaM) kinase inhibitors STO-609 acetate and PD 150606 cell permeable calpain inhibitor. Treatment of SH-SY5Y and SH-SY5Y_R with modulators of calcium signaling revealed THAPS, CPZ, as well as 2APB and CYCLA as potentially active compounds in CDDP resistant NB cells since their combination with CDDP or TOPO showed significant additive effects, revealing the potential role of the regulation of sarco/endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) pump, IP3 receptors and mitochondrial calcium flux in enhancement of chemotherapy. The other calcium modulators did not cause increased cytotoxity in SH-SY5Y_R cells, with STO-609 acetate and PD 150606 demonstrating additive effects with CDDP or TOPO only in the parental SH-SY5Y cells. Additional research is required to experimentally validate these findings in independent NB models with acquired resistance to chemotherapy as well as the functional roles of modified calcium signaling in CDDP-resistance and characterize their potential relevance for in vivo treatment of chemotherapy-resistant NB.

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**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.04/F9

**Topic:** B.14. Neuro-Oncology

**Support:** NPRP-60893021

**Title:** Alterations in phenotype and intracellular calcium ($[Ca^{2+}]_i$) management in chemoresistant neuroblastoma cells following cisplatin treatment

**Authors: *D. BUSSELBERG**[1], J. E. MCCALLUM[1], E. VARGHESE[1], A. M. FLOREA[2]
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**Abstract:** Neuroblastoma (NB) is a childhood tumor that is often treated with cisplatin (CDDP) as a first line chemotherapy. Secondary effects resulting chemotherapy is the acquired drug resistance that leads to poor patient survival that still represents major challenges towards effective chemotherapy. Intracellular calcium ($[Ca^{2+}]_i$) is a universal second messenger that regulates many cellular processes including apoptosis. Several chemotherapeutic agents including CDDP have shown to modulate $[Ca^{2+}]_i$ and apoptosis in neuroblastoma. Here we analyze gene expression and specific functional characteristics of resistant NB cells associated with $[Ca^{2+}]_i$ handling in sensitive and CDDP-resistant subline of NB cells (SH-SY5Y).
The trypan blue cell viability test showed that in CDDP-resistant SH-SY5Y, the CDDP exposure resulted lower cytotoxicity as compared to the parental CDDP sensitive cells ($\leq$1μM; $p<0.01$). The mRNA expression of a selected genes related to drug transport ABCB1, ABCC1, ABCG2, ATP-7a and LRP were analyzed. LRP demonstrated a highly significant 3-fold increase in basal CDDP-resistance ($p<0.001$). A scratch-wound healing assay reveled that CDDP exposure could not block migratory capacity of CDDP-resistant cells, as with CDDP-sensitive migratory capacity ($P<0.01$). Furthermore, plasma membrane drug transporter function was measured via a Hoechst dye exclusion assay, determined by verapamil inhibition. Verapamil could not inhibit ABC efflux transporter function in CDDP-resistant SH-SY5Y cells treated with CDDP. Finally, single cell $[Ca^{2+}]_i$ was measured using the calcium sensitive fluorescent dye Fluo-4AM, over 3h. A more pronounced increase of was observed in CDDP-resistant cells, compared to sensitive SH-SY5Y cells. Thapsigargin, a SERCA blocker and verapamil an L-type channel blocker were used to monitor calcium influx to ER stores or from an extracellular source. Results indicate that CDDP-resistant cells cisplatin mediated increases in $[Ca^{2+}]_i$ were most compromised following depletion and block of ER calcium stores.
Together, results characterize a chemoresistant NB phenotype that has improved capacity for survival, cell motility and drug efflux. Future investigations will assess if disruption of intracellular calcium storage, initially induced following CDDP exposure, might compromise chemotherapy resistant NB cell viability.

**Disclosures: D. Busselberg:** None. **J.E. McCallum:** None. **E. Varghese:** None. **A.M. Florea:** None.

**Poster**

**566. Brain Tumor Biology**

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**Program#/Poster#:** 566.05/F10

**Topic:** B.14. Neuro-Oncology

**Support:** Gel-E Life Sciences funding

Regenerative Engineering in Medicine (REM) partnership seed grant

UGA-OVPR Startup Funds

**Title:** The small molecule GAG-antagonist surfen decreases glioma infiltration *In vitro* and attenuates tumor growth in a rodent model of glioma

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**Abstract:** Glioblastoma multiforme (GBM) is a stage four astrocytoma comprising a majority of primary malignant brain tumor diagnoses in adults in the U.S. Conventional therapies are ineffective, leading to patient death within 15 months of diagnosis. Chondroitin sulfate proteoglycans (CSPGs) and their glycosaminoglycan (GAG) side chains are key components of brain extracellular matrix (ECM) implicated in promoting tumor invasion. We hypothesize that glioma invasion is triggered by selective expression of oversulfated CS-GAGs in tumor microenvironment, and preventing glioma cell signaling with extracellular CS-GAGs will restrict invasion. Microfluidics devices were used to present mono- and oversulfated CS-GAG matrices to F98 rat glioblastoma cells and cell infiltration *in vitro* was quantified. Cell infiltration was compared across unsulfated (HA), 4,6-sulfated CS-GAG (CS-E) matrices, and media only controls. The small molecule GAG-antagonist (surfen) was introduced to inhibit GAG signaling. Focal adhesion protein colocalization was quantified within antagonist-containing and control hydrogels to determine the influence of CS-GAGs on cell invasion. *In vivo* tumor inductions in rats were performed stereotactically to induce frontal lobe tumors to mimick human GBM. F98 cells in media only or containing surfen were inoculated to evaluate effects of surfen on glioma invasion over 21 days. MR imaging was used to track progress of tumor volume and angiogenesis.
Our results demonstrated enhanced preferential cell invasion into hydrogel matrices containing disulfated CS-E compared to unsulfated hydrogels ($p<0.05$). F98 cells invading into CS-E hydrogels displayed enhanced colocalization ($p<0.05$) of focal adhesion proteins compared to cells within unsulfated hydrogels. This effect was significantly reduced in cells within CS-E

hydrogels containing surfen ($p<0.05$). Rats inoculated with F98 cells developed diffusely invasive tumors 14 days post-induction when compared to surfen treated rats, which developed smaller tumors with more defined margins ($p<0.05$). These results suggest that sulfated CS-GAGs may directly induce tumor invasion, and that this signaling mechanism can be disrupted by surfen. Our results also suggest that heightened presence of extracellular CS-E induced enhanced cellular migration in a GAG sulfation-dependent manner, and that perturbing cellular interactions with CS-E significantly reduced tumor invasion. Identification of the role of CS-GAGs in glioma behavior would advance our understanding of glioma invasion, and contribute to development of novel therapeutic interventions for this intractable form of brain cancer.

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**Poster**

**566. Brain Tumor Biology**

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**Topic:** B.14. Neuro-Oncology

**Support:** 1.Stem Cell and Translational Research, the National Key Research and Development Program of China (2016YFA0101001)

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3.National Natural Science Foundation of China (31471016)

**Title:** Tspo deficiency promotes glioma development

**Authors: *D. WANG**, Y. FU, H. WANG, H. CHEN, Y. HU, J. ZHANG
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**Abstract:** Glioma is the most common type of primary tumor in brain with a high morbidity and mortality. Previous studies indicated that the 18 kDa translocator protein (TSPO) was highly expressed in glioma tissue, especially in the malignant glioma tissues. However, the roles of TSPO in glioma development are unclear. In this study we investigated the functions of TSPO in glioma development by using in vivo mouse model by intracranial xenograft glioma in TSPO knockout mice. The results showed that TSPO deficiency promoted the growth of glioma. The volume of glioma with TSPO deletion grew bigger than that of WT glioma. In addition, the expression of CD31 and other angiogenic factors was increased in the TSPO-KO glioma, indicating an enhanced angiogenesis in TSPO KO glioma. We also found more $P2Y12^{+}$ microglia/macrophages infiltrated in TSPO-KO glioma compared to WT glioma. Our findings

demonstrate that TSPO deficiency promotes glioma progression, and TSPO may be a novel target for glioma therapy.

**Disclosures:** **D. Wang:** None. **Y. Fu:** None. **H. Wang:** None. **H. Chen:** None. **Y. Hu:** None. **J. Zhang:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.07/F12

**Topic:** B.14. Neuro-Oncology

**Title:** The N - alkyl carbazole derivative 4OHCARB6BSAL induces neuroblastoma cell death by promoting p53 protein translocation into the nucleus

**Authors: *S. L. NORI**[1], A. SANTORO[1], M. ZANETTI[2], V. NICOLIN[2]
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**Abstract:** Neuroblastoma (NB) is a childhood tumor of neural crest origin presenting along the sympathetic chain especially in the adrenal medulla or paraspinal ganglia. More than half of children diagnosed with high-risk NB either do not respond to conventional therapies or relapse after treatment, and nowadays chemotherapy has not yet made a significant effect on the survival of high-risk NB children. An attractive feature of NB is that the p53 protein, whose alterations are well known to play a crucial role in malignant transformation, is rarely mutated at the time of diagnosis and it appears that p53 dysfunction can result mostly from a nonfunctional conformation or cytoplasmic sequestration of the wild-type protein. Therefore, pharmacological approaches aimed at targeting the p53 protein by promoting its translocation into the nucleus could improve NB therapeutic strategies. In this study, we investigated the cytotoxicity of a new N-alkyl carbazole derivative named 4OHCARB6BSAL (Saturnino et al., Eur J Med Chem. 2013, 60:112-119) in NB SK-N-AS cells using DAPI staining and explored its potential mechanism of action by analyzing cell morphology and p53 distribution by immunofluorescence microscopy during the treatment. Results show that the compound is able to significantly inhibit the number of SK-N-AS cells in a concentration range from 10 to 50 µM and a time treatment of 48 h. Particularly it reduces cell survival of about 25 % at 10 µM, whereas at 50 µM only about a 3% of cells were viable. The cytotoxic activity of the carbazole derivative was associated to a change of cell shape and cytoskeletal organization, as revealed by β-tubulin staining, compared to untreated (control) cells. On the other hand, we also observed a change in cell distribution of the phosphorylated (Ser15) p53 protein that appeared concentrated in the nucleus of the cells as a consequence of the treatment. Interestingly, the distribution pattern of p53 protein was comparable to that induced by the MDM2 antagonist Nutlin-3 (Van Maerken et al., Mol Cancer

Ther. 2011, 10:983-993) used as positive control. Moreover, to provide further insights we also detected STAT3 expression and distribution in NB cells. Results show a general low expression of this protein even though an increase in immunofluorescence intensity in the nucleus has been detected. Altogether, these data suggest that 4OHCARB6BSAL can induce cell death by targeting p53 protein and might be involved in the activation of differentiation pathways by modulating STAT3 protein expression.

**Disclosures: S.L. Nori:** None. **A. Santoro:** None. **M. Zanetti:** None. **V. Nicolin:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

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**Program#/Poster#:** 566.08/G1

**Topic:** B.14. Neuro-Oncology

**Support:** Grants-in Aids for Scientific Research from the Japan Society for Promotion of science (16J40093)

**Title:** Plasticity of cognitive functions after resection of glioma: new evidence from voxel-based lesion symptom mapping

**Authors: *C. NIKI**[1], T. KUMADA[2], T. MARUYAMA[1], M. TAMURA[1], Y. MURAGAKI[1]
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**Abstract: Background and aims:** The aim of this study was to evaluate changes of cognitive function of glioma patients from pre-operation to 6 months after surgery and identify brain regions that are related to recovery of cognitive function or continuous impairments after resection of glioma. **Method:** We recruited 25 patients with glioma in the left hemisphere (15 male, 10 female, mean age=41.2, SD=10.4, all right-handed). Cognitive test battery composed of six cognitive tasks such as memory, attention was administered at pre-operative stage, 1 month and 6 months after a resective surgery. A factor analysis was performed to their pre-operative cognitive performance to extract cognitive factors common to the six cognitive tasks, then examined the neural correlates for the extracted factors using voxel-based lesion-symptom mapping(VLSM) method. To reveal differences of brain regions related to impairments of cognitive performance between 1 month and 6 months after surgery, VLSM were performed twice, for performance of 1 month and that of 6 months later. **Results:** Four main cognitive factors were extracted by a factor analysis. VLSM analyses revealed that the first component affected verbal memory and verbal fluency tasks and corresponded with anterior parts of middle temporal (MTG) and superior temporal gyri (STG) at 1 month later. Lesions to MTG found at 1 month later was disappeared at 6 months later, whereas those to STG were revealed at both 1 month and 6 months later. The second component, affecting letter-digit substitution and digits

forward and backward tasks, revealed around the posterior STG, including Wernicke's area, the middle STG, the supramarginal and the angular gyri at both 1 month and 6 months later. The third and the fourth components affected each task, concept of shifting and Stroop color-word tasks. For the third component, cognitive performance was improved from 1 month to 6 months later and STG found at 1 month disappeared at 6 months later, whereas regions around the supramarginal and the angular gyri were found only at 6 months later. Cognitive performance of the fourth component also improved from 1 month to 6 months later and the inferior frontal lobe found in analyses at 1 month disappeared at 6 months later, although the middle frontal regions were found only at 6 months later. **Discussion:** Results showed that brain regions associated with cognitive impairments in the early stages recovered 6 months later, suggesting plasticity of higher cognitive function after resection of glioma. Results also suggests that some brain regions might be amenable to recovery of cognitive function following resection of glioma than others.

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**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

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**Program#/Poster#:** 566.09/G2

**Topic:** B.14. Neuro-Oncology

**Support:** NIH Grant R01CA200624-02

**Title:** Implications of the inhibitors of HuR multimerization in glioma treatment

**Authors: *N. FILIPPOVA**, X. YANG, L. B. NABORS
Univ. of Alabama At Birmingham, Birmingham, AL

**Abstract:** The mRNA binding protein HuR expression is consistently elevated in tumor tissue and promotes HuR cytoplasmic localization essential for HuR dependent oncogenic transformations. In our previous report, we performed a detailed analysis of HuR multimerization in samples of primary brain tumors, patient-derived xenolines (PDGx), and the established glioma U251 cell line. We found a significant increase in HuR multimerization with the enhancement of tumor grade.
In our current work, by utilizing the HuR/split Firefly luciferase assay in U251 cells, we discovered several new compounds inhibiting HuR multimerization. The tanshinone group compound 15,16 dihydrotanshinone-I (DHTS), the compound #5 and the JSI-124 are among on them. The HuR specific regulator MS-444 has been utilized as the control. The $IC_{50}$ for the inhibition of HuR-dimerization are: 20$\pm$6 uM (n=4), 1.5h of treatment for DHTS; 21$\pm$6 uM (n=4), 6h of treatment for compound #5 and 0.9$\pm$0.1 uM (n=4), 6h of treatment for JSI-124. We

found that the exposure of the established glioma cell lines and human derived PDGx to the above compounds leads to the ultimate reduction of tumor cell survival and proliferation. The most pronounced effect on the decrease of cancer cell survival and proliferation was observed with the JSI-124, the main mechanisms of action of which consist of the inhibition of HuR dimerization and the inhibition of JAK/STAT3 pathways. The JSI-124 effectively inhibits TMZ resistant and non resistant cancer cell growth and works well in combination with the irradiation cell treatment. In the cytotoxicity assay the JSI-124 $IC_{50}$ for the stem cell population isolated from PDGx cell lines was in 0.05-0.1 uM range. The JSI-124 growth inhibitory effect depends from the cell density and declines with the increase of cell density. The mouse GL261 cells with the HuR knockdown exhibit higher sensitivity to the JSI-124 compared to the sh-control cells, the difference increases with an increase in cell density. Several glioma cell lines with high levels of HuR and PDL1 expression exhibit significant reduction of HuR and PDL1 levels following the JSI-124 treatment. In the glioma mouse immunocompetent model the evaluation of the source and the concentration of the PDL1 and the cytokine profile in the tumor micro environment and CSF prior and after the disruption of the HuR function is in progress. Overall, our work confirmed that the inhibition of HuR dimerization in the glioma cell lines decreases cell proliferation, enhances cancer cell sensitivity to the chemotherapeutics and may affect cancer cell immune defense axes through the regulation of PDL1 expression and the cytokines profile.

**Disclosures:** **N. Filippova:** None. **X. Yang:** None. **L.B. Nabors:** None.

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**566. Brain Tumor Biology**

**Location:** Halls A-C

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**Program#/Poster#:** 566.10/G3

**Topic:** B.14. Neuro-Oncology

**Title:** Bradykinin induces cell migration and interleukin-8 production through the bradykinin B1 receptor in glioblastoma

**Authors: *Y.-S. LIU**[1,2], C.-F. TSAI[3], D.-Y. LU[1,4]
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**Abstract:** Glioblastoma (GBM) is the most aggressive brain tumor, with a poor prognosis because of the ease with which tumor cells migrate to surrounding normal brain. Recent studies have shown that bradykinin is involved in progressions of various types of cancer. However, the molecular mechanisms and pathologic roles underlying bradykinin-induced GBM migration remain unclear. In this study, we found that bradykinin induced GBM migration in a dose-

dependent manner. Analysis of cytokine array showed that bradykinin increased production of interleukin-8 (IL-8) in GBM. Moreover, bradykinin induced IL-8 mRNA and protein expression were observed in GBM as well. Knockdown of IL-8 attenuated bradykinin-induced migration in GBM. Bradykinin-induced IL-8 protein expression was repressed by the administration of a bradykinin B1 receptor (B1R) antagonist, but not the bradykinin B2 receptor (B2R) antagonist. Activations of FAK and STAT3 were observed time-dependently after stimulation by bradykinin. In addition, inhibition of B1R by desBK effectively antagonized the bradykinin-induced phosphorylation of FAK. Moreover, treatment with FAK or STAT3 inhibitor reduced the bradykinin-induced IL-8 protein expression. Taken together, these results indicate that bradykinin-induced IL-8 expression through B1R, FAK and STAT3 resulting in contributing to promote GBM migration.

**Disclosures: Y. Liu:** None. **C. Tsai:** None. **D. Lu:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.11/G4

**Topic:** B.14. Neuro-Oncology

**Title:** Sirtuins mediated metabolic stress in HIV associated brain lymphoma

**Authors: *D. PATEL**[1], P. R. GUDA[1], S. RAY[1], R. SUBEDI[1], M. GHOSH[1], G. ASEMU[2], T. K. MAKAR[1], J. L. BRYANT[2]
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**Abstract:** Inhibition of histone deacetylases (HDACi) is one of the treatments of many types of cancer, including brain lymphoma. Among the HDACs, Class III HDACs, also known as sirtuins (SIRTs), are unique in that their function is directly related to the cell's metabolic state through their dependency on the co-factor NAD(+).The role of SIRTs in cancer is extremely complex, with different functions. Mammalian SIRTs (SIRT1-7) differ according to cellular localization and biologic functions. Among these, SIRT -3, -4, and -5 are located in the mitochondria and the rest are cytosolic. Mitochondrial Sirt3 regulates multiple cellular and physiologic processes, including cell cycle, gene expression, cell viability, stress response, metabolism, and energy homeostasis. Recent research suggests that Sirt3 influences tumors by regulating the metabolic state of the cell. So far nothing is known about the role of sirtuins in the regulation of HIV-associated brain B cell lymphoma. In this study, we examined the relation between SIRTs and the hippocampal changes of HIV-associated brain B cell lymphoma. Recently we developed the HIV-1 Tg26 mouse model of brain B cell lymphoma similar to what is seen in human HIV primary central nervous system lymphoma (PCNSL) which is a malignant diffuse large B cell lymphoma that occurs in 3-5 % of HIV patients. The hippocampus plays a crucial role in

cognitive function. Memory deficits are characteristic of HIV-associated neurocognitive disorders and involved with hippocampal pathology in the hippocampus region of these mice, NAMPT (NAD(+) biosynthesizing enzyme), SIRT1 (The NAD(+)-dependent deacetylase), SIRT3 (mitochondrial deacetylase) and PGC1α (a key enzyme involved in mitochondrial biogenesis and function) expressing cells were increased, Our finding suggests NAMPT/SIRT1/SIRT3/PGC1α signaling is activated in the hippocampus of PCNSL in the HIV-1 TG26 mouse model. The identification of metabolic changes in the hippocampus of these mice may provide novel insight into the basic mechanisms underlying key cognitive deficit associated with the disease

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**Poster**

**566. Brain Tumor Biology**

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**Topic:** B.14. Neuro-Oncology

**Support:** PhD fellowship 254080 to AGGR

Conacyt 255087 to AO

Soluciones para un México Verde to AO

PRODEP 185471 PTC 112358

**Title:** Anti-cancer activity of brown seaweed Egregia menziesii's extracts against nervous system cell lines

**Authors: A. G. GUTIÉRREZ-RODRÍGUEZ**[1], R. MÉNDEZ[2], S. HUERTA[3], R. TOVAR[2], L. C. R. HERNÁNDEZ-KELLY[5], L. E. AGUILAR-ROSAS[6], A. ORTEGA[7], T. OLIVARES-BAÑUELOS[6], *R. C. ZEPEDA[4]
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**Abstract:** Gliomas are malignant and infiltrative brain tumors, which treatment generally includes surgery, radiation, and chemotherapy. However, brain tumor’s treatment commonly is difficult to achieve and drugs are non-selective, causing side effects and chemotherapy tolerance. Seaweeds are used since ancient times to treat several kinds of tumors, and it has been described

that seaweeds are rich in natural products with several biological activities, including anti-cancer activity. Egregia menziesii is a brown seaweed that belongs to the Lessoniaceae family and it is distributed in the north Pacific coast of Mexico, which biological activity has not been investigated yet. Since, some Lessoniaceae family members are showed anti-proliferative activity, the aim of this work was to evaluate the anti-cancer activity of Egregia menziesii extracts against cell lines of nervous system lineage. Specimens of this brown seaweed were collected in Campo 5 Punta Banda, Baja California, Mexico, and hexanic, chloroformic, and methanolic extracts were obtained by maceration. MTT proliferation assay was used to evaluate cell viability in C6, N1E-115, MIO-M1, U737 cell lines, and the non-cancerous Bergmann glia primary culture; after 4, 24, and 48h exposure. Our results showed that the hexanic and chloroformic extracts have a potent anti-proliferative capacity in C6 glioblastoma cells (IC50= 2.3 y 2.0 µg/ml, at 24h exposure) respectively, without affecting the Bergmann glia cell's viability. In conclusion, these results show the first approach to evaluate the anti-cancer activity of the Mexican algae E. menziesii.

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**Poster**

**566. Brain Tumor Biology**

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**Program#/Poster#:** 566.13/G6

**Topic:** B.14. Neuro-Oncology

**Support:** Australian Postgraduate Award

Australian Research Council

**Title:** Does dual pharmacological inhibition of aquaporin-1 water and ion channel activity inhibit glioma cell migration?

**Authors: *M. DE IESO**[1], J. PEI[1], M. KOURGHI[1], S. NOURMOHAMMADI[1], J. E. HARDINGHAM[2], A. J. YOOL[1]
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**Abstract:** Cancer is a leading cause of death, primarily due to metastasis. Aquaporin-1 (AQP1) is one of fifteen classes of water channels in mammals and is well known for roles in fluid absorption and secretion in the kidney, brain, and vascular system. AQP1 has also been shown to act as a cGMP-gated ion channel, essential for cerebrospinal fluid production. AQP1 is

upregulated in a subset of aggressive cancers including many gliomas, and some colorectal cancers, and expression has been shown to increase with the grade of the neurological malignancy. Interestingly, when AQP1 is transfected into an AQP1-null glioma cell line in vitro, cell migration is potentiated compared to wild type. This potentiation of cell migration cannot be replicated with the transfection of other mammalian aquaporins, suggesting a unique migration-enhancing property of AQP1. These findings imply that AQP1 may play an important role in facilitating cell migration and motility in glioma cells. Moreover, upregulation of AQP1 in glioma tumours may also contribute to vasogenic brain oedema, and the acidification of the extracellular space – likely contributing factors to the invasive potential of these cancer cells. Our research group recently discovered two novel inhibitors (AqB011 and bacopaside II) that selectively block the AQP1 ion and water channels respectively, and applied individually were found to inhibit cell migration in HT29 colon cancer cells highly expressing AQP1, without evidence of cytotoxicity at effective doses. Cell migration was quantified using a circular wound closure assay and live cell tracking. Dual treatment produced a block of 81%, which was significantly greater than the block produced by AqB011 (38%) or bacopaside II (44%) alone. Additionally after AQP1 was knocked down via short interfering RNA, the effects of both AqB011 and bacopaside II on cell migration were nullified, suggesting specific action on the AQP1 channel. These results were the first to suggest that the AQP1 water and ion conductance may exhibit a coordinated role in facilitating cell migration in AQP1-dependent cancer cell lines. Work here tests the idea that blocking both AQP1 water and ion channels in glioma cell line U87-MG will inhibit cell migration and invasion in an in vitro model, consistent with previous results from the HT29 cell line. Western blot analysis for AQP1 expression in U87-MG cell line showed levels of AQP1 similar to that of HT29, thus a reasonable candidate to test the AQP1 inhibitors. More work needs to be conducted to test specificity of the AQP1 inhibitors, and to test how AQP1 water and ion conductance may exhibit a cooperative role in facilitating cancer cell migration and invasion.

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**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.14/G7

**Topic:** B.14. Neuro-Oncology

**Support:** NSFC 81622014

**Title:** Pericyte dysfunction in radiation induced brain injury

**Authors: *Y. TANG**[1], J. CHENG[2], Y. GUAN[2], Y. LI[2], J. CAI[2], X. WANG[2]
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**Abstract:** Radiation-induced brain injury (RI) is a severe complication of radiotherapy for head and neck tumor. However, the pathogenesis of RI remains unclear.
We found that after radiation, there is severe Blood brain barrier (BBB) disruption, which is in line with literatures. It has been known that pericytes play key role in maintaining the integrity of BBB. Therefore, we detect the expression of soluble platelet-derived growth factor receptor-β (PDGFR-β), a hallmark of pericytes, in the cerebrospinal fluid of patients after radiotherapy, and discover that expression of PDGFR-β is significantly higher among patients with RI than those without RI ($P < 0.05$).The level of PDGFR-β is positively correlated with the area of edema and severity of brain injury (measured by SOMA score), suggesting involvement of pericytes.
Further we aim to elucidate how pericytes function in RI through animal model. We find that after whole brain radiation of 30Gy, fluorescent dye exudation increases significantly using two-photon microscopy imaging, suggesting increase of BBB permeability and BBB disruption after radiation. In addition, we also found that blood flow slows down, diameter of microvessels narrows and density of microvessels decreases in cortex and hippocampus. Moreover, decrease of pericytes' density, coverage rate and its detachment from endothelial cells are significant. Expression of PDGFR-β in cortex and hippocampus is reduced. Nevertheless, detailed signaling pathway needs further investigation. Our previous work confirmed that microglia activate and secrete large amounts of inflammatory mediators such as TNF-α, IL-6 and IL-1β after radiation. And it has been reported in vitro that pericytes transform to "harmful cells" in respond to inflammatory mediators such as TNF-α or IL-6. Thus we speculate that there might be direct crosstalk between pericytes and microglia after radiation.
Next we will try to clarify the mechanism of vascular injury after radiation and the signaling pathway, and verify the scientific hypothesis that there is crosstalk between pericytes and microglia.

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**Poster**

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**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.15/G8

**Topic:** B.14. Neuro-Oncology

**Title:** Oral administration of a novel histone deacetylase 6 inhibitor MPTOB291 reduces glioblastoma volume and changes gene expression in tumor surrounding stromal tissue in orthotopic xenograft mouse model

**Authors: *B. BATSAIKHAN**[1], K.-S. HUNG[2], J.-Y. WANG[1,2]
[1]Grad. Inst. of Med. Sci., Taipei Med. Univ., Taipei, Taiwan; [2]Dept. of Neurosurg., Wan Fang Hospital, Taipei Med. Univ., Taipei, Taiwan

**Abstract:** Histone deacetylase (HDAC) inhibitors have emerged as a new class of anti-tumor agents for various types of tumors, including glioblastoma. We have recently found that a novel HDAC 6 inhibitor MPTOB291 has anti-tumor effects on glioma cell death, cell cycle arrest, migration *in vitro* and on tumor growth, angiogenesis *in vivo*. To futher examine anti-tumor effects of this novel drug on tumor microenvironment, we examined the expression of mRNAs and lncRNAs in orthotopic xenografts in mice by using cDNA microarray. MRI and bioluminescence imaging analyze show that tumor growth in animals was inhibited by MPTOB291 and that tumor volume determined by MRI and bioluminescence signal determined by *in vivo* imaging system (IVIS-200) were highly correlated ($R=0.839$, $p<0.05$). Our microarray data showed that a number of mRNAs and lncRNAs were up- and down-regulated by MPTOB291 compared to those in sham xenograft and xenograft treated with vehicle drug. Gene Ontology (GO) enrichment analysis and KEGG pathway analysis suggest that treatment of MPTOB291 promotes protective processes which are related to glioma pathoway and protective pathways in tumor surrounding tissue (TSST) such as MAPK signaling pathway ($p<0.05$). To evaluate a role of interaction between mRNAs and lncRNAs on glioma microenvironment, we found physical and functional relationships of top 10 up- and down- regulated mRNAs and lncRNAs. Data show that nearby located-mRNAs of top ten up- and down-regulated lncRNAs were expressed similarly with those expression-changed lncRNAs. Highly correlated ($p<0.05$) lncRNAs with top ten up- and down-regulated mRNAs were also expressed similarly with those coding transcripts. Our study have shown that lncRNA and mRNA interaction plays a role in tumor microenvironment; and the novel HDAC inhibitor, MPTOB291 may decrease tumor growth *in vivo* by changing gene expression in tumor surrounding stromal tissue.

**Disclosures: B. Batsaikhan:** None. **K. Hung:** None. **J. Wang:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.16/G9

**Topic:** B.14. Neuro-Oncology

**Title:** Caffeine modulates the hypoxic/angiogenic pathway in glioblastoma cells

**Authors: *G. MAUGERI**[1], A. D'AMICO[2], D. RASÀ[1], S. CAVALLARO[3], V. D'AGATA[1]
[1]BIOMETEC, Univ. of Catania, Catania, Italy; [2]San Raffaele Open Univ. of Rome, Rome, Italy; [3]Italian Natl. Res. Council, Catania, Italy

**Abstract:** Glioblastoma multiforme (GBM) represents the most aggressive primary brain tumor in adults including extensive hypoxic areas. The latter is one of the hallmarks of this cancer. It triggers hypoxia-inducible factors (HIFs) expression leading to accumulation of pro-angiogenic factors such as vascular endothelial growth factor (VEGF). Previous papers have shown that the most common ingested psychoactive drug in the world, caffeine, reduces proliferation of GBM, by inducing apoptotic cell death. In the present study, it has been investigated whether caffeine effect is mediated through the modulation of HIFs and VEGF expression. Results have shown that caffeine significant reduced HIF-1α and VEGF expression in GBM cells exposed to hypoxia. Its role is mediated by inhibition of PI3K/Akt and MAPK/ERK signaling pathways, which are implied in HIFs regulation. These data give new insight into the antitumor activity of caffeine, counteracting hypoxia event in GBM.

**Disclosures: G. Maugeri:** None. **A. D'Amico:** None. **D. Rasà:** None. **S. Cavallaro:** None. **V. D'Agata:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.17/G10

**Topic:** B.14. Neuro-Oncology

**Title:** TAK1 inhibitor 5Z-7-oxozeaenol sensitizes glioma to chemotherapy

**Authors: *E. ESPOSITO**, M. CAMPOLO, M. LANZA, G. CASILI, I. PATERNITI, A. FILIPPONE, S. CUZZOCREA
Univ. of Messina, Messina, Italy

**Abstract:** Gliomas are the most common primary brain tumors of the central nervous system. Despite relevant progress in conventional treatments, the prognosis of such tumors remains almost invariably dismal. NF-κB activation is one of the resistance mechanisms for cancer cells to escape from chemotherapy-induced cell-death. TAK1 is an essential component in genotoxic stresses-induced NF-κB activation; however, the role of TAK1 in the development of chemoresistance in glioma remains unknown. Using a panel of glioma cell lines, U138 and A172, we found that TAK1 inhibitor 5Z-7-oxozeaenol significantly augmented the cytotoxic effects of temozolomide (TMZ) on glioma cell lines. TAK1 inhibition also enhanced the inhibitory effect of TMZ on anchorage independent growth. Treatment of glioma cells with 5Z-7-oxozeaenol blocked TMZ- induced NF-κB activation, reduced cytokines expression and

enhanced TMZ-induced apoptosis in both cell lines.
Together, our results provide a proof-of-concept that TAK1 inhibition significantly increases the sensitivity of glioma cells to chemotherapy-induced cell-death and can serve as an effective adjunct to current chemotherapeutic regimens for high risk diseases.

**Disclosures:** **E. Esposito:** None. **M. Campolo:** None. **M. Lanza:** None. **G. Casili:** None. **I. Paterniti:** None. **A. Filippone:** None. **S. Cuzzocrea:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.18/H1

**Topic:** B.14. Neuro-Oncology

**Title:** Tumor microtubes are present also in glioma cell line cultures

**Authors: *V. TORRE**[1,2], X. LI[1,2,3], V. FARZAMRAD[1,4], D. POZZI[1], Y. YANG[3,2]
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**Abstract:** When primary glioma cell lines derived from patients are implanted in the brain of animal laboratories, a multitude of extremely long (up to .500 μm) and very thin (1-2 μm thick) membrane protrusions are observed (Osswald et al 2015). These protrusions are referred to as tumor microtubes (TMs) and are thought to be a major player of the malignancy of brain tumors. We found that similar structures can be seen in vitro when glioma cell lines are cultured and stained with the fluorescent dye membrane marker Vybrant DiI. Indeed, live cell imaging of U87 cell lines (Fig.1) shows the presence of very thin processes connecting different glioma cells at distances varying from 10 to 500 μm. These thin processes are not visible with usual bright field illumination and presumably for this reason were not seen before. These processes are very similar to the TMs previously described (Osswald et al 2015) and will be referred to in the same way. In our experiments a fluorescent image was acquired every minute, so that we could observe the dynamics of these TMs over time. In vitro TMs provide connections between distant glioma cells which remain stable for several hours even when the cells move and proliferate. TMs appear to be formed when glioma U87 cells divide. Similar structures are also observed in cultures of astrocytes, but in this case these thin and long structures are not stable and disappear in less than 10 minutes. Cultures of glioma U87 and of U251 cell lines exhibit also large and slow calcium transients during which the relative change of fluorescence ΔF/F could be 100 %. When glioma cells form tumorspheres, these calcium waves become less evident.
Osswald M et al. (2015) Brain tumour cells interconnect to a functional and resistant network. Nature. 528(7580):93-98.

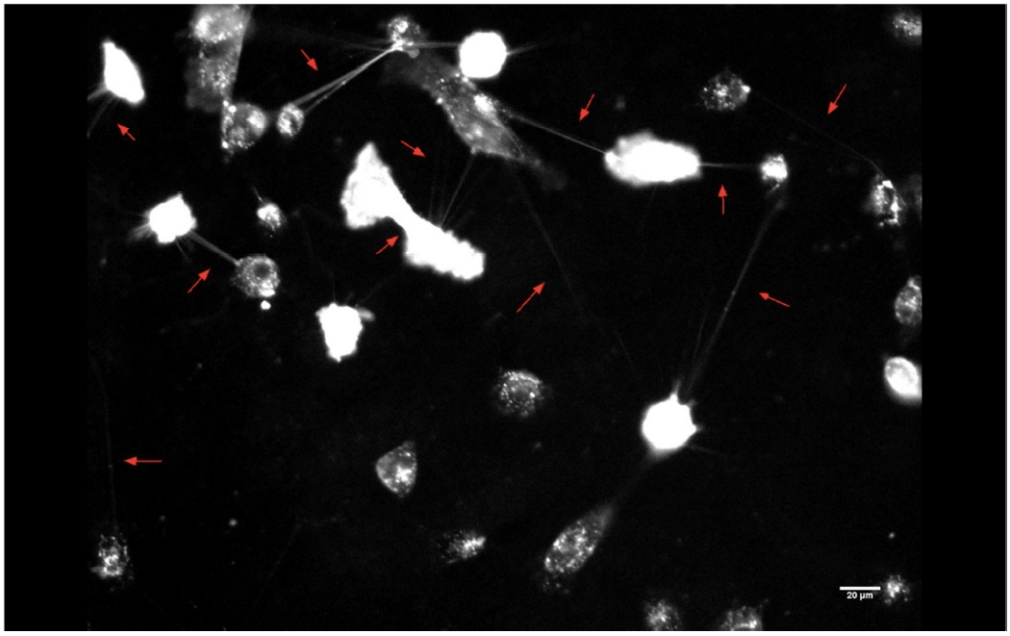


*Fig.1: A fluorescence image of a glioma U87 culture, stained with the membrane marker Vybrant DiI. Red arrows indicate TMs providing stable links between glioma cells. TMs are stable structures observed for several hours.*

**Disclosures: V. Torre:** None. **X. Li:** None. **V. Farzamrad:** None. **D. Pozzi:** None. **Y. Yang:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.19/H2

**Topic:** B.14. Neuro-Oncology

**Support:** NIH Grant R00NS082379

**Title:** Inhibition of prostaglandin E2 receptor EP2 suppresses malignant gliomas

**Authors: J. QIU**[1,2], Q. LI[1], K. BELL[1], E. ZHANG[3], J. YU[3], Z. SHI[2], *J. JIANG[1]
[1]James L. Winkle Col. of Pharm., Univ. of Cincinnati, Cincinnati, OH; [2]Dept. of Cell Biol. and Inst. of Biomedicine, Jinan Univ. Col. of Life Sci. and Technol., Guangzhou, China; [3]Dept. of Intrnl. Med., Univ. of Cincinnati Col. of Med., Cincinnati, OH

**Abstract:** Glioma constitutes about 80% of all primary malignant brain tumors in humans, and 82% of these cases are classified as the WHO grade IV tumor - glioblastoma multiforme (GBM). Despite current combined treatment engaging surgical resection, chemotherapy and radiation therapy, the median survival of GBM patients is only about one year, with less than 3-5% of

patients surviving over five years. Malignant gliomas grow relentlessly and impinge on vital neural structures, leading to profound neurological impairments. As such, over 80% of glioma patients experience seizures during the course of their illness. Developing new therapeutics with adequate efficacy for this devastating brain condition and the associated comorbidity is an urgent unmet need. Cyclooxygenase-2 (COX-2) is commonly induced in gliomas, and its expression level is highly positively correlated with the tumor grade and mortality of human patients. Mounting evidence from animal and human studies suggests that the elevated COX-2 is a key driving force for glioma growth. However, COX-2 inhibitors in malignant glioma treatment has been limited due to their well-documented vascular toxicity and recent inconsistent outcomes from a number of population studies. It thus has been proposed that targeting the downstream prostanoid receptors might provide more specificity than blocking the entire COX cascade. As a major enzymatic product of COX-2, prostaglandin E2 ($PGE_2$) directly mediates inflammatory reactions during the pathogenesis of tumors and other chronic conditions, and facilitates the disease progression via its four G protein-coupled receptors (GPCRs) - EP1, EP2, EP3 and EP4. However, which type of EP receptor is involved in COX-2/$PGE_2$-mediated glioma growth remains largely unknown. Using a combination of pharmacological and genetic strategies, we show that the $PGE_2$ receptor EP2 is a dominant Gαs-coupled receptor that mediates COX-2/$PGE_2$-initiated cAMP signal pathways in human malignant glioma cells, such as LN229 and SF767, and is essentially involved in the proliferation, migration and invasion of these brain tumor cells *in vitro, in vivo* and *in situ*. Our results together suggest that $PGE_2$ signaling via EP2 receptor increases the malignant potential of human glioma cells and might represent a novel molecular target for malignant gliomas.

**Disclosures: J. Qiu:** None. **Q. Li:** None. **K. Bell:** None. **E. Zhang:** None. **J. Yu:** None. **Z. Shi:** None. **J. Jiang:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.20/H3

**Topic:** B.14. Neuro-Oncology

**Support:** NMSU Manasse Endowment

**Title:** Effects of serum-free media on the growth of astrocytoma in hydrogel and monolayer environments

**Authors: *M. P. JOGALEKAR**[1], E. E. SERRANO[2]
[1]Mol. Biol., [2]Biol., New Mexico State Univ., Las Cruces, NM

**Abstract:** Grade IV astrocytoma is a type of invasive primary malignant tumor. Preliminary work from our laboratory has shown that astrocytoma cells form distinct morphologies when cultured in different microenvironments - monolayer or hydrogel - in media supplemented with serum. This pilot study was designed to evaluate the growth of astrocytoma in serum-free conditions. To this end, we established the grade IV astrocytoma cell line, CCF-STTG1, in monolayer and in hydrogel (Geltrex$^{TM}$) using media containing either fetal calf serum, or serum-free media with growth factors. Phase contrast and epifluorescence microscopy of monolayer cultures showed that astrocytoma cells appeared dispersed and flattened in presence of serum, while the cells aggregated when cultured in serum-free media. In contrast, astrocytoma grown in hydrogel culture, with or without serum, formed compact cell clusters interconnected by strands of extended cells. Preliminary analysis suggests cell proliferation is augmented in serum-free medium as compared to cultures where media contain serum. These findings suggest that the combination of hydrogel and serum-free media with growth factors can provide a unique model for examining cancer progression in vitro. Future studies will continue to investigate different hydrogels for their potential to support astrocytoma cell growth in serum-free conditions.
Research supported by the NMSU Manasse Endowment Fund.

**Disclosures:** **M.P. Jogalekar:** None. **E.E. Serrano:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.21/H4

**Topic:** B.14. Neuro-Oncology

**Support:** APS-Rita Allen Foundation

**Title:** Reciprocal facilitation of proliferation and migration between Schwann cells and cancer cells through Adenosine A2B receptor

**Authors: *Y. YE**[1], E. SALVO[2]
[2]Bluestone Ctr. for Clin. Res., [1]New York Univ., New York, NY

**Abstract:** Neural invasion (NI) occurs in 85% of head and neck squamous cell carcinoma (HNSCC) cases. NI contributes to cancer pain and is a route for metastasis dissemination. Schwann cells have been recently shown to play an active role in facilitating neural invasion by cancer cells. Cancer microenvironment contains high levels of adenosine which is accumulated during cancer induced hypoxia, inflammation, and ischemia. Adenosine and its pro-inflammatory receptor A2B in cancer contribute to cancer growth and metastasis. We hypothesize that cancer derived adenosine stimulates Schwann cells to proliferate and migrate towards cancer through A2B receptor, and controlling A2B receptor on both cancer and

Schwann cells will inhibit cancer progression (*i.e.*, cancer growth and NI). Using an *in vitro* co-culture system, we demonstrate that Schwann cells (RSC96) and oral cancer cells (HSC-3) can reciprocally promote proliferation, migration, and invasion, which are critical cellular events that lead to NI. A2B receptors are expressed on both HSC-3 and RSC96 cells and treatment with the A2B receptor antagonist PSB603 significantly reduced RSC96 and HSC-3 cell proliferation and migration towards each other, whereas the A2B receptor agonist BAY60-6583 promoted cell proliferation in both cell lines. Together, these results suggest an important role of A2B receptors in Schwann cell interaction with oral cancer. Whether A2B receptor holds the key for NI *in vivo* needs to be determined.
This work is supported by American Pain Society & Rita Allen Foundation.

**Disclosures: Y. Ye:** None. **E. Salvo:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.22/H5

**Topic:** B.14. Neuro-Oncology

**Support:** WUSTL Chancellor's Graduate Fellowship

**Title:** The use of rs-fMRI to identify glioblastoma infiltration for preoperative planning

**Authors: *A. G. DANIEL**[1], J. L. ROLAND[2], J. S. SHIMONY[3], E. C. LEUTHARDT[2,1]
[1]Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO; [2]Neurolog. Surgery, [3]Mallinckrodt Inst. of Radiology, Washington Univ. Sch. of Med., Saint Louis, MO

**Abstract:** Despite advancements in neuro-oncology, glioblastoma, the most common primary brain tumor, has a median survival of 15 months with optimal surgical and medical treatment. This is related to the observation that microscopic tumor cells extend beyond the borders defined by gadolinium enhancement. The diffuse tumor extent leads to debate regarding the amount of tissue that should be resected to limit recurrence. Tissue that when resected leads to an overt deficit, termed eloquent, is a well-accepted limit. However, non-eloquent tissue beyond gadolinium enhanced borders may have variable tumor involvement. In addition, tumor infiltration may also be heterogeneous within these borders. Resting-state functional magnetic resonance imaging (rs-fMRI) can be used to identify language, sensory and motor cortex yet limited studies have evaluated rs-fMRI to assess tumor involvement. In this study, we evaluated the effects of glioblastoma on standard rs-fMRI connectivity measures between tumor and peri-tumor regions to distant areas in 6 patients. Distinct patterns emerged within these regions and their contralateral homologues. Inter-hemispheric correlations suggested persistence of functional connectivity despite tumor infiltration. These results indicate a potential role for rs-

fMRI to improve the delineation of areas associated with glioblastoma and functional tissue, which may better inform preoperative planning.

**Disclosures: A.G. Daniel:** None. **J.L. Roland:** None. **J.S. Shimony:** None. **E.C. Leuthardt:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.23/H6

**Topic:** B.14. Neuro-Oncology

**Title:** BPM 31510, a clinical stage metabolic modulator, alters mitochondrial bioenergetics and resensitizes Temozolomide resistant glioma lines to TMZ induced cell death

**Authors: T. DADALI**, P. AWATE, S. MOGRE, A. DIERS, S. GESTA, V. K. VISHNUDAS, *K. THAPA, N. NARAIN, R. SARANGARAJAN
Berg, LLC, Framingham, MA

**Abstract:** Glioblastoma multiforme (GBM) is an aggressive malignancy with limited treatment options and high probability of acquiring chemoresistance. Upon acquisition of Temozolomide (TMZ) chemoresistance, glioma undergo mitochondrial electron transport chain (ETC) remodeling, correlating chemoresistance and alterations in metabolism at the mitochondrial level. BPM31510, a metabolic modulating agent in clinical trials for solid tumors and GBM, selectively targets and reprograms cancer cells to execute mitochondrial-dependent apoptosis. Here, we sought to investigate the mechanism of action of BPM31510 in TMZ chemo-sensitive and resistant glioma models. BPM31510 demonstrated anti-cancer activity in human GBM cell lines in vitro (U251-MG, U87-MG), and long-term survival was achieved in ~20% of BPM31510-treated rats bearing orthotopically implanted C6 glioma allografts, indicating efficacy in the chemo-sensitive/naïve setting. A model of acquired TMZ chemoresistance (TMZ-R) was then generated by progressive adaptation of parental U251-MG and U87-MG cells to incremental doses of TMZ. Treatment with BPM31510 decreased viability in TMZ-R U-251 and U-87 MG cell lines. In addition, combinational treatment with BPM31510 sensitized parental cells to TMZ, and importantly, resensitized TMZ-R cells to TMZ (250-1000 μM), indicating BPM31510 retains anti-cancer activity in a TMZ chemoresistant setting. Given the critical role of ETC remodeling in TMZ chemoresistance, we next examined the impact of BPM31510 treatment on mitochondrial respiration. As expected, alterations in mitochondrial respiration characterized by an increase in succinate (Complex II) and glycerol-3-phosphate (Complex III)-fueled respiration were observed in TMZ-R cells compared to parental controls. BPM31510 exposure (EC50) did not impact pyruvate/malate (Complex I) driven respiration in either parental of TMZ-R U251-MG. In contrast, BPM31510 consistently decreased succinate- and

glycerol-3-phosphate driven respiration in both parental and TMZ-R U251-MG, indicating that BPM31510 disrupts ETC Complex function dependent on a functional Q-pool and that TMZ-dependent ETC remodeling remains sensitive to BPM31510. Together this data demonstrate that BPM31510, by altering Q-pool dependent mitochondrial function, may provide a potential therapeutic strategy to treat GBM in both chemosensitive and resistant settings.

**Disclosures:** **T. Dadali:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **P. Awate:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **S. Mogre:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **A. Diers:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **S. Gesta:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **V.K. Vishnudas:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **K. Thapa:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **N. Narain:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC. **R. Sarangarajan:** A. Employment/Salary (full or part-time):; BERG, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BERG, LLC.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.24/H7

**Topic:** B.14. Neuro-Oncology

**Title:** A rapid, pH-sensitive screening method to detect internalization of cell surface markers for development of antibody-based pharmaceuticals to treat brain tumors

**Authors: P. A. SHRAMM**[1], L. ANCHETA[2], D. HIGGINS[1], *D. A. LAPPI[3]
[1]Advanced Targeting Systems, San Diego, CA; [2]Cytologistics, LLC, San Diego, CA; [3]Veiove Animal Hlth., San Diego, CA

**Abstract:** Some of the most potent treatments for cancers have been antibodies to cell surface proteins that cause tumor cell proliferation. Examples are cetuximab (antigen: EGFR) approved for colorectal cancer and Trastuzumab (ERBB2) for breast cancer. These antibodies have more than one effect on the cancer cell, but one of the most important is that, upon binding to the cell surface antigen, the complex is internalized by so-called antibody mediated internalization. As such, the mitogenic cell surface protein no longer plays a role in cancer cell division. Despite the blood brain barrier challenging systemic treatment for brain tumors, intracerebroventricular injection can produce similar results. For example, Gholamin *et al*., (Sci Transl Med 9:381, 2017) and Kang *et al.* (Sci Rep 6:34922, 2016) reported down-regulation of brain tumor mitogenic agents through antibody-mediated endocytosis. The quick and efficient screening of antibodies that internalize effectively is vital for determining suitability of an antibody as a therapeutic targeting agent. Here we describe a method for the efficient determination of internalization of cell surface molecules by antibodies using a pH-dependent fluorescent reporter cross-linked to a secondary antibody in a plate-based assay with visualization of internalization in hours. This conjugate is comprised of an affinity-purified monovalent secondary antibody against both the heavy and light chain of human or mouse IgG and is conjugated to a pH - dependent fluorescent reporter. The fluorescence from this reporter increases intensity as the pH of its surroundings becomes more acidic, as evident when exposed to the environment inside a cell (thereby providing evidence of internalization). A successful assay protocol has been developed to provide an $EC_{50}$ by way of a fluorescence-detecting plate reader, which could be used to explore antibody candidates as therapeutics in a quick and reproducible manner.

**Disclosures: P.A. Shramm:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **L. Ancheta:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **D. Higgins:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **D.A. Lappi:** F. Consulting Fees (e.g., advisory boards); Advanced Targeting Systems.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.25/H8

**Topic:** A.07. Developmental Disorders

**Support:** NIH T32 CA 9657-25

Jacobs Research Foundation

**Title:** Characterization of an alternatively spliced NTRK2 variant in cancer: Employing novel reagents to uncover novel functions

**Authors: *S. S. PATTWELL**[1,2], B. G. HOFFSTROM[2], N. E. BOIANI[2], H. BOLOURI[2], S. ARORA[2], T. A. GOODPASTER[2], J. RANDOLPHI-HABECKER[2], E. C. HOLLAND[1,2]
[1]Human Biol., [2]Fred Hutchinson Cancer Res. Ctr., Seattle, WA

**Abstract:** Most known for their essential roles in the development and maintenance of the nervous system, the neurotrophins consist of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), NT-3, NT-4 and their respective tropomyosin receptor kinases (Trks) TrkA, TrkB, and TrkC along with the low affinity nerve growth factor receptor, p75. In addition to their roles in neuronal survival, proliferation, differentiation, and apoptosis, Trks exert diverse effects on cellular outcomes through their interactions with ion channels, G-protein coupled receptors (GPCRs), and activation of downstream signaling cascades such as extracellular related kinase (ERK) and phosphatidylinositol-3 kinase (PI3K). Prior to Trks' established roles in neurobiology, oncogenic Trk was discovered as tropomyosin 3 (TPM3)-TrkA fusion and as a result, oncological studies of the neurotrophin family have since embarked on the quest for identifying additional Trk fusions. Putative oncogenic Trk fusions have been observed in various cancer types, but their clinical significance remains unclear and these fusions tend to occur at very low frequencies below 1-2%. The low incidence of these fusions combined with significant overexpression of various Trks in a multitude of cancers raises the complex possibility that another aspect of Trk biology, aside from kinase-domain fusions, may be at play. Basic scientific and clinical investigation surrounding Trks' role in cancer has been hindered due to the nonspecific nature of antibodies and kinase inhibitors, combined with a lack of precise exon-specific expression data from patient populations. Alterations in RNA splicing are being appreciated for their contributions to cancer, and as TRK genes have multiple splice variants, we sought to investigate exon-specific expression of neurotrophin receptors in human gliomas via RNA-Seq data obtained from The Cancer Genome Atlas (TCGA). We have created an antibody specific to an alternatively spliced NTRK2 variant and have used it to detect protein expression in human gliomas. Forced expression of this neurotrophic receptor splice variant in mouse models contributes to the formation of gliomas and other tumors, within and outside the central nervous system, highlighting the potential role for alternative splicing of neurotrophin receptors in oncogenesis.

**Disclosures: S.S. Pattwell:** None. **B.G. Hoffstrom:** None. **N.E. Boiani:** None. **H. Bolouri:** None. **S. Arora:** None. **T.A. Goodpaster:** None. **J. Randolphi-Habecker:** None. **E.C. Holland:** None.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.26/H9

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** This work was supported by the Field Neurosciences Institute, St. Mary's of Michigan and the Neuroscience Program at Central Michigan University

Verdure Science (Noblesville, IN) for donating the solid-lipid curcumin particles

**Title:** Curcumin GBM: Solid lipid curcumin particles kill more human glioblastoma cells than does dietary curcumin

**Authors: *P. MAITI**[1], G. DUNBAR[2]
[1]Psychology and Neurosci. Program, Central Michigan University/St. Mary's of Michigan, Saginaw, MI; [2]Psychology and Neurosci. Program, Central Michigan University/St. Mary's of Michigan, Mt. Pleasant, MI

**Abstract:** Glioblastoma mutiforme (GBM) is the most prevalent, aggressive brain cancer, characterized by necrosis surrounding the brain area. Current palliative therapies available are surgical treatment, radiation therapy, and chemotherapy, but these treatments are unable to stop completely the progression of this disease, so alternative therapies are needed. Curcumin (Cur), a natural polyphenol, has potent anti-cancer effects against several cancers. It is a potent inhibitor for tumor growth and possesses promising pro-apoptotic activities. Unfortunately, its poor solubility and rapid degradation make it less attractive for GBM therapy. Recently, we have shown that solid lipid curcumin particles (SLCPs) have greater bioavailability and brain tissue penetration than observed with administration of dietary Cur. The present study characterizes the comparative cell viability, cell death, and DNA fragmentation in culture human GBM cells (U87MG) after treatment with different dose and duration of either dietary Cur or SLCPs. We have investigated cell viability by MTT assay, mode of cell death by annexin-V staining, DNA fragmentation by TUNEL staining, and single cell gel electrophoresis (Comet assay), and also nuclear morphology by propidium iodide following administration of 25 μM dietary Cur or SLCPs for 24-, 48-, and 72-h. We observed that SLCPs showed significantly less cell viability, greater cell death, and DNA fragmentation in comparison to dietary Cur. Further, our comet analysis revealed longer DNA tails and more number of fragmented nuclear lobes when SLCP was used, in comparison to dietary Cur. Taken together, our *in vitro* work suggests that use of SLCPs may be a promising strategy for reversing or preventing GBM growth and differentiation, as compared to using dietary Cur *in vitro*. **Keywords:** Glioblastoma mutiforme, curucmin, apoptosis, DNA fragmentation, comet assay, TUNEL staining, cell death

**Disclosures:** **P. Maiti:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Verdure Science donated solid lipid curucmin particles for this study. **G. Dunbar:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Verdure Science, Indianapolis.

**Poster**

**566. Brain Tumor Biology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 566.27/H10

**Topic:** A.07. Developmental Disorders

**Support:** NIH Grant 7R01NS036692-16

**Title:** Enhanced peritumoral hyperexcitability in a pediatric glioma model: The role of KCC2

**Authors: *S. L. CAMPBELL**[1], A. NYITRAY[3], L. CHAUNSALI[2], H. SONTHEIMER[4]
[1]Virginia Tech. Carilion Res. Inst., [2]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [3]Virginia Tech. Carilion Sch. of Med., Roanoke, VA; [4]Sch. of Med. and Res. Inst., Virginia Tech. Sch. of Neurosci., Roanoke, VA

**Abstract:** Pediatric high-grade gliomas are highly aggressive brain tumors with high mortality and morbidity. High-grade gliomas are less frequent in children and widely understudied. When gliomas occur in the cortex of pediatric patients, they often present with seizures, called tumor-associated seizures. We previously showed that in vivo implantation of adult patient-derived tumors with high expression of cystine/glutamate transporter system xc (-) (SXC) induced seizures and displayed peritumoral hyperexcitability in adult animals. In addition we found that GABAergic disinhibition contributed to the disease pathophysiology where peritumoral neurons exhibited elevated intracellular Cl(-) concentration and consequently depolarizing, excitatory gamma-aminobutyric acid (GABA) responses. In these adult neurons, the plasmalemmal expression of KCC2- a potassium-chloride transporter, which establishes the low Cl(-) concentration required for GABA receptor-mediated inhibition, was significantly decreased. KCC2 expression is developmentally regulated and causes Cl(-) to be depolarizing early in development. It is unknown whether the expression of KCC2 is altered in pediatric glioma and contributes to peritumoral hyperexcitability. Changes in Cl(-) homeostasis in the developing brain could affect therapeutic strategies to treat tumor-associated seizures in younger patients. The objective of this study is to determine if glioma induces changes in KCC2 expression early in development and if those changes affect peritumoral hyperexcitability. Electrophysiological recordings from pups implanted with pediatric glioma cells revealed that peritumoral neurons are hyperexcitability. Pediatric peritumoral neurons displayed spontaneous epileptiform events and decreased action potential (AP) threshold. Compared to sham controls, peritumoral neurons also fired more action potentials and displayed a depolarization block in response to small current

injections. The mRNA (SLC7A11) and protein level of SXC was lower in our pediatric glioma cells compared to adults. There was also a decrease in KCC2 expression in the pediatric peritumoral cortex compared to sham controls. Together our data suggest that a high expression of SXC on pediatric glioma cells is not required for pediatric peritumoral hyperexcitability. Furthermore, the effect of the decrease in KCC2 expression is dependent on the excitatory state of GABA, inhibitory or excitatory, therefore more studies are warranted to determine the contribution of KCC2 to pediatric peritumoral hyperexcitability.

**Disclosures: S.L. Campbell:** None. **A. Nyitray:** None. **L. Chaunsali:** None. **H. Sontheimer:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.01/H11

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

**Support:** University of Bristol PhD studentship

BRACE project grant

**Title:** VEGFR1 and VEGFR2 in Alzheimer’s disease and vascular dementia

**Authors: *R. HARRIS**, S. MINERS, S. ALLEN, S. LOVE
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**Abstract:** Brain ischaemia is the defining pathological process in vascular dementia (VaD); however, cerebral blood flow is also reduced in Alzheimer’s disease (AD) and there is evidence that the hypoperfusion contributes to tissue damage. Vascular endothelial growth factor-A (VEGF) is a pro-angiogenic factor expressed in response to tissue hypoxia. VEGF receptor 2 (VEGFR2) mediates the actions of VEGF on endothelial cells leading to the formation of blood vessels. VEGF receptor 1 (VEGFR1) has limited kinase activity and its soluble form (sVEGFR1) acts as a negative regulator of VEGF. We previously reported that increased VEGF protein in AD was not associated with an increase in microvessel density. Aβ42 was shown to bind to VEGFR2 and block signalling in vitro providing a possible mechanism for reduced angiogenesis; however, there are no studies of VEGFR1 or VEGFR2 in AD brain. We have investigated the expression of these receptor proteins in parietal cortex and white matter in AD, VaD and controls, and related this to VEGF, microvessel density and Aβ levels.Samples of medial parietal cortex and white matter were dissected from 49 AD, 19 VaD and 37 control brains from the South West Dementia Brain Bank, UK. Total VEGFR1 was measured by dot blot. VEGFR1 subtypes were measured by western blot. Total VEGFR2, VEGF and von Willebrand factor

(VWF, a marker of microvessel density) were measured by ELISA. Aβ40 and Aβ42 had previously been measured in parietal cortex from the same brains. Human brain microvascular endothelial cells (HBMECs) were used to investigate VEGFR signalling. Total VEGFR1 levels were reduced in AD compared to control though an increase in the level of sVEGFR1 was observed in AD. VEGFR2 level was similar in the three groups, as was VWF level, in agreement with previous data. VEGFR2 level positively correlated with both VEGF and VWF. It also correlated positively with soluble Aβ40 but not Aβ42. Elevated VEGF fails to increase microvessel density in AD despite normal VEGFR2 level. This suggests that VEGFR2 signalling is defective in AD. In combination with an increased proportion of a negative regulator of VEGF, this may contribute to reduced angiogenesis observed in AD. Further research using is needed to elucidate the underlying mechanisms.

**Disclosures: R. Harris:** None. **S. Miners:** None. **S. Allen:** None. **S. Love:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.02/H12

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

**Support:** Alzheimer's Association Research fellowship (AARF-16-443173)

**Title:** Role of Unc5c, an Alzheimer’s risk gene in late-onset Alzheimer’s Disease in a novel mouse model

**Authors: *D. KARUNAKARAN**[1], R. J. VASSAR[1], R. J. WATTS[2,3], J. K. ATWAL[2]
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**Abstract:** Alzheimer’s disease (AD) is characterized by amyloid plaques, neurofibrillary tangles, and synaptic and neuronal loss. Recently, a rare autosomal dominant coding mutation, T835M, was discovered in the Un-coordinated 5c (*UNC5C*) netrin receptor gene that segregated with late-onset AD (LOAD). T835M alters a conserved amino acid in the hinge region of the UNC5C death domain, suggesting the mutation may increase apoptosis. Interestingly, *UNC5C* rare coding mutations near amino acid 835 (T837K, S843G, Q860H) have also been found associated with AD, suggesting that this region of UNC5C is critical for AD risk and pathogenesis. Indeed, in primary hippocampal neurons, overexpression of UNC5C T835M increased cell death in response to neurotoxic stimuli including beta-amyloid (Aβ). These results suggest a mechanism by which UNC5C T835M may confer increased risk of LOAD, however the effects of this mutation in an AD animal model have not yet been explored. Toward this end, we generated a mouse knock-in (KI) model of Unc5c T835M and crossed it with the 5XFAD mouse model of

amyloid pathology and neuron loss. Our preliminary results in mice suggest that UNC5C T835M promotes neuron loss in the presence of Aβ pathology. We hypothesize that the UNC5C T835M mutation predisposes to LOAD by exacerbating neuronal death, as observed in the 5XFAD brain, via increased sensitivity to Aβ-induced neurotoxicity and UNC5C death domain activation. We are further investigating mechanisms of cell death and distal phenotypes in 5XFAD; Unc5c T835M KI mice by biochemical, cellular, and behavioral approaches. Although neuron loss is a cardinal feature of AD, the molecular mechanism of cell death in AD is still unclear. We expect our results to provide valuable insight into the role of UNC5C T835M mutation in Aβ- and Tau-associated cell death, and thereby identify novel therapeutic targets to prevent neuron loss in AD.

**Disclosures: D. Karunakaran:** None. **R.J. Vassar:** None. **R.J. Watts:** None. **J.K. Atwal:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.03/I1

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

**Support:** NIH/NIA Grants P30AG10161 and RF1AG15819.

University of Minnesota College of Pharmacy Melendy/Peters Research Scholarship

University of Minnesota Academic Health Center Faculty Research Development Program

**Title:** Dysregulation of protein prenylation in aging and Alzheimer’s Disease

**Authors: *A. JEONG**[1], M. DISTEFANO[2], D. A. BENNETT[4], G. WOOD[3], L. LI[1]
[1]Exptl. and Clin. Pharmacol., [2]Chem., [3]Pharmacol., Univ. of Minnesota-Twin Cities, Minneapolis, MN; [4]Rush Alzheimer's Dis. Center, Rush Univ., Chicago, IL

**Abstract:** Alzheimer’s disease (AD) is the leading cause of age-related dementia, and the 6th leading cause of death in the U.S. Despite its prevalence, the pathogenesis of AD is not fully understood. Emerging evidence suggests that protein prenylation, an important posttranslational modification, may be dysregulated in aging, contributing to the development of AD. Many proteins, including the Ras superfamily of small GTPases, undergo prenylation catalyzed by farnesyl transferase (FT) and geranylgeranyl transferases (GGT-1 and GGT-2). These small GTPases are involved in regulating diverse cellular processes. Recent findings have shown that the levels of isoprenoids are increased in the brain of AD patients. Animal studies also showed elevated isoprenoids in aged mouse brains. In addition, genetic downregulation of protein

prenylation reduces amyloid pathology and neuroinflammation in a transgenic mouse model of AD. This study aims to determine the dynamic changes of prenylated proteins in human brains and their association with aging and AD pathology. For the aging study, postmortem frozen frontal cortical tissues (BA46/BA9) from cognitively normal individuals across the lifespan were obtained via the NIH NeuroBioBank. For the AD study, postmortem frozen tissues from the dorsolateral prefrontal cortex were obtained from the Religious Orders Study, which included age- and sex-matched human subjects with a spectrum of cognitive function from individuals with no cognitive impairment (NCI), mild cognitive impairment (MCI), to AD dementia. The brain tissue samples were subjected to subcellular fractionation and immunoblotting analysis. We found that compared to the young age group (20-29 years), the levels of FT were increased in both the middle age group (50-59 years) and the old age group (74-91 years), whereas the levels of GGT-1 were only increased in the old age group. In the brain of individuals with AD dementia, only the levels of FT, but not GGT-1, were increased. Consistent with the upregulation of FT in AD dementia, we found a significant increase in membrane-associated (farnesylated) H-Ras in the brains from individuals with MCI and AD dementia compared to age- and sex-matched individuals with NCI. Further, the levels of farnesylated H-Ras correlate with the tangle pathology in the brain. These findings suggest that protein prenylation is upregulated in the brain during aging, and in particular, abnormal upregulation in protein farnesylation may contribute to the pathogenic process of AD dementia.

**Disclosures: A. Jeong:** None. **M. Distefano:** None. **D.A. Bennett:** None. **G. Wood:** None. **L. Li:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.04/DP05/I2 (Dynamic Poster)

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

**Title:** Neurons are not postmitotic: DNA replication, mitotic entry and initiation of cell division amidst canonical mitotic checkpoint regulation

**Authors: *C. C. WALTON**, I. PATIÑO-PARRADO, E. BARRIO-ALONSO, J. M. FRADE
Cajal Institute, CSIC, Madrid, Spain

**Abstract:** Neurons are the paradigmatic postmitotic cell. Indeed, after several years of work, we have never found any trace of cell cycle activity in cultured NeuN+/MAP2+ neurons. However, there is evidence of cell-cycle re-entry in Alzheimer’s disease. Here we show that the canonic cell cycle checkpoints are active in NeuN+/MAP2+ neurons and that their suppression results in a functional cell-cycle. Ectopic expression of endogenous cell cycle regulators in mouse hippocampal neurons maintained in culture for 16-17 days (i.e. at postsynaptogenesis stages of

development) induced BrdU incorporation in 48%, 58%, and 33% of neurons at, respectively, 24, 36, and 48 hours post transfection (hpt). The decline of BrdU incorporation observed at 48 hpt coincided with cell death, likely triggered by the mitotic DNA damage response (DDR) since widespread DNA double strand breaks (DBS) were present in transfected neurons, as assessed by Histone H2AX phosphorylation. Indeed, inhibition of the apoptotic DBS branch with a dominant-negative form of p53 dramatically reduced the proportion of active caspase-3-positive neurons. After apoptosis rescue, 41%, 59% and 61% of neurons incorporated BrdU at 24, 48, and 72 hpt respectively, and neurons progressed into G2 phase (G2). At 36 hpt, 46% of neurons were in late G2, as evidenced by the presence of phosphoHistone H3 (pH3) specific foci, suggesting the G2/M checkpoint prevents M-phase (M) entry of neurons with DNA damage. Abrogation of this checkpoint with MK1775, a Wee1 inhibitor, increased neurons in M from 2% to 31% as assessed by nuclear pH3 staining. Based on chromatin morphology and pH3 staining, 23% of transfected neurons were in prophase, 4% in prometaphase and 4% in metaphase. Prometaphase onset was further confirmed by anillin staining, which shifts from the nucleus to the cell cortex in this stage. The spindle assembly checkpoint (SAC), which arrests cells at metaphase and prevents anaphase and cytokinesis onset (cell division), appears to be also present in neurons. By overcoming the SAC with the Mps1 inhibitor AZ3146 we found neurons attempting division joined by a nucleoplasmic bridge with chromatin stretching between two nuclei. Midbody formation, the last stage of cytokinesis before abscission, was confirmed with anillin staining. In corollary, insofar it affords efficient mitotic checkpoints, we propose that the proper assembly of mitotic machinery is what renders neurons apparently postmitotic, a status that may be applicable to other postmitotic cells. Finally, to the degree in which mitotic machinery can be efficiently manipulated, one may consider the possibility of postmitotic tissue regeneration.

**Disclosures: C.C. Walton:** None. **I. Patiño-Parrado:** None. **E. Barrio-Alonso:** None. **J.M. Frade:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.05/I3

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

**Support:** R21DC014357

R01DC014723

**Title:** Fus1 KO mice as new model for sAD: Dysfunction of the electron transport chain and compensatory enhanced blood volume responses in the olfactory bulb

**Authors: *G. CORONAS-SAMANO**[1], K. L. BAKER[1,2], A. GUMASTE[1,2], W. J. T. TAN[3], A. V. IVANOVA[3], J. V. VERHAGEN[1,2]
[1]The John B. Pierce Lab., New Haven, CT; [2]Neurosci., [3]Surgery, Yale Sch. of Med., New Haven, CT

**Abstract:** Aging is a complex biological process in humans characterized by a progressive impairment of sensory function, motor skills and cognition over time. Mitochondrial dysfunction has been proposed to play a critical role in the aging process via oxidative stress, energy deficiency and calcium dysregulation. Mitochondrial dysfunction also has been implicated in age-related neurodegenerative diseases such as sporadic Alzheimer's disease (sAD). Fus1/Tusc2 is a mitochondrial tumor suppressor protein regulating numerous mitochondrial activities. Knockout of Fus1 in mice (Fus1 KO) results in mitochondrial dysfunction, chronic inflammation, and premature aging. Our *in silico* analysis showed that Fus1 levels are significantly decreased in aging brains and further decreased in the brains of AD patients, thus strongly suggesting a link between Fus1 and sAD. Genes co-expressed with Fus1 are involved in mitochondrial respiration and neurodegenerative diseases including AD. Our analysis of mitochondrial respiration in WT and Fus1 KO embryonic fibroblasts and epithelial cells using high resolution respirometry (Oxygraph-2k) showed significantly lower respiratory reserve capacities in both types of KO cells suggesting an intrinsic defect in the ability of their mitochondria to produce additional ATP in response to increased energy demands. Our recent RNA microarray analysis of hippocampi and olfactory bulbs (OB) from 7 m.o. Fus1 KO mice showed significant alterations in Alzheimer's-associated processes and pathways. We have previously demonstrated that 4 months old Fus1 KO female mice have increased oxidative stress, disrupted metabolic homeostasis, autophagy, PKC and calcium signaling in the hippocampus and/or OB. Behaviorally they performed poorly in the hidden cookie task and the Morris water maze. Given that loss of odor identification is an early marker for sAD and that we found deficits in odor-guided behavior in Fus1 KO mice, we performed intrinsic optical imaging of odor-induced blood volume responses at hemoglobin's isosbestic wavelength (565nm). We found a ~76% increase in peak blood volume response in KO mice over WT ($P<0.001$) in large and small dorsal blood vessels. These findings support the hypothesis that mitochondrial dysfunction in Fus1 KO mice negatively affects energy homeostasis, the physiology and function of the olfactory bulb, and other functions that parallels sAD. Thus, the Fus1 KO mouse is a useful model for studying aging and aging-associated disorders such as sAD characterized by learning and memory decline and impaired mitochondrial activities.

**Disclosures: G. Coronas-Samano:** None. **K.L. Baker:** None. **A. Gumaste:** None. **W.J.T. Tan:** None. **A.V. Ivanova:** None. **J.V. Verhagen:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.06/I4

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

**Support:** NIH RO1 (AG051538)

National Natural Science Foundation (NSFC) of China (No. 81528007)

**Title:** Delta-secretase phosphorylation by SRPK2 enhances its enzymatic activity, provoking the pathogenesis in Alzheimer’s disease

**Authors: *Z. WANG**[1,2], K. YE[1]
[1]Dept. of Pathology and Lab. Med., Emory Univ., Atlanta, GA; [2]Dept. of Pathophysiology, Key Lab. of Ministry of Educ. of Neurolog. Diseases, Tongji Med. College, Huazhong Univ. of Sci. and Technol., Wuhan, China

**Abstract:** Delta-secretase, a lysosomal asparagine endopeptidase (AEP), simultaneously cleaves both APP and Tau, controlling the onset of pathogenesis of Alzheimer’s disease (AD). However, how this protease is post-translationally regulated remains unclear. Here we report that Serine-arginine protein kinase 2 (SRPK2), a kinase phosphorylating serine/arginine (SR) domain-containing proteins involved in pre-mRNA splicing machinery, phosphorylates delta-secretase and enhances its enzymatic activity. SRPK2 phosphorylates the serine 226 residue on delta-secretase and accelerates its autocatalytic cleavage, leading to its cytoplasmic translocation and escalation of enzymatic activities. Delta-secretase is highly phosphorylated in human AD brains, tightly correlated with strong SRPK2 activity. Overexpression of phosphorylation mimetic (S226D) in young 3XTg mice strongly promoted APP and Tau fragmentation, and facilitated amyloid plaque deposits and neurofibrillary tangles (NFT) formation, resulting in cognitive impairment. Conversely, viral injection of the non-phosphorylatable mutant (S226A) into 5XFAD mice, an aggressive model of AD, resulted in decreased APP and Tau proteolytic cleavage and attenuated AD pathologies, and a reversal of cognitive defects. Our findings support that delta-secretase phosphorylation by SRPK2 plays a critical role in regulating this crucial protease, aggravating AD pathogenesis.

**Disclosures: Z. Wang:** None. **K. Ye:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.07/I5

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

**Support:** Arizona Biomedical Research Commission Early Investigator Award

Barrow Neurological Foundation Grant

**Title:** Amyloid beta-induced alterations in basal forebrain cholinergic intrinsic excitability are sub-region specific

**Authors: *A. A. GEORGE**[1], H. A. BIMONTE-NELSON[3], R. J. LUKAS[4], P. WHITEAKER[2]
[1]Neurobio., [2]Div. Neurobiol, Barrow Neurolog. Inst., Phoenix, AZ; [3]Arizona State Univ., Tempe, AZ; [4]Barrow Neurol Inst., Phoenix, AZ

**Abstract:** Alzheimer's disease (AD), a progressive neurodegenerative disorder, is one of the most common causes of mental deterioration in the elderly. Hallmarks of AD pathology include alterations in brain regions associated with higher cognitive functions. Several studies have correlated the severity of cognitive decline in AD with a loss of basal forebrain cholinergic neurons (BFCNs). Mechanisms underlying cholinergic neurodegeneration and subsequent memory impairments remain unknown. However, interactions between amyloid-β (Aβ), a suspected etiopathogenic agent in AD, with a nicotinic acetylcholine receptor subtype containing α7 subunits (α7*-nAChR) trigger hippocampal pyramidal neuronal homeostatic instability. Recently, a nAChR subtype containing α7 and β2 subunits has been identified on BFCNs. These heteromeric α7β2-nAChRs have different pharmacological properties from those of homomeric α7-nAChRs and are highly sensitive to functional inhibition by Aβ. Toward understanding the roles played by α7β2-nAChRs in BFCN function we used organotypic basal forebrain slice cultures and whole-cell patch clamp electrophysiology to investigate Aβ-induced alterations in BFCN intrinsic excitability. Whole-cell current clamp recordings were taken from cholinergic neurons within the medial septal-diagonal band (MSDB), the horizontal diagonal band (HDB), and the nucleus basalis (NB). We demonstrate that chronic incubation (9 days) with oligomeric or fibrillar forms of $A\beta_{1\text{-}42}$ increases MSDB and HDB cholinergic action potential firing rates (mean increase of 64 ± 8% and 25 ± 3.5%, respectively). Additionally, the magnitude of the medium afterhyperpolarization (mAHP) phase following spike generation (mean reduction of 44 ± 6.5% and 15 ± 2.5%, respectively) was lower when compared to control conditions (scrambled version of $A\beta_{1\text{-}42}$). No significant changes in action potential firing rates or mAHP magnitudes were observed in cholinergic neurons within the NB following chronic Aβ administration. Using nAChR β2 subunit knockout mice, we demonstrate normalization of Aβ-induced alterations in firing rate and mAHP of BFCNs within the MSDB. These preliminary findings suggest that

specific forms of $A\beta_{1-42}$ alter cholinergic intrinsic excitability by interacting with β2-containing nAChR subtypes. These interactions may be uniquely specific to certain cholinergic circuits within the basal forebrain and suggest novel and potentially productive therapeutic strategies to combat neurodegeneration in a brain region affected early in AD. This work was supported by the Arizona Biomedical Research Commission (AAG) and the Barrow Neurological Foundation (AAG).

**Disclosures: A.A. George:** None. **H.A. Bimonte-Nelson:** None. **R.J. Lukas:** None. **P. Whiteaker:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.08/I6

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

**Support:** R00-AG031293, Strom Estate funds, University of Minnesota Foundation (SEL)

**Title:** Bidirectional modulation of Alzheimer phenotype by alpha-synuclein in mice

**Authors: *G. BOYLE**[1], M. LACROIX[1], M. SHERMAN[1], F. AMAR[1], M. LEE[1], T. COLE[2], S. LESNE[1]
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Ionis Pharmaceuticals, Carlsbad, CA

**Abstract:** Despite the presence of Lewy bodies in more than half of Alzheimer's disease (AD) cases, the functional role of α-synuclein (αSyn) in AD remains unclear. Here we report that genetic regulation of wild-type αSyn expression modulates several key components of the phenotype displayed by APP transgenic mice, including survival, pathology and cognitive deficits. Overexpression of human αSyn had no bearing on premature mortality and led to a marked reduction in amyloid deposition. Counter-intuitively, deficits in spatial memory were exacerbated in young bigenic animals. By contrast, ablating *SNCA* in APP mice abolished early mortality, increased plaque burden and improved memory retention. To demonstrate the translational impact of these results, we will lower αSyn expression using antisense oligonucleotides in APP mice and determine whether cognitive deficits are alleviated. Altogether, this complementary, bidirectional genetic evidence suggests that αSyn may be an intrinsic component of AD and a novel therapeutic target.

**Disclosures: G. Boyle:** None. **M. LaCroix:** None. **M. Sherman:** None. **F. Amar:** None. **M. Lee:** None. **T. Cole:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Provided drug (alpha-synuclein ASO). **S. Lesne:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.09/I7

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

**Support:** Easton Center For Research on AD, fund JV-68800

**Title:** Allosteric BACE inhibition by Deformable Nanovesicle-delivered sAPPα *In vitro* and *In vivo*

**Authors: *J. J. CAMPAGNA**, A. IVANOVA, P. SPILMAN, H. GALLO, D. BAI, V. JOHN
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**Abstract: Background.** A hallmark of Alzheimer's disease (AD) brain tissue is the presence of plaques largely composed of amyloid-β (Aβ) peptide. Aβ is derived by BACE1 cleavage of amyloid precursor protein (APP) to generate soluble APPβ (sAPPβ) and the β C-terminal fragment (βCTF), followed by cleavage of βCTF by γ-secretase to produce Aβ. Alternatively, APP is cleaved by ADAM10 to produce soluble APPα (sAPPα) and the α C-terminal fragment (αCTF). We recently reported that sAPPα acts an as endogenous inhibitor of BACE activity and that the mechanism is likely allosteric, that is, sAPPα interacts with an 'exosite' site remote from the catalytic dyad to exert its effect (Libeu et. al., JAD, 2015). BACE inhibition as a target for new AD therapeutic development is still of interest, and allosteric inhibition is appealing since it has the potential to be both substrate- (APP) and enzyme- (BACE) selective. Therefore, sAPPα - or a small molecule mimetic - may be a potential therapeutic for AD that has a lower risk of undesirable off-target effects due to its selectivity. Since sAPPα is a large protein and subject to proteolysis, we encapsulated it in deformable nanovesicles (DNVs) to increase the likelihood of brain delivery. This gave us an opportunity to perform proof-of-concept *in vivo* studies of allosteric BACE inhibition and to ascertain sAPPα's potential as a therapeutic.
**Methods.** Recombinant human sAPPα (hsAPPα) was encapsulated in DNVs (DNV-hsAPPα) using a microfluidic reactor-based method we recently developed (Subbiah et. al., J. of Drug Delivery 2017). DNV-hsAPPα ability to lower sAPPβ and Aβ levels was first assessed *in vitro* in Chinese Hamster Ovary cells stably transfected with huAPP (CHO-7W). We next treated human iPSC-derived neurons with DNV-hsAPPα to determine dendritic outgrowth and sAPPβ levels; used a Caco-2 cell permeability assay of both free- and DNV-hsAPPα to predict BBB penetrance; and then confirmed brain permeability in a pharmacokinetic (PK) study by injecting wildtype mice and rats with DNV-hsAPPα intravenously (IV). AlphaLISAs (Perkin-Elmer) specific for human sAPPα, sAPPβ, and Aβ were used for all studies.
**Results.** DNV-hsAPPα reduced sAPPβ and Aβ in both CHO-7W cells and human iPSC-derived neurons. Compared to free hsAPPα, DNV-hsAPPα showed greater Caco-2 cell permeability, and

we were able to detect husAPPα in rodent brain after IV DNV-hsAPPα delivery.
**Conclusions.** Encapsulation of hsAPPα in DNVs is an efficient way to deliver hsAPPα in rodent AD models and to conduct proof-of-concept testing of allosteric inhibition of BACE *in vivo*. These studies could enable development of DNV-hsAPPα as a potential promising new therapeutic approach for AD.

**Disclosures: J.J. Campagna:** None. **A. Ivanova:** None. **P. Spilman:** None. **H. Gallo:** None. **D. Bai:** None. **V. John:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.10/I8

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

**Support:** Cure Alzheimer Fund

VA VHA I21BX002215

**Title:** Neurofilament light chain is increased in induced pluripotent stem cell-derived three dimensional neurons from Alzheimer patients

**Authors: W. XIA**[1], M. CHEN[2], *T. D. STEIN[3]
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**Abstract:** Mass Spectrometry (MS)-based analysis of human tissue from peripheral and central nervous system has contributed to our current understanding of the relationship between blood and brain proteins in Alzheimer disease (AD) patients. We have collected peripheral blood mononuclear cells (PBMC) from AD patients and control subjects, converted PBMC to induced pluripotent stem cell (iPSC) lines, and further differentiated iPSC into human three-dimensional (3D) neurons. At autopsy, postmortem brain tissue specimens from AD subjects were collected. Liquid chromatography/MS was used to analyze 3D neurons labelled with isobaric mass tags for relative protein quantification. Our study revealed a group of differentially expressed proteins that are associated with AD, including those proteins involved in modulating γ-secretase activity for Aβ generation. Importantly, our unbiased proteomic profiling showed a specific increase of neurofilament light chain in 3D neurons derived from AD subjects compared to controls. Previous reports from others have shown that neurofilament light chain was increased in cerebrospinal fluid (CSF) and blood of patients with AD, dementia with Lewy bodies, idiopathic Parkinson’s disease, multiple system atrophy, progressive supranuclear palsy, or corticobasal

syndrome. Exploration of tauopathy was carried out in 3D neurons by quantification of total tau and phosphorylated tau at residue 181 and 231 using phosphor-tau specific ELISAs. We found that phosphorylation of tau protein was altered among those iPSC-derived 3D neurons from AD patients. In conclusion, our 3D neuronal culture provides an in vitro platform to mimic the brain environment. Similar to findings in human blood and CSF, the detection of neurofilament light chain increased in AD neurons illustrates its physiological relevance in AD pathogenesis.

**Disclosures: W. Xia:** None. **M. Chen:** None. **T.D. Stein:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.11/I9

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

**Support:** NIH intramural program

**Title:** Proteolytic cleavage modulates TREM2 function and regulates gene expression for microglia lineage

**Authors: *J. SIMA**
NIA/NIH, Baltimore, MD

**Abstract:** Triggering receptor expressed on myeloid cells-2 (TREM2) variants have been shown to be linked to late onset Alzheimer disease (AD) and its proteolytic cleavage is also implicated for its function in AD. However, the molecular features of TREM2 cleavage in microglia remain unclear. Here, found that the c- terminal fraction (CTF) of TREM2 had two isoforms and each sequence was determined by Edman sequencing. Furthermore, we found that a small fraction of TREM2 CTF entered into nucleus. Overexpression of TREM CTF affected the morphology both in primary mouse microglia and in microglia cell lines. Next, we found several genes involved in cell lineage determination were regulated by TREM2 CTF in cell culture. Currently, we are analyzing the mechanism of nuclear TREM2 on the gene regulation and microglia morphogenesis. Overall, our findings imply a new function of TREM2 on gene regulation in microglia.

**Disclosures: J. Sima:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.12/I10

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

**Support:** NIA Grant P01AG14449

NIH Grant R01AG043375

NIH Grant P30AG010161

**Title:** Frontal cortex epigenetic dysregulation during the progression of Alzheimer’s disease

**Authors: *L. MAHADY**[1,2], M. NADEEM[1], B. HE[1], S. PEREZ[1], M. MALEK-AHMADI[3], J. MIGUEL[1], E. MUFSON[1]
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**Abstract:** Epigenetic proteins including histone deacetylases (HDACs) and sirtuins (SIRTs) are dysregulated in the entorhinal and parietal cortices in Alzheimer’s disease (AD). Whether alterations in HDAC and SIRT protein levels occur in the frontal cortex and are associated with pathological and cognitive changes during AD progression remains unknown. Here we performed quantitative western blotting to examine alterations in HDACs 1-4, 6, and SIRT1 in frozen frontal cortex tissue obtained from subjects who died with a premortem clinical diagnosis of no cognitive impairment (NCI, n=14), mild cognitive impairment (MCI, n=13), mild/moderate AD (mAD, n=13) and severe AD (n=8, sAD) from the Rush Religious Orders Study (RROS) and the Rush ADC, respectively. Groups were matched by age, gender, and postmortem interval. Western blot analysis revealed a significant increase in levels of HDAC1 and HDAC3 proteins in the frontal cortex of MCI (HDAC1, $p<0.001$; HDAC3, $p=0.02$) and mAD (HDAC1, $p<0.001$), with a decrease in HDAC1 ($p=0.05$) and HDAC3 ($p=0.04$) in sAD compared to NCI subjects. HDAC2 levels remained stable ($p=0.62$) across clinical groups. HDAC4 levels were significantly increased in MCI ($p<0.001$) and mAD ($p=0.04$) compared to NCI, and did not change significantly in sAD compared to control levels. HDAC6 levels increased across disease progression (NCI<MCI, $p=0.01$; NCI, mAD<sAD, $p<0.001$), while SIRT1 decreased significantly in MCI ($p=0.03$), mAD ($p<0.001$), and sAD ($p<0.001$) compared to NCI subjects. HDAC1 was negatively correlated with perceptual speed ($r=-0.45$, $p<0.05$), while levels of SIRT1 were positively correlated with perceptual speed ($r=0.61$, $p<0.001$), episodic memory ($r=0.36$, $p<0.05$), global cognitive score ($r=0.40$, $p<0.05$), and mini-mental state examination ($r=0.37$, $p<0.05$). There was a positive correlation between frontal cortex neurofibrillary tangle (NFT) counts and HDAC1 ($r= 0.37$, $p=0.02$), while SIRT1 levels were negatively correlated (r=-

0.31, p=0.05) with these lesions. These results indicate that specific HDAC protein levels are altered in the frontal cortex of prodromal AD and may contribute to cortical genomic and cellular instability during disease progression.

**Disclosures: L. Mahady:** None. **M. Nadeem:** None. **B. He:** None. **S. Perez:** None. **M. Malek-Ahmadi:** None. **J. Miguel:** None. **E. Mufson:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.13/J1

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

**Support:** MoE Tier 2 R-143-000-589-112 (YL)

NMRC 1222-2009 (CML)

**Title:** Fluorogenic probes to monitor cytosolic phospholipase $A_2$ activity

**Authors: *C.-M. LOW**[1], C. NG[3], T. KWOK[3], F. TAN[2], Y. LAM[3]
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**Abstract:** Arachidonic acid (AA) is a precursor of a family of lipid mediators, including prostaglandins, that regulates a wide variety of physiological responses and disease pathogenesis. The biosynthesis of AA occurs mainly through the activation of phospholipase A2 (PLA2) which catalyzes the hydrolysis of the sn-2 ester bond of glycerophospholipids to release AA. Mammalian cells have diverse forms of PLA2 including the secreted small molecular weight sPLA2, the larger cytosolic Ca2+-dependent cPLA2 and Ca2+-independent iPLA2. Although sPLA2 could participate in the release of arachidonate during injury, the main gatekeeper for the enzymatic conversion of arachidonate is cPLA2. Given that cPLA2 is an ubiquitous enzyme which is highly selective for glycerophospholipids containing AA, there has been much interest to design and identify cPLA2 inhibitors to better understand the molecular mechanism regulating this enzyme and to develop efficacious therapeutics for the treatment of cPLA2-upregulated diseases such as neuroinflammation and Alzheimer's disease. To this end, we are interested to identify novel chemical tools, particularly fluorescent probes which could provide a means to monitor cPLA2 activity. Arachidonyl trifluoromethyl ketone (AACOCF3) is a potent inhibitor of cPLA2 which served as the template for modifications in this study. Arachidonic acid derivatives equipped with either one or two fluorescent groups attached to the tip of the alkyl chains were synthesized and shown to function as inhibitor and substrate probes of cPLA2. The inhibitor probe 7OHCou-AACF3 has an IC50 of 12.5 +- 1.0 uM which is comparable with AACOCF3. It

is able to quench iNOS production via its role as an inhibitor and is capable of imaging the increase in cPLA2 protein levels. We have also developed another probe Flu7OHCou as a cPLA2-selective fluorogenic substrate and demonstrated its use for inhibitor screening assays. Taken together, we report two novel fluorogenic probes capable for determining cPLA2 activity as well as cellular imaging.

**Disclosures:** **C. Low:** None. **C. Ng:** None. **T. Kwok:** None. **F. Tan:** None. **Y. Lam:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.14/J2

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

**Title:** Analysis of physicochemical interaction GM1 ganglioside cluster containing lipid membrane with amyloid beta peptides: Alzheimer's related protein

**Authors: *M. VAHED**[1], T. HOSHINO[1], S. NEYA[1], M. VAHED[2], M. KATSUMI[3]
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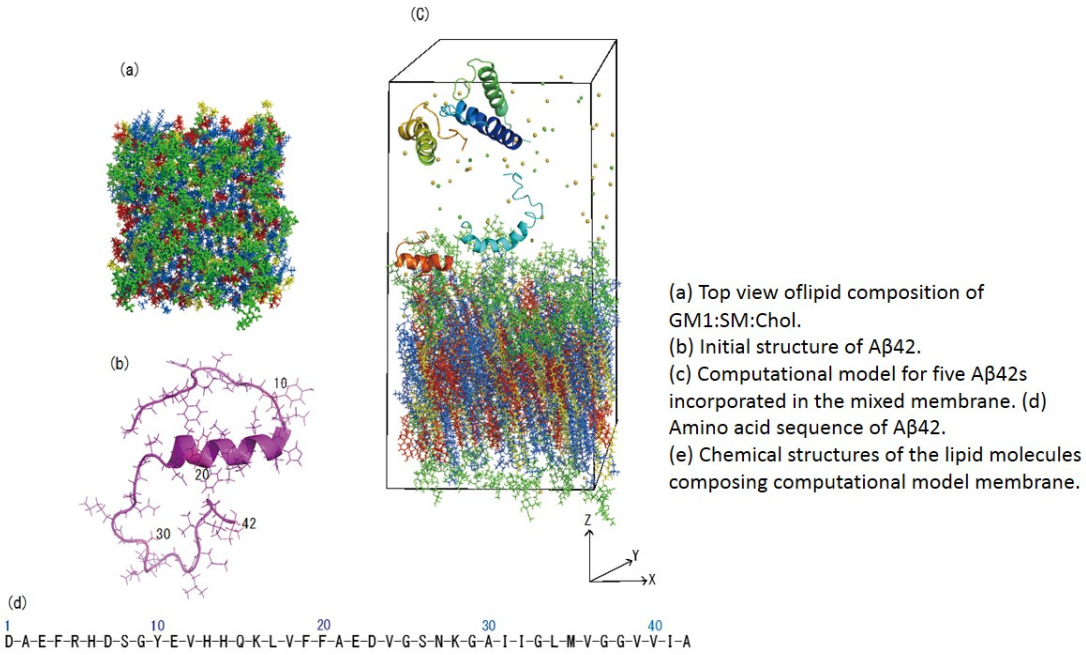
**Abstract:** The Cluster of GM1-ganglioside lipid membrane play an important role to interaction with amyloid beta (Aβ) in the initial stages of Alzheimer's disease pathology. In this work, molecular dynamics (MD) simulations for five Aβ42 were performed to investigate the behaviors of Aβ42 on GM1-ganglioside-containing lipid membrane. As far the computational model, the initial atom coordinate of Aβ42 were extracted from one of the conformations which had been determined by solution nuclear magnetic resonance (NMR) spectroscopy (PDB accession code: 1Z0Q/1IYT). A computational model for mixed membrane was composed of 48 monosialotetrahexosylganglioside (GM1), 96 sphingomyelin (SM) and 96 cholesterol (CHL). A 1000ns simulation was executed with NAMD 2.9 programs to analyze the probability of the Aβ binding to the mixed lipid membrane. The hydrogen bond occupancy was calculated using visual molecular dynamics (VMD) software. The results showed that binding affinity of Aβs were increases with GM1 in lipid membrane, suggesting the involvement of OH−π and CH-π interaction between the aromatic side chains of sugar carbohydrate moieties of GM1 with aromatic rings of Aβs. The secondary structures of five Aβ42 through MD simulations were examined, which indicated that the most frequently structure was helix. Cluster dendrogram analysis of structure Aβ42 represents the conformation changes during interaction with lipid membrane.




(a) Top view oflipid composition of GM1:SM:Chol.
(b) Initial structure of Aβ42.
(c) Computational model for five Aβ42s incorporated in the mixed membrane. (d) Amino acid sequence of Aβ42.
(e) Chemical structures of the lipid molecules composing computational model membrane.

**Disclosures:** **M. Vahed:** None. **T. Hoshino:** None. **S. Neya:** None. **M. Vahed:** None. **M. Katsumi:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.15/J3

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

**Support:** NIH 2P01AG014930

**Title:** Dramatic changes in mitochondria and calcium homeostasis during differentiation of induced pluripotent stem cells

**Authors:** ***A. THAKKAR**[1], H. CHEN[1], X. HUI[1], D. PAULL[2], H. ZHOU[2], A. LI[2], S. NOGGLE[2], G. GIBSON[1]
[1]Weill Cornell Medicine,Burke Med. Res. Inst., White Plains, NY; [2]New York Stem Cell Fndn. Res. Inst., New York, NY

**Abstract:** Reduction in glucose metabolism and changes in calcium are the hallmarks of neurodegenerative diseases like Alzheimer’s disease (AD). Internal calcium stores are exaggerated in fibroblasts from patients with AD and in multiple models of AD. Activities of the alpha-ketoglutarate dehydrogenase complex (KGDHC), a rate-limiting enzyme of the

tricarboxylic acid cycle, are diminished in AD brain and in models of plaques and tangles. Reducing KGDHC increases the internal calcium stores similarly to cells from AD patients. Assessing the importance of the abnormalities in the disease process and their therapeutic importance has not been possible because it is unknown if they occur in human neurons, and how the calcium and mitochondria interact in human neurons. The emergence of research with fibroblast derived induced pluripotent stem cells (iPSCs) from humans will allow questions to be answered that have always eluded the field. Research on mitochondria (e.g., KGDHC) or internal calcium homeostasis in iPSCs and their interactions in iPSCs or iPSC derived cells is surprisingly limited.

The goal of these studies is to provide a foundation for subsequent research on calcium and mitochondria in AD. iPSCs that were generated from fibroblasts derived from a 75-year-old healthy male were positive for the pluripotent markers Oct4 and SSEA4. These cells were differentiated to neural stem cells (NSCs) that were positive for nestin, SOX2 and SOX1. The activities of KGDHC in iPSCs were approximately equal to those in fibroblasts and about 50% lower than in a standard mouse neuroblastoma cell line (N2a). The activity declined about 50% upon differentiation to NSCs. In-situ measurement of KGDHC activity showed a reduction of 45-75% upon differentiation of iPSCs to NSCs. The subunit proteins of the complex (E1k, E2k and E3) in iPSCs were also diminished by differentiation to NSCs. Calcium regulation in iPSCs and NSCs was studied by measuring cytosolic free calcium in response to depolarization with KCl (50 mM) and by measuring internal calcium stores in the endoplasmic reticulum that are sensitive to bradykinin (BRCS). Depolarization did not increase calcium in either iPSC or NSC. In iPSCs, BRCS increased in a dose-dependent manner in response to bradykinin. BRCS declined by 50% from one to two days in culture. BRCS was six times higher in iPSCs than NSCs under similar conditions.

The data demonstrate that KGDHC and BRCS change dramatically upon differentiation of iPSCs to NSCs. Productive use of iPSCs and iPSC-derived NSCs and neurons for studies of neurodegeneration require a deeper understanding of how mitochondria and energy metabolism change with differentiation.

**Disclosures:** **A. Thakkar:** None. **H. Chen:** None. **X. Hui:** None. **D. Paull:** None. **H. Zhou:** None. **A. Li:** None. **S. Noggle:** None. **G. Gibson:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.16/J4

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

**Support:** NIH Grant RF1AG053981

NINDS R25 Grant

Alzheimer's Association Grant AARF-16-441393

**Title:** Exploring the role of BRCA1 in neurodegenerative disease

**Authors: *N. M. SHANBHAG**, M. D. EVANS, L. MUCKE
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**Abstract:** The accumulation of DNA damage is emerging as a driving force behind normal aging as well as neuronal degeneration. As a testament to this, mutations in DNA repair proteins underlie diseases that can manifest neurodegenerative phenotypes, such as ataxia telangiectasia and xeroderma pigmentosum. It is therefore possible that acquired deficits in DNA repair could contribute to late onset neurodegenerative disorders such as Alzheimer's disease (AD). Suberbielle et al. (Nat. Commun. 2015) recently demonstrated reduced cerebral levels of the DNA repair factor BRCA1 in a mouse model of AD and in postmortem brain tissue from humans with AD. Experimental depletion of BRCA1 in neurons of the dentate gyrus in wildtype mice increased levels of neuronal DNA double strand breaks and caused deficits in spatial learning and memory. These data suggest that BRCA1 may be critical for DNA integrity in the adult brain, and that BRCA1 depletion in AD may impact cognitive performance.
To further explore the role of BRCA1 in the adult central nervous system, we developed several lines of transgenic mice with alterations in BRCA1 levels or function. These include mice with constitutive and inducible cell type-specific reductions in BRCA1, overexpressing BRCA1, or carrying mutations in distinct functional domains of BRCA1. Our preliminary data suggest that 50% reduction of BRCA1 in the brain can lead to deficits in spatial learning and memory in mice. Ongoing studies aim to clarify the roles of BRCA1 and other DNA repair factors in the adult brain and in AD, how BRCA1 depletion leads to cognitive deficits, and whether these deficits can be prevented or reversed by increasing BRCA1 expression.

**Disclosures:** **N.M. Shanbhag:** None. **M.D. Evans:** None. **L. Mucke:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.17/J5

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

**Support:** P50 AT008661-01 from the NCCIH

Senior VA Career Scientist Award

**Title:** Role of polyphenols in kinase complex mammalian target of rapamycin complex 1 (mTORC1) - Dependent protein translation as novel mechanisms in promoting resilience against sleep deprivation - Induced cognitive impairment

**Authors: *T. FROLINGER**, A. SHARMA, S. DE BOER, A. BELL, S. SIMS, G. M. PASINETTI
Neurol., Icahn Sch. of Medicine, Mount Sinai Med. Cent, New York, NY

**Abstract:** Sleep deprivation is a common problem in our society resulting in deleterious consequences, including impaired memory and cognitive performance. Long term memory consolidation and late-phase long-term potentiation (L-LTP) requires active protein synthesis in the hippocampus. The kinase complex mammalian target of rapamycin complex 1 (mTORC1) regulates protein synthesis by phosphorylating and inhibiting the eukaryotic translation initiation factor 4E-binding protein (4EBP) and activation of the ribosomal protein S6 kinase (S6K), resulting in the binding of the eukaryotic initiation factor 4E (eIF4E) to elF4G to initiate translation. Sleep deprivation impairs L-LTP and mTORC1-dependet protein translation in the hippocampus. We showed the dietary supplementation with a standardized bioactive dietary polyphenol preparation (BDPP) rescued hippocampal-dependent long term memory impairment in a mouse model of sleep deprivation. Based on this evidence we investigated the role of BDPP in mTORC1 pathways in the molecular mechanisms mediating the cognitive resilience to sleep deprivation. We found BDPP treatment prevented the SD-induced down regulation of eIF4E expression and up regulated the interaction between eIF4E and eIF4G, an important step towards translation initiation. In addition we identified the specific brain bioavailable bioactive phenolic metabolites derived from BDPP; Quercetin-glucuronide and 3-hydroxybenzoic acids to increase eIF4G expression and down regulate 4EPB expression in embryonic primary cortico-hippocampal neurons. We also found treatment with Quercetin-glucuronide attenuates SD-induced impairment in protein synthesis-dependent L-LTP. Collectively, these findings demonstrate that BDPP regulation of mRNA and protein translation is a critical mediator of resilience against the memory deficits caused by sleep deprivation.

**Disclosures: T. Frolinger:** None. **A. Sharma:** None. **S. de Boer:** None. **A. Bell:** None. **S. Sims:** None. **G.M. Pasinetti:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.18/J6

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

**Support:** PRIN- PRIN2015W729WH

**Title:** Multiple Herpes Simplex Virus-1 (HSV-1) reactivations induce oxidative damages in mouse brains

**Authors: *M. FABIANI**[1], A. TRAMUTOLA[2], M. E. MARCOCCI[1], F. DI DOMENICO[2], M. PERLUIGI[2], A. T. PALAMARA[1,3,4], G. DE CHIARA[5]
[1]Dept. of Publ. Hlth. and Infectious Dis., [2]Dept. of Biochem. Sci., Sapienza Univ. of Rome, Rome, Italy; [3]Pasteur Inst. – Cenci-Bolognetti Fndn. Sapienza Univ. of Rome, Rome, Italy; [4]IRCCS San Raffaele Pisana, Roma, Italy; [5]Inst. of Translational Pharmacol., Natl. Res. Council, Rome, Italy

**Abstract:** Several evidence support the role of oxidative stress in Alzheimer disease (AD) pathophysiology. In particular, many redox proteomics studies on AD cerebral tissues led to the identification of oxidatively modified proteins that were consistent with biochemical or pathological alterations of the disease (Nunomura et al, 2001; Zhu et al, 2004; Smith et al, 2007; Droge et al, 2007). Interestingly, HSV-1, a neurotropic virus able to establish a lifelong latent infection in trigeminal ganglion followed by periodic reactivations, has been reportedly linked both to AD (Piacentini et al, 2015) and to oxidative stress conditions (Nucci et al, 2000; Palamara et al, 1995). Herein we design *in vivo* studies to investigate whether multiple HSV-1 reactivations induced in the brain the accumulation of oxidative stress hallmarks, particularly those correlated to AD. To this aim, BALB/c mice were inoculated via snout abrasion with HSV-1, virus reactivation was periodically induced by thermal stress, and virus replication in the brain was analyzed through PCR and RT-PCR analysis of viral TK gene and ICP4 mRNA. Oxidative stress marker levels, i.e. 4-hydroxynonenal (HNE, marker of lipid peroxidation), 3-nitrotyrosine (3NT, marker of protein nytrosylation) and carbonylated proteins, were measured in brains of mice undergone multiple HSV-1 reactivations by western blotting. In addition, redox proteomic was used to identify those HNE-modified proteins mostly modulated by recurrent HSV-1 reactivations into the brain. Following several cycle of viral reactivation, we found in mouse brains: 1) viral TK and ICP4 genes in cortex and hippocampal tissues, indicating that HSV-1 is able to reach and replicate in those brain regions mostly affected during AD; 2) increased levels of HNE, 3-NT, and protein carbonylation, indicating generalized conditions of oxidative stress; 3) thirteen HNE-modified proteins whose levels were significantly modulated in the cortex of HSV-1 infected mice compared to control mice. Interestingly, all these proteins are involved in important cellular processes, such as energy metabolism, protein folding, cell structure, and signal transduction, suggesting that their oxidative modification may affect brain physiology. Some of these proteins are reported to be significantly HNE-modified in AD brains compared to matched controls. In addition, these mice showed several signs of neurodegeneration (De Chiara et al, SfN abstract 2017). Overall, these data support the hypothesis that repeated HSV-1 reactivation into the brain may concur to neurodegeneration also inducing oxidative damages.

**Disclosures: M. Fabiani:** None. **A. Tramutola:** None. **M.E. Marcocci:** None. **F. Di Domenico:** None. **M. Perluigi:** None. **A.T. Palamara:** None. **G. De Chiara:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.19/J7

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

**Support:** NIH/NINDS Grant R01NS093362

The Bluefield Project to Cure FTD

**Title:** Progranulin is proteolytically cleaved into stable, lysosomal granulins that are haploinsufficient in frontotemporal dementia with GRN mutations

**Authors: *C. J. HOLLER**[1], G. TAYLOR[1], Q. DENG[2], T. L. KUKAR[1]
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**Abstract:** Frontotemporal dementia (FTD) is the second most common neurodegenerative disease in people <65 years old. Heterozygous mutations in the progranulin gene (*GRN*) leading to progranulin (PGRN) haploinsufficiency are a major cause of familial and sporadic FTD. It is currently unknown how loss of PGRN leads to neurodegeneration, but mounting evidence suggests PGRN plays a critical role in maintaining proper lysosome function in the brain. This concept is supported by several lines of evidence. First, homozygous *GRN* mutations in humans leads to a complete loss of PGRN protein and manifests as a lysosomal storage disease called neuronal ceroid lipofuscinosis (NCL), characterized by lysosome dysfunction and neurodegeneration. Second, FTD patients with *GRN* mutations as well as *Grn*-deficient mice also display lysosomal storage disease phenotypes. Third, two receptors - sortilin and the prosaposin/cation-independent mannose 6-phosphate receptor/low density lipoprotein receptor-related protein complex - have been demonstrated to bind and traffic PGRN to lysosomes. Finally, variants in *TMEM106B*, which encodes a lysosomal trafficking protein, are a significant risk factor for FTD-*GRN*. PGRN is a secreted glycoprotein that can be cleaved into seven ~6 kDa proteins called granulins (GRNs), however little is known about the production of GRNs or their levels in disease due to lack of specific tools. To overcome this gap, we have identified and validated antibody-based tools for the detection of endogenous human GRNs which have previously never been reported. Using these tools, we find that endocytosed PGRN is rapidly processed into stable GRNs within lysosomes. Processing of PGRN into GRNs is conserved between humans and mice and is dependent on cysteine proteases. Further, alkalizing agents or expression of TMEM106B in cells cause lysosome dysfunction and inhibit processing of PGRN into GRNs. Finally, multiple GRNs are haploinsufficient in FTD-*GRN* patient derived cells and brain tissue. Our findings suggest that GRNs may be the functional units of PGRN responsible for maintaining lysosome homeostasis, and their deficiency may underlie development of lysosomal storage disease and neurodegeneration. Future experiments will determine if

individual GRNs can rescue lysosomal dysfunction phenotypes in cellular and animal models of PGRN deficiency.

**Disclosures:** **C.J. Holler:** None. **G. Taylor:** None. **Q. Deng:** None. **T.L. Kukar:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.20/J8

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

**Support:** NIH Grant AG051266

BrightFocus Foundation

Alzheimer's Association

**Title:** Aβ proteolytic degradation at the N- and C-terminus modulates the mechanisms of brain clearance and amyloid formation

**Authors:** ***J. GHISO**[1], E. CABRERA[1], P. MATHEWS[2], E. MEZHERICHER[1], T. BEACH[3], T. A. NEUBERT[1], A. ROSTAGNO[1]
[1]New York Univ. Langone Med. Ctr., New York, NY; [2]Nathan Kline Inst., Orangeburg, NY; [3]Banner Sun Hlth. Res. Inst., Sun City, AZ

**Abstract:** High degree of Aβ heterogeneity - going far beyond the classic Aβ40/Aβ42 dichotomy and particularly associated with a plethora of C-terminal truncations - is a consistent finding in proteomic analysis of CSF collected from healthy controls as well as from AD individuals and transgenic mouse models. Biochemical analysis of parenchymal brain deposits largely expands the Aβ heterogeneity, revealing numerous post-translational modifications and multiple truncations at both N- and C-terminal ends of the molecule likely reflecting local action of resident enzymes. Notably, the relevance of these N- and C-terminal truncated species for the mechanism of AD pathogenesis remains largely understudied. Utilizing specifically designed differential tissue extraction protocols, synthetic homologues of intact and truncated Aβ peptides as well as novel antibodies recognizing specific truncations we compared their solubility properties, self-oligomerization propensity, predisposition for fibrillization, topographic localization, and *in vivo* brain clearance characteristics. The findings clearly indicate that in AD and transgenic mouse brain specimens N- and C-terminal Aβ truncated forms exhibit differential fractionation characteristics. Water-soluble brain extracts were largely enriched in C-terminal fragments - closely resembling the heterogeneity of the CSF Aβ peptidome - whereas N-terminal truncated fragments in brain homogenates primarily required formic acid for solubilization in line with their preferential topographic association with amyloid plaque cores. Biophysical

studies using synthetic homologues confirmed the differences in solubility and contrasting oligomerization/ fibrillization characteristics of the various Aβ truncated derivatives whereas intracerebral injections of monomeric and oligomeric radiolabeled homologues revealed striking differences in their brain clearance characteristics. While soluble derivatives exhibited a fast brain removal, oligomerization largely increased brain retention, a characteristic particularly evident in fragments truncated at Phe4, topographically abundant in fibrillar, thioflavin positive plaque cores. Taken together, our data indicate that Aβ degradation at its C-terminal-end generates highly soluble fragments associated to catabolic/clearance mechanisms whereas truncations at the N-terminus favor oligomerization and brain retention, with the potential to exacerbate the process of amyloid formation and self-perpetuate the amyloidogenic loop.

**Disclosures: J. Ghiso:** None. **E. Cabrera:** None. **P. Mathews:** None. **E. Mezhericher:** None. **T. Beach:** None. **T.A. Neubert:** None. **A. Rostagno:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.21/J9

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

**Support:** National Key Basic Research Program of China (2013CB530900)

**Title:** AnkyrinG disruption and axon initial segment breakdown lead to neuronal polarity loss in Alzheimer's disease transgenic mouse models

**Authors: *F. MA**[1], K. HERRUP[2]
[1]Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong; [2]Hong Kong Univ. of Sci. & Technol., Kowloon, Hong Kong

**Abstract:** Mature neurons are highly polarized cells that typically have multiple dendrites and a single axon. The axon and dendrites have different sets of characteristic resident proteins. This asymmetric distribution of the cellular components is defined as neuronal polarity. Nearly every aspect of neuronal development, neuronal signaling and neuronal plasticity depends on the accurate localization of these proteins. Therefore, polarity is critical for neurons to form a functional network. In the past decade, the mechanisms that underlie the development of neuronal polarity have received a lot of attention, but the maintenance of such polarity is often overlooked. It is known now that the axon initial segment (AIS) serves as a critical physical barrier at the junction of the axon with the cell body for maintaining the polarity. The AIS itself has a number of specialized proteins, one of which is a unique scaffold protein Ankyrin G (AnkG) that governs the assembly and stabilizes the initial segment. However, neuronal polarity is not immutable. Under certain pathological conditions, neurons fail to maintain their polarity

and immunocytochemistry reveals the mis-localization of some normally polarized proteins. In the two different transgenic mouse models of Alzheimer's disease, APP K670/M671 (R1.40) mice and APP K670/M671 /PS1 dE9 (PAB) mice, we observed a portion of neurons in the cortex undergoes a loss of polarity as the brains age. The length of AnkG-labeled AIS in the neocortex of these mice as well as the levels of AnkG protein throughout the life span of both wildtype and the AD models were also examined. AnkG levels in the neocortex of R1.40 mice were significantly different from wildtype beginning at six months of age. This is consistent with the hypothesis that the loss of polarity is associated with the pathogenic process of Alzheimer's disease. Using ß-amyloid peptide and lipopolysaccharide to mimic AD chemistry *in vitro* mimicked these findings and lead to the collapse of neuronal polarity. Together, these results suggest that the loss of neuronal polarity is correlated with Alzheimer's disease and may be an important factor in the neurodegeneration process.

**Disclosures: F. Ma:** None. **K. Herrup:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.22/J10

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

**Title:** Interaction of Aβ, oxidative stress, and PKCε in hippocampal neurons and microvascular endothelium in Alzheimer's disease

**Authors: *J. HONGPAISAN**[1], A. SEN[1], T. J. NELSON[1], D. L. ALKON[2]
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**Abstract:** Oxidative stress and cerebrovascular damage have been implicated in Alzheimer's disease (AD). Reduced brain levels of protein kinase C epsilon (PKCε) have been found in autopsy samples from AD victims. In the present study, reciprocal interactions among Aβ, oxidative stress, and PKCε were studied in microvascular endothelium and pyramidal neurons in the hippocampus. PKCε and mitochondrial manganese-superoxide dismutase (MnSOD) were depressed in microvascular endothelium of CA1 areas of autopsy-confirmed AD hippocampus. Microvascular density was correlated with PKCε level. Increases in Aβ and decreases in PKCε, MnSOD, and brain-derived neurotrophic factor (BDNF) levels were found in pyramidal neurons. In cultured human primary hippocampal neurons, oxidative stress, induced with t-butyl hydroperoxide, reduced neuron density and the levels of MnSOD and BDNF. These changes were reversed with the PKCε activators bryostatin and DCPLA-ME, indicating that MnSOD and BDNF expression are PKCε-dependent. Oxidative stress reduced levels of amyloid precursor protein (APP) but increased Aβ; both were prevented by PKCε activators, suggesting activation of the amyloidogenic pathway (normally inhibited by PKCε) and/or a decrease in Aβ

degradation (normally activated by PKCε). In cultured neurons, siRNA suppression of PKCε produced effects similar to oxidative stress. PKCε, MnSOD, and vascular endothelial growth factor (VEGF) levels were also reduced in hippocampal neurons and microvascular endothelium, as well as microvascular loss, in 5–6 month old Tg2576 AD transgenic mice. These changes were prevented by PKCε activators. These results suggest that a decrease in PKCε suppresses MnSOD, VEGF, and BDNF expression. An imbalance among PKCε, Aβ, and ROS may contribute to vascular dysfunction in AD.

**Disclosures:** **J. Hongpaisan:** None. **A. Sen:** None. **T.J. Nelson:** None. **D.L. Alkon:** A. Employment/Salary (full or part-time):; NeuroDiagnostics LLC.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.23/J11

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

**Support:** NIH 2R01AG037637-07

**Title:** Staging Alzheimer’s disease-like pathology in 3xTg-AD mice

**Authors: *R. BELFIORE**[1,2], E. FERREIRA[1], R. VELAZQUEZ[1], C. BRANCA[1], N. DAVE[1], A. RODIN[1], A. CACCAMO[1], S. ODDO[1,3]
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**Abstract:** Animal models of AD represent an invaluable tool to evaluate potential therapeutic compounds and to study mechanisms underlying the pathogenesis of the disease. 3xTgAD mice are a widely used animal model of AD. As reported in 2003, these mice are characterized by the accumulation of plaques, tangles, and cognitive decline. Currently, 3xTg-AD mice are being used by more than 100 investigators throughout the world. Such widespread use has led to the generation of multiple independent colonies. Converging evidence indicates that the phenotype of 3xTg-AD mice has shifted over the years, and contradicting reports about onset of pathology and cognitive deficits are apparent in the literature. Given the widespread use of these mice, it is imperative to re-define the progression of AD-like pathology. We employed a cross-sectional approach using 3-, 6-, 12-, 16-, and 20-month-old 3xTg-AD and wildtype mice (n = 15/age group/genotype). Per each age-group, we tested mice in a battery of cognitive and non-cognitive tasks, including the Morris water maze, open field, novel object recognition, contextual fear conditioning, and Rotarod. We will present a comprehensive, age-dependent assessment of the cognitive changes of 3xTg-AD mice and the associated neuropathological phenotype, including soluble and insoluble Aβ and tau levels in different brain regions. The data presented here will

serve as a benchmark for investigators in the field using these mice. In addition, our results will facilitate the design of preclinical studies in which these mice are used to test new therapeutic approaches.

**Disclosures:** **R. Belfiore:** None. **E. Ferreira:** None. **R. Velazquez:** None. **C. Branca:** None. **N. Dave:** None. **A. Rodin:** None. **A. Caccamo:** None. **S. Oddo:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.24/J12

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

**Support:** NIH 1R21NS096375-01A1

**Title:** Acute knockdown of tau in the adult hippocampus impairs spatial learning and memory

**Authors: *R. VELAZQUEZ, JR**[1], E. FERREIRA[1], A. TRAN[1], O. SALVATORE[1,2]
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**Abstract:** Misfolded and hyperphosphorylated tau accumulates in several neurodegenerative disorders collectively known as tauopathies. Evidence indicates that tau-mediated neuropathology may occur by both toxic gain-of-function and by loss-of-function. While a wealth of data is available on the role of tau in the formation of toxic inclusions, less is known about its function in the otherwise healthy adult brain. Furthermore, being that anti-tau therapies are quickly approaching clinical trials, fully understanding the involvement of tau in the healthy brain remains an important question. To date, it has been difficult to elucidate the role of tau in learning and memory during adulthood as data obtained from tau knockout mice are confounded by robust developmental compensation of other microtubule-binding proteins such as microtubule-associated protein 1A. Thus, to fully understand tau's role in learning and memory, manipulations need to be done solely in adult mice to remove any developmental compensations. Here, we generated an adeno-associated virus (AAV) expressing a doxycycline (doxy)-inducible short-hairpin (Sh) RNA targeted to tau, herein referred to as AAV-shRNAtau. We stereotaxically and bilaterally injected 8-month-old C57x129 mice with either the AAV-shRNAtau or an AAV expressing a scramble shRNA sequence (AAV-shRNA-CTL; n =17/group). During the surgeries, mice were kept off doxy to keep the expression of the tau shRNA off. Seven days after the injections, all animals were put on doxy, to induce expression of the tau and control shRNAs. One month after the start of the doxy administration, we tested all mice in the rotarod and Morris water maze, to assess motor coordination, and spatial learning and memory, respectively. Following testing, we harvested tissue from mice to assess spine density, microtubule assembly

and axon morphology. Our results show that reducing tau in the adult hippocampus produces significant impairments in motor coordination, endurance and spatial memory. Collectively, these findings illustrate the importance of the tau protein in both non-cognitive and cognitive functions. These results may have a major impact on tauopathies and associated therapies as we have directly addressed important questions regarding the role of tau in adult brains.

**Disclosures:** **R. Velazquez:** None. **E. Ferreira:** None. **A. Tran:** None. **O. Salvatore:** None.

**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.25/K1

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

**Support:** NIH 2R01AG037637-07

**Title:** Central insulin dysregulation precedes peripheral insulin resistance in two mouse models of Alzheimer’s disease

**Authors: *A. L. TRAN**[1], R. VELAZQUEZ, Jr[1], E. ISHIMWE[2], L. DENNER[3], N. DAVE[1], O. SALVATORE[1,4], K. T. DINELEY[2]
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**Abstract:** Type 2 diabetes (T2D) is one of the most prevalent risk factors that contributes to Alzheimer’s disease (AD). Brain and peripheral insulin dysregulation have been demonstrated in subjects with both mild cognitive impairment (MCI) and early AD. However, it is not certain whether changes in brain insulin signaling occur before or after the onset of peripheral insulin resistance. Here, we examined peripheral glucose metabolism and brain insulin signaling in the Tg2576 and 3xTg-AD mouse models of AD at ages with different degree of AD-like pathology. We first assessed peripheral insulin resistance via a glucose tolerance test in 10- and 16-month-old 3xTg-AD and age-matched wildtype (WT) mice. Our results show that fasting glucose levels and the ability to restore elevated glucose levels were significantly different between WT and 3xTg-AD mice at 16 months of age, but non-significantly different at 10 months of age. In contrast, as previously reported, peripheral insulin resistance in Tg2576 is first detected at 9 months of age. Immunoblot results of brain homogenates show reductions in IRS-1 and PI3K levels in the older age groups of 3xTg-AD (16-month old) and Tg2576 (9-month old), illustrating dysregulated brain insulin signaling. Additionally, downstream target PDK1 was dysregulated in both 5-month-old Tg2576 and 10-month-old 3xTg-AD mice, prior to peripheral

insulin resistance. Notably, 10-month-old 3xTg-AD mice show decreased activity of IRS-1 and PI3K, while Tg2576 mice show dysregulation of these two markers only after the onset of peripheral insulin resistance. This finding may be attributed to the 3xTg-AD mice developing both Aβ and tau pathology while Tg2576 mice only develop Aβ plaques. AKT, an integral component of the insulin signaling pathway was dysregulated in both mouse models after the onset of peripheral insulin resistance. Lastly, GSK-3β, a convergent target of the PDK1/AKT pathways and a key negative regulator of IRS-1, was upregulated consecutively with peripheral insulin resistance. Collectively, our data show evidence that various brain insulin signaling abnormalities are evident months before peripheral insulin resistance in two mouse models of AD. Furthermore, these brain insulin dysregulations are more apparent in 3xTg-AD mice which features both hallmark pathologies of AD. This work suggests that early AD may reflect engagement of different signaling networks that influence brain metabolism, which in-turn alters peripheral insulin signaling.

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**Poster**

**567. Alzheimer's Disease: Biochemistry Approaches and Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 567.26/K2

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

**Support:** NIH 2R01AG037637-07

**Title:** Mechanisms of neuronal loss in Alzheimer’s disease

**Authors: *A. CACCAMO**[1], C. BRANCA[1], I. S. PIRAS[2], E. FERREIRA[1], M. J. HUENTELMAN[2], W. S. LIANG[2], B. READHEAD[3], J. T. DUDLEY[3], E. E. SPANGENBERG[4], K. N. GREEN[4], R. BELFIORE[1,5], W. WINSLOW[1], S. ODDO[1]
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**Abstract:** Neuronal loss is a major pathological feature of Alzheimer’s disease (AD). Nevertheless, the mechanisms underlying this key event in the disease pathogenesis are not clear. Necroptosis, a programmed form of necrosis, is performed by the mixed lineage kinase domain-like (MLKL) protein, which is activated by receptor-interactive protein kinases (RIPK) 1 and 3. Necroptosis is activated in various neurodegenerative disorders including multiple sclerosis and amyotrophic lateral sclerosis. However, it remains to be determined whether necroptosis plays a role in AD. Here we show that necroptosis is activated in human AD brains and its activation

correlates with brain weight and cognitive scores. We also show that necroptosis is also activated in 5xFAD mice, which are characterized by marked neuronal loss. Using complementary in vitro and in vivo approaches, we found that reducing necroptosis activation rescues AD-related neuronal loss. Overall, these data provide the first direct evidence that necroptosis is a mechanism involved in neurodegeneration in AD.

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**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.01/K3

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

**Support:** NINDS/NIH Grant R01NS082730-01

NIA/NIH Grant R01AG044372

**Title:** Characterizing the interaction between tau and protein phosphatase 1

**Authors: *K. CHRISTENSEN**[1], B. COMBS[2], C. RICHARDS[3], N. M. KANAAN[4]
[2]Col. of Human Medicine, Translational Sci. and Mol. Med., [3]Dept. of Translational Sci. and Mol. Med., [4]Translational Sci. & Mol. Med., [1]Michigan State Univ., Grand Rapids, MI

**Abstract:** Axonal degeneration and synapse loss are key traits of Alzheimer’s disease, with several lines of evidence suggesting these may be the earliest degenerative events. Additionally, growing evidence suggests tau’s functional capabilities are more diverse than previously thought, and may include regulation of signaling pathways. Our group previously showed that the N-terminus of tau can activate a pathway involving protein phosphatase-1 (PP1) that causes axonal transport deficits, but it remains unclear whether tau directly or indirectly interacts with and activates PP1. Here, we tested the hypothesis that tau activates PP1 through a direct interaction. Tau constructs used include full-length wild-type (hT40), pseudophosphorylated-AT8 (psAT8), and hTt40 and psAT8 tau with amino acids 2-18 deleted (Δ2-18), as well as the three PP1 isoforms (α, β and γ isoforms). HEK293T cells were transfected with plasmids coding HaloTag-PP1 and NanoLuciferase-tau for use in pulldown and NanoBRET experiments. PP1 activity was measured using recombinant PP1 and tau proteins and in an *in vitro* PP1 activity assay. NanoBRET results show that each of the tau constructs tested interact with all three PP1 isoforms. Pulldown experiments confirmed that tau directly interacts with each PP1 isoform, and

each tau construct increased the activity of all PP1 isoforms. Interestingly, tau showed a stronger interaction and activation of PP1α and PP1γ compared to PP1β. These results indicate that tau and PP1 can directly interact, and that this interaction increases PP1 activity. Future studies will further characterize this interaction in the context of tau-mediated toxicity in neurons.

**Disclosures: K. Christensen:** None. **B. Combs:** None. **C. Richards:** None. **N.M. Kanaan:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.02/K4

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

**Title:** Regulation of tau sumoylation by SUMO ligases and proteases

**Authors: H. WADA**, *T. NIIKURA
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**Abstract:** The microtubule-associated protein tau is mainly expressed in the axon and associated in neurodegenerative disorders including Alzheimer's disease (AD). AD is neuropathologically characterized by intraneuronal neurofibrillary tangles (NFT), which are formed by the abnormal hyperphosphorylated tau that is aggregated and accumulated in the soma. In addition to phosphorylation, other post-translational modifications were found in tau, including ubiquitination, acetylation, methylation, and glycosylation. However, functional effects of these are largely unknown. Sumoylation is a process in which, Small Ubiquitin-like Modifier (SUMO) is conjugated to lysine residues of target proteins, and is mediated by enzymes: E1, E2, and E3. PIAS (protein inhibitor of activated STAT) protein family, with four PIAS genes in mammals, function as SUMO E3 ligase. Additionally, six SENP (sentrin-specific protease) family proteins have been identified as desumoylation enzymes. The balance of activities between sumoylation and desumoylation enzymes influences the rate of sumoylation. It has been previously shown that sumoylation and phosphorylation in tau have positive correlation, but it is still unclear how it is regulated. In this study, we identified enzymes that regulate sumoylation and desumoylation of tau. We found that PIAS2 functioned as tau specific sumoylation enzyme, and SENP1 and 2 functioned as tau specific desumoylation enzyme. Consistently, we verified that tau colocalized with SENP1 or PIAS2 in HEK293 cells. Further, we identified at least one new sumoylation site in tau protein. Our findings suggest that these sumoylation-regulating proteins can be new targets to understand the pathogenesis of the tau-related neurodegenerative disorders.

**Disclosures: H. Wada:** None. **T. Niikura:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.03/K5

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

**Support:** P20GM103430

**Title:** Modulation of hyperphosphorylated tau accumulation by chaperone proteins in a neuroblastoma cell culture model of Alzheimer's disease

**Authors: *S. MAY**[1], N. H. ZAWIA[2], J. L. CAMBERG[3]
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**Abstract:** Alzheimer's disease is characterized by intracellular and extracellular aggregates of impaired, misfolded proteins, including tau and amyloid-beta. To prevent aggregate accumulation, healthy cells have a robust, extensive network of molecular chaperone proteins that maintain protein homeostasis. Molecular chaperone proteins assist in the folding of nascent polypeptides, refolding and reactivation of misfolded or partially unfolded proteins, and targeting of irreversibly misfolded proteins for degradation. Here, we investigated the role of chaperone proteins Hsp70, Hsp90, and Hsp110 in tau neuroblastoma SH-SY5Y cells treated with the phosphatase inhibitor okadaic acid. Accumulation of hyperphosphorylated tau was monitored over time in undifferentiated and differentiated cells. Initially, we observed that Hsp70 expression levels increase after administration of okadaic acid to cells. Additional experiments to knockdown and overexpress chaperone proteins will determine if changes in the molecular chaperone landscape lead to changes in the rate of tau accumulation. These studies investigate the involvement of molecular chaperone proteins in the pathogenesis of Alzheimer's disease and may provide insight into new therapies for targeting tau protein misfolding and aggregation.

**Disclosures: S. May:** None. **N.H. Zawia:** None. **J.L. Camberg:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.04/K6

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

**Support:** SFB 944

**Title:** Quantitative live cell imaging reveals critical influence of a highly conserved pseudorepeat region on tau's interaction with neuronal microtubules

**Authors: *R. BRANDT**, B. NIEWIDOK, M. IGAEV, F. SUENDERMANN
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**Abstract:** The tau proteins are generated by alternative splicing from a single gene and are the major microtubule-associated proteins in neuronal axons. In healthy brains, tau remains enriched in the axon however the distribution changes during Alzheimer's disease and other tauopathies, where tau localizes to the somatodendritic compartment. Tau's redistribution is associated with hyperphosphorylation, reduced binding to microtubules (MTs) and aggregation into filaments. Tau's MT interaction is thought to be mediated by the microtubule-binding region (MBR), which contains three or four repeat regions. Regions flanking the MBR may modulate tau's interaction with MTs. The functional organization of tau has been mainly studied *in vitro* or in non-neuronal cells. Although, it has become evident that tau shows an unexpectedly high dynamic interaction with MTs - a feature that has been termed "kiss and hop" behavior - at conditions of high MT density that prevail in the axon [1]. It is unclear, how "kiss and hop" is regulated and what is the effect of flanking regions in authentic axons. To scrutinize the functional organization of tau's MT interaction in axon-like processes, we employed a refined fluorescence decay after photoactivation (FDAP) approach [2]. Tau was N-terminally tagged with a photoactivatable variant of GFP and a panel of C-terminal tau deletion constructs was expressed in neuronally differentiated PC12 cells [3]. A defined region of interest within a process was activated and the fluorescence intensity distribution as a function of time monitored. The decay transient was fitted by a mathematical model to yield mobility and binding properties of the tagged protein. Our findings show that - in contrast to previous *in vitro* data - tau isoform variation had only a minor influence on MT binding. Notably, the presence of a conserved pseudorepeat region (PRR) in tau's C-terminus robustly enhanced MT binding by a greater-than-sixfold decrease of the dissociation rate. Bioinformatic analysis showed that the PRR is highly conserved across vertebrates, is also present in MAP2 and the non-neuronal MAP4, and constitutes a separate group in nearest-neighborhood cluster analysis. Our data indicate that under conditions of high MT density in the axon, tau's MT binding and localization are crucially affected by the presence of the PRR and indicate that conditions that affect the integrity of the PRR could have a critical role in triggering the redistribution of tau during tauopathies. References: [1] Janning et al. (2014) Mol. Biol. Cell 25:3541-51. [2] Igaev et al. (2014) Biophys. J. 107:2567-78. [3] Niewidok et al. (2016) Mol. Biol. Cell 27:3537-49.

**Disclosures:** **R. Brandt:** None. **B. Niewidok:** None. **M. Igaev:** None. **F. Suendermann:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.05/K7

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

**Support:** NHMRC

**Title:** LKB1 and tau phosphorylation in Alzheimer's disease

**Authors: A. VOLKERLING**[1], M. BI[1], F. DELERUE[1,2], *S. CHUA[1], L. M. ITTNER[1,2,3], A. ITTNER[1]
[1]Dementia Res. Unit, Sch. of Med. Sci., [2]Transgenic Animal Unit, UNSW, Sydney, Australia; [3]Neurosci. Res. Australia, Sydney, Australia

**Abstract:** Alzheimer’s disease (AD) and frontotemporal lobar dementia with tau pathology (FTLD-tau) are the most prevalent forms of dementia with tauopathy. They are characterised by the presence of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau, as well as neuronal dysfunction that is partially due to defective neuronal metabolism. Liver kinase B1 (LKB1) is a crucial regulator in neuronal energy homeostasis via activating AMP-activated protein kinase (AMPK). During neuronal development, LKB1 plays a vital role in regulating neuronal polarity via specific phosphorylation of tau. However, whether this pathway is involved in neuronal dysfunction in tau pathology remains unknown. Our experiments show that LKB1 function is seen to be impaired when naturally occurring tau phosphorylation is dysregulated. This in turn has downstream effects on brain metabolism and cognition. Hence, we propose that site specific phosphorylated tau is able to inhibit LKB1-mediated AMPK activation in neurons. Furthermore, the LKB1-tau pathway may serve as a future therapeutic target in tau-related neurodegeneration.

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**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.06/K8

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

**Support:** FCT MD/PhD Scholarship # PD/BD/105938/2014

**Title:** Implication of Rab35/ESCRT pathway in Tau proteostasis and its impact on the stressed brain

**Authors: *J. V. SILVA**[1,2,3], S. QUINTREMIL[1], C. CUNHA[2,3], T. MEIRA[4,3,2], C. DIOLI[2,3], J. SILVA[2,3], I. SOTIROPOULOS[2,3], C. WAITES[1,4]
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**Abstract:** Exposure to chronic stress and high glucocorticoid (GC) levels leads to cognitive decline and neuronal atrophy. Previously, GC were shown to induce Alzheimer's disease (AD)-related pathomechanisms, including the intracellular accumulation of hyperphosphorylated Tau protein, suggesting that dysregulation of Tau proteostasis is a major causative factor for stress-induced brain pathology. However, the mechanisms underlying Tau turnover and degradation are still poorly understood. Here, we have identified a novel molecular pathway that mediates the degradation of Tau in hippocampal neurons. This pathway comprises the small GTPase Rab35 and the endosomal sorting complex required for transport (ESCRT) machinery, which catalyzes the biogenesis of multivesivular bodies (MVBs) for delivery of cargo to lysosomes. Interestingly, we find that the Rab35/ESCRT pathway is negatively regulated by GC, which are known to impair Tau degradation and lead to Tau accumulation, hyperphosphorylation, and synaptic missorting. Furthermore, we show that stimulating this pathway via Rab35 overexpression can rescue GC-induced Tau accumulation, thus supporting the relevance of the Rab35/ESCRT pathway for Tau pathomechanisms, and identifying a promising therapeutic target for the treatment of stress-related cognitive decline and other tauopathies, such as AD.

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**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.07/K9

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

**Support:** AG054025

NS094557

**Title:** P53 and Tau in Alzheimer's Disease

**Authors: *K. FARMER**[1], P. SARKAR[2], R. KAYED[2]
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**Abstract:** The homotetrameric tumor suppressor, p53, acts as a master regulator of cell cycle control, apoptosis, and DNA repair. DNA damage is one of the earliest pathological changes in Alzheimer's disease (AD) and a reduction in the DNA damage response significantly increases neurodegeneration. P53 has been shown to play a neuroprotective role in tauopathies. Previous studies have also implicated the microtubule-associated protein tau as a factor in DNA damage in neurodegenerative diseases. Depending on phosphorylation state, nuclear tau has been proposed to play a protective role against DNA damage. In addition, tau has been shown to induce chromatin relaxation, which subsequently leads to DNA damage and global changes in transcription. As p53 becomes activated due to DNA damage, it may be possible that an interaction between tau and p53 occurs in the early stages of AD. In addition, tau oligomers have been found to develop toxic gain of function and are one of the major contributors to neuron death in AD. Tau oligomers have also been found to sequester and/or cause the aggregation of other proteins. Thus, we hypothesize that p53 may be sequestered by tau oligomers, inhibiting its function. Neurons in Alzheimer's disease (AD) have also been shown to re-express factors that promote cell cycle progression and undergo cell cycle re-entry just before massive neuronal death in AD. This would suggest a reactivation of the cell cycle—in which p53 is critically involved—in the process of neurodegeneration. We evaluated total p53, functional phospho-p53, and tau aggregation status in AD patients and Tg2576 mice by immunohistochemistry using both commercial and novel antibodies with conformational epitopes common to oligomers of aggregated proteins. We found that phospho-p53 levels are decreased in human AD brain in comparison to control brain. We also found that phospho-p53 colocalizes with tau oligomers in human AD brain, highlighting a potential overlap between DNA damage response and AD. Our findings suggest that tau oligomers may be interacting with p53 and causing a down regulation of functional p53 in AD. This may suggest that tau may be interacting in DNA damage response signaling and therefore affecting cell death. These results may have implications for a number of other neurodegenerative disorders. Therefore, further research is needed to understand the underlying signaling pathways of tau and p53 interaction.

**Disclosures:** **K. Farmer:** None. **P. Sarkar:** None. **R. Kayed:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.08/K10

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

**Title:** Seaweed reduces phosphorylation of tau protein in the olfactory bulb

**Authors: M. IMAI**[1], F. KAWAKAMI[2], *H. AKITA[4], K. YOSHINAGA[5], T. KAHARA[5], H. MARUYAMA[3]
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**Abstract:** Background Recently, diet habit changes to high fatty food, obesity, diabetes and lifestyle diseases are increasing in Japan. There was reported that development of Alzheimer's disease is high risk in diabetes mellitus patients compare to normal person. However, the clear mechanism of Alzheimer's onset has not been elucidated. The present study was investigated the seaweed of Japanese traditional diet to effect on prevention and onset of Alzheimer's disease. We have already obtained data showing the prevention and improvement effect of diabetes by improving glucose metabolism abnormality by seaweed ingestion. Material and Method The mice were divided into four groups as high fat diet, normal diet, high fat diet with 1% seaweed, and normal diet with 1% seaweed. In addition, all groups were fed ad libitum for 6 months. Phosphorylation of tau protein in mouse olfactory bulb and cerebral cortex part was analyzed by western blotting method. Results and Discussion It was found that phosphorylation of tau was suppressed in both of high fat diet and normal diet with seaweed. This result suggested that feeding of seaweed suppresses tau phosphorylation and it might be related to reduce the onset of Alzheimer's disease. Furthermore, seaweed components related to inhibition of phosphorylation of tau are identified while focusing on signal systems such as AKT and GSK-3β, used cultured cell HEK293 overexpression of tau.

**Disclosures: M. Imai:** None. **F. Kawakami:** None. **H. Akita:** None. **K. Yoshinaga:** None. **T. Kahara:** None. **H. Maruyama:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.09/K11

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

**Title:** Soluble tau oligomer species detection by western blot and mass spectrometry

**Authors: *A. FRANCOIS**[1], V. PASTEAU[1], R. BILLIRAS[1], K. ALBINET[1], G. ROLLIN-JEGO[1], F. IOP[1], C. BARDET[2], T. FORTIN[2], F. PANAYI[1], C. LOUIS[1], A. GOBERT[1]
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**Abstract:** Alzheimer Disease and other tauopathies show abnormal aggregation of tau protein into paired helical filaments (PHFs) and neurofibrillary tangles. Initially small, but soluble oligomers turn subsequently into insoluble PHFs. It is hypothesized that small molecules, such as leucomethylthioninium bismethanesulfonate salt (LMTM), are able to prevent aggregation. The present study aimed at monitoring tau oligomer formation after acute treatment with LMTM. Two complementary techniques, Western blot (WB) and mass spectrometry (MS), were compared, analysing samples from wild-type (WT) and tau transgenic mice (P301S). LMTM was administered subcutaneously (10 mg/kg) to 9 months old WT (n=9) and P301S (B6:C3-Tg(Prnp-MAPT*P301S)PS19Vle/J, n=9) mice. Animals were sacrificed 1 hour after treatment and brains dissected. Brain samples were homogenized and proteins separated using denaturing or semi-native polyacrylamide gel electrophoresis (PAGE) for visualization of tau monomers or oligomers, respectively. For MS, gel bands were excised (according to molecular weight standards), crushed, trypsin digested and heavy peptides added for quantitation. Light and heavy peptides were screened in LC-SRM mode using a triple quadrupole mass spectrometer. Initially, optimal brain structure and age was determined. Total tau was detected in decreasing amounts in: hippocampus ≥ frontal cortex > brain stem ≥ spinal cord in P301S and WT mice, though to a lower extent. Starting at 9 months (P301S), a doublet band (~65 kDa) appears in denaturing PAGE, likely reflecting a different conformation of tau. This observation matches with temporal changes in phosphorylation and behavioural status. Similar levels of Mono-, di-, and trimers were quantified using semi-native PAGE in both strains, with or without LMTM treatment. However, quantification by MS of LMTM samples revealed a 2-fold increase in the ratio monomer/oligomer in WT mice. No change in ratio was observed in treated P301S mice that typically express high levels of tau, suggesting the acute LMTM dosing was too low to prevent tau oligomerization. Absolute quantification with MS is able to reveal changes in amounts of tau oligomers *vs* monomers after LMTM treatment, whereas WB is not sensitive enough to reveal such changes.

**Disclosures:** **A. Francois:** None. **V. Pasteau:** None. **R. Billiras:** None. **K. Albinet:** None. **G. Rollin-Jego:** None. **F. Iop:** None. **C. Bardet:** None. **T. Fortin:** None. **F. Panayi:** None. **C. Louis:** None. **A. Gobert:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.10/K12

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

**Support:** ARC Discovery Project grant DP170100781

**Title:** Phosphorylation of the carboxyl terminal tail of tau drives its insolubility

**Authors: W. S. LEE**[1], D. C. TAN[1], M. BI[3], A. VAN HUMMEL[2], S. IPPATI[5], *L. M. ITTNER[1], Y. D. KE[4]
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**Abstract:** Tau-containing neurofibrillary tangles are a neuropathological hallmark of Alzheimer's disease, progressive supranuclear palsy and other forms of frontotemporal dementia. Tau is a multi-domain protein, comprising an amino terminal proline-rich projection domain, followed by 3 or 4 microtubule-binding repeats and a carboxyl terminal tail region (CTTR). In disease, tau becomes phosphorylated at both physiological and 'pathological' sites, resulting in a state referred to as hyper-phosphorylated. Several of the 'pathological' phosphorylation sites that have been associated with advanced disease are located within the protein's CTTR. However, little is known about the functional relevance of the CTTR. To obtain insight into the role of the CTTR, we asked if there are specific interaction partners of the CTTR and how hyper-phosphorylation might impact on such interactions. Therefore, we substituted the serine residues of human tau at the position 396,404,409 and 422 by aspartic acid to mimic phosphorylation (PM tau) and used this variant as a bait for yeast-two-hybrid screening compared to the non-mutant tau (WT tau) sequence of the CTTR. We identified 21 candidates that interacted preferentially with PM tau, 18 candidates that interacted preferentially with WT tau and 7 that interacted with both tau variants. Differential cluster analysis of interaction candidates identified two major clusters, namely molecular chaperones and members of the ubiquitin proteasome system. Using co-immunoprecitation (CoIP), we confirmed interaction of several E3 ubiquitin ligases and molecular chaperones with full-length tau. Then we used differential extraction with buffers of increasing stringencies to determine the effects of the identified E3 ubiquitin ligases and chaperones on tau solubility. We found that differential interaction mediated by phosphorylation of sites in the CTTR accelerated formation of insoluble tau aggregates in transfect cells. Taken together, we found that phosphorylation of tau in the CTTR contributes to the protein's quality control and possibly its deposition in disease.

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**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.11/DP06/L1 (Dynamic Poster)

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

**Support:** MGH

**Title:** Tau protein disrupts nucleocytoplasmic transport in Alzheimer's disease

**Authors: *B. EFTEKHARZADEH**[1], J. G. DAIGLE[2], S. WEGMANN[3], S. DUJARDIN[4], A. B. SCHMIDER[5], M. D. GODIN[5], M. MAESAKO[5], S. DEVOS[5], R. E. BENNETT[6], J. MERTENS[7], R. J. SOBERMAN[5], F. H. GAGE[8], J. D. ROTHSTEIN[9], B. T. HYMAN, MD,PhD[5]
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**Abstract:** The mechanism of tau induced neurotoxicity is unknown. Nucleocytoplasmic transport (NCT), an essential process for cell survival, is impaired in cells with cytoplasmic protein aggregates such as mutant huntingtin and TDP-43 due to impairment of nuclear pore complexes (NPCs). We tested the hypothesis that soluble, high molecular weight bioactive tau disrupts neuronal NCT. The effect of cytoplasmic tau on NPCs was tested in wild type (WT) and tau P301S overexpressing (PS19) neurons in vitro and in vivo, and in human control and Alzheimer autopsy samples. Five lines of evidence support the idea that tau correlates with disruption of NCT. NCT was evaluated in the presence of different tau species using a fluorescence-based nuclear pore integrity reporter, the nuclear: cytoplasmic ratio of endogenous RanGTP, and a dextran exclusion assay. In cells having tau aggregates or high molecular weight soluble tau, we observe abnormal distribution of NPCs and disrupted NCT. Both spectral FRET and FLIM demonstrated close proximity of aggregated tau and NPCs, suggesting a direct protein-protein interaction of tau with NPC constituents. Nuclei from affected areas in AD brain have abnormal dextran exclusion, and altered RAN GTP nuclear/cytoplasmic distribution. The extent of aberrant nuclear pore morphology and nuclear membrane leakiness correlate with Braak staging in AD brains. We suggest that abnormal cytoplasmic soluble or aggregated tau disrupts NCT, likely through direct interactions of tau with NPCs. We believe that tau-induced impairment of nuclear transport may contribute to tau-related neurotoxicity.

**Disclosures: B. Eftekharzadeh:** None. **J.G. Daigle:** None. **S. Wegmann:** None. **S. Dujardin:** None. **A.B. Schmider:** None. **M.D. Godin:** None. **M. Maesako:** None. **S. DeVos:** None. **R.E. Bennett:** None. **J. Mertens:** None. **R.J. Soberman:** None. **F.H. Gage:** None. **J.D. Rothstein:** None. **B.T. Hyman:** None.

**Poster**

**568. Tau Biochemistry and Physiology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 568.12/L2

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

**Support:** NIH Grant R01GM101066

**Title:** Regulation of microtubule dynamics by Tau

**Authors: *R. ALI**[1], C. L. BERGER[2]
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**Abstract:** The microtubule associated protein, Tau, is implicated in a class of neurodegenerative diseases known as “Tauopathies”. One of the common feature of these disease states is misregulation of axonal transport. Thus to better understand the pathology of these diseases it is important to understand the role that Tau plays in regulation of axonal transport. Tau is known to regulate microtubule dynamics *in vitro*, which is thought to be an important function in stabilizing the microtubule tracks required for efficient axonal transport; however the mechanisms by which Tau regulates microtubule dynamics are not well understood. Moreover, six isoforms of Tau are expressed in adult human brain, and the isoform specificity of Tau’s function is also not completely clear. To address these issues, we use total internal reflection fluorescence (TIRF) microscopy to examine the dynamics of individual microtubules in the absence and presence of different isoforms of Tau. We are currently working with 3RS-, 3RL- and 4RL isoforms of Tau. 3RS- and 3RL-Tau contain three microtubule-binding repeats in the C-terminal microtubule-binding domain, but differ in the number of N-terminal acidic inserts that they contain (0 and 2, respectively) in the N-terminal projection domain. 4RL-Tau contains four microtubule-binding repeats and 2 N-terminal acidic inserts in the N-terminal projection domain. Our findings show that 3RS- and 3RL tau isoforms reduce microtubule catastrophe frequencies but have no effect on microtubule rescue frequencies, whereas, 4RL-Tau increases microtubule rescue frequency but has no effect on microtubule catastrophe frequency. 3RL-Tau and 4RL-Tau increase microtubule growth velocity and 3RL-Tau has no effect on it. We further demonstrate that Tau reduces the rate at which microtubule tip structure evolves while it is growing, such that the microtubule tip becomes tapered at a slower rate in the presence of Tau compared to the no Tau control. This is a previously unknown mechanism by which Tau can alter microtubule catastrophe frequency. Currently, work is on-going with other isoforms of Tau. In summary, we are elucidating in detail the role of Tau in regulation of microtubule dynamics in an isoform specific manner, leading to new insight as to how misregulation of Tau’s function affects microtubule dynamics and in turn axonal transport during the disease process.

**Disclosures:** **R. Ali:** None. **C.L. Berger:** None.

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.01/L3

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

**Support:** Cell and Tissue Engineering NIH BiotechnologyTraining Grant (T32-GM008433)

George W. Woodruff School of Mechanical Engineering at Georgia Institute of Technology startup funds

**Title:** Cerebrovascular pericytes in an Alzheimer's microenvironment secrete cytokines and modulate endothelial barrier function

**Authors: *L. WEINSTOCK**[1], L. WOOD[2]
[1]Dept. of Biomed. Engin. and Inst. for Bioengineering and Biosci, [2]Mechanical Engin. and Inst. for Bioengineering and Biosci., Georgia Inst. of Technol., Atlanta, GA

**Abstract:** Postmortem Alzheimer's disease (AD) brain tissues exhibit extensive vascular changes including loss of blood-brain barrier (BBB) function. Moreover, increased hippocampal vascular permeability has been reported in patients with mild cognitive impairment, suggesting that vascular dysfunction may play a pathogenic role. Yet, the mechanisms promoting vascular dysfunction remain poorly understood. Cerebral pericytes encircle the vascular endothelial layer on the parenchymal side and are responsible for regulating blood-brain barrier function. In this work, we hypothesized that pericytes become dysfunctional in response to the AD microenvironment and in turn promote loss of barrier function. Pericytes give structural support to the endothelial layer in the microvasculature, help maintain vascular coupling, and are immunoactive. Pericyte dysfunction has also been correlated with AD onset and in AD comorbidities, e.g. diabetes and traumatic brain injury**.** Our data indicate that amyloid beta, key inflammatory cytokines (e.g., TNF-α), and high insulin/glucose do not affect cerebral pericyte viability or migration in 2D and 3D cell cultures. However, TNF-α and high glucose, but not Aβ alone, stimulated broad up-regulation of pericyte-expressed inflammatory cytokines. The cytokines that correlated most strongly with pro-inflammatory and diabetic conditions include MCP-3, G-CSF, IL-8, and RANTES, which are known to promote immune cell chemotaxis and angiogenesis. Pericyte-conditioned culture medium reduced expression of endothelial adherens junction proteins VE-Cadherin and PECAM in TNF-α and high glucose/insulin conditions significantly more than when the conditions were directly applied to endothelial cells. These data suggest that pericyte response to the AD microenvironment may promote leakiness of the blood-brain barrier. Given the chemotactic nature of the cytokines secreted by pericytes in these

conditions, our data further suggest that pericyte signaling may play an important role in regulating activation and recruitment of neural glia and extravasation of circulating immune cells at the blood-brain barrier.

**Disclosures: L. Weinstock:** None. **L. Wood:** None.

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.02/L4

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

**Support:** BrightFocus A20152965

NIH UL1TR001108

NIH R01 AG023012

**Title:** CCR2-mediated peripheral macrophage recruitment is essential for regulating tau pathological outcomes

**Authors: *T. MCCRAY**[1], V. JADHAV[1], C. M. MILLER[3], G. E. LANDRETH[2], B. T. LAMB[4], S. PUNTAMBEKAR[1], S. M. BEMILLER[5]
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**Abstract:** It is widely accepted that there exists a profound role for inflammation in the pathogenesis of Alzheimer’s disease (AD). This inflammatory component can play a dichotomous role in modifying the various hallmarks of AD, including the deposition of amyloid beta (Aβ) and microtubule associated protein tau (MAPT; Tau). Acute inflammation can be beneficial to pathology by increasing the clearance and processing of toxic misfolded proteins, but left chronically unchecked can be detrimental to other aspects of disease including oxidative stress, and exacerbating hyperphosphorylation and aggregation of intracellular tau. Further nuancing the inflammatory contribution to disease are the non-redundant roles for distinct myeloid cell populations in the brain which remain poorly understood. It has been shown throughout the course of numerous CNS diseases including multiple sclerosis and AD, that pathogenesis can involve the recruitment of several populations of peripheral immune cells which can uniquely shape pathology. Until now, little has been known regarding the presence or role of peripheral inflammatory cell populations throughout tauopathy. Here, using a variety of transgenic mouse models including *Cx3cr1*$^{GFP/+}$*;Ccr2*$^{RFP/+}$ mice, along with bone-marrow chimerism studies, we demonstrate a significant region-specific increase in infiltrating

inflammatory cells in the brains of hTau mice compared to B6 controls beginning at 6-months and persisting through 18-months of age. This phenomenon is abrogated upon deletion of myeloid CCR2 which results in choroid plexus cell accumulation, but not entry of peripheral cell populations into the diseased parenchyma. This results in heightened tau pathology which is most notable in white matter regions. These results demonstrate a clear role for peripheral immune cell populations in the pathogenesis of AD related tauopathy, and represents a unique therapeutic target which is accessible without the need access the brain through the blood brain barrier.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.03/L5

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

**Support:** Erb family donation to the Beaumont Foundation

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**Title:** Specific serum antibody binding to phosphorylated and non-phosphorylated tau in non-cognitively impaired, mildly cognitively impaired, and Alzheimer’s disease subjects

**Authors: *A. C. KLAVER**[1], M. P. COFFEY[2], D. A. BENNETT[3], D. A. LOEFFLER[1]
[1]Dept. of Neurol., [2]Dept. of Biostatistics, Beaumont Res. Inst., Royal Oak, MI; [3]Rush Alzheimer's Dis. Ctr., Chicago, IL

**Abstract:** Tau vaccination and systemic administration of anti-tau antibodies prevent pathology and cognitive impairment in mouse models of tauopathy, suggesting that therapies which increase anti-tau antibodies might slow development and/or progression of Alzheimer’s disease (AD). The extent to which individuals with no cognitive impairment (NCI) possess serum anti-tau antibodies, and whether their concentrations of these antibodies differ from the anti-tau antibody levels found in persons with mild cognitive impairment (MCI) or AD, are unclear. We developed an ELISA to measure serum anti-tau antibody levels; this ELISA accounted for antibody polyvalent binding and for the possibility that antibody binding to phosphorylated tau peptides could be due to binding to non-phosphorylated epitopes on those peptides. Specific IgG

and IgM binding to phosphorylated ("pTau;" phosphorylated at Serine-199 and Serine-202) and non-phosphorylated ("non-pTau") tau 196-207 was measured in serum samples from subjects with NCI, MCI, or AD (n = 10/group). RESULTS: Specific pTau and non-pTau IgG antibodies were detected in most subjects. Mean pTau IgG increased in MCI subjects by 53% and 70% vs. AD and NCI subjects respectively (both $p < 0.05$), while no statistically significant differences were found between groups for non-pTau IgG ($p = 0.052$), pTau IgM, or non-pTau IgM antibody levels. Non-pTau IgG levels were negatively associated with global cognition (Spearman rho = -0.50). These findings suggest that serum IgG and IgM to both phosphorylated and non-phosphorylated tau are likely to be present in older persons regardless of their cognitive status. Further, serum concentrations of anti-phospho-tau IgG may be increased in individuals with MCI.

**Disclosures:** **A.C. Klaver:** None. **M.P. Coffey:** None. **D.A. Bennett:** None. **D.A. Loeffler:** None.

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.04/L6

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

**Support:** R01AG043522-05

**Title:** Bexarotene modifies hippocampal neuroinflammation to enhance and sustain cognitive improvements in an AD mouse model

**Authors: *B. CASALI**, E. G. REED-GEAGHAN, G. E. LANDRETH
Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, OH

**Abstract:** Treatment of Alzheimer's disease (AD), a neurodegenerative disorder characterized by pathological hallmarks of beta-amyloid (Aβ) plaque deposits, inflammation, and cognitive decline, remains a clinical obstacle in part due to lack of targeted therapeutics. Our lab established that prolonged treatment with nuclear-receptor agonist bexarotene reversed cognitive deficits in a murine model of AD without notable modification of plaque burden. These conclusions suggest that nuclear receptor agonists exert salutary effects on cognition through alternative mechanisms not fully dependent on plaque burden changes. To elucidate alternative mechanisms, we treated one-year old APP/PS1 mice with bexarotene for two-weeks and then we discontinued bexarotene treatment for two weeks. In both groups of mice, we administered the novel-object recognition test to monitor cognitive improvements, and we analyzed amyloid pathology at the conclusion of the study. Similar to previous findings, bexarotene enhanced cognition; strikingly, we observed sustained improvements in cognition in mice discontinued

from bexarotene. Cognitive improvement in both treatment groups was independent of changes in plaque burden and insoluble Aβ species. Following bexarotene treatment and its discontinuation, we observed significant reductions in microglial reactivity exclusively in the hippocampus of APP/PS1-treated mice. Our findings demonstrate that bexarotene may selectively modify neuroinflammation in a regional-specific manner to reverse hippocampal-dependent cognitive deficits in AD mice, and they may lend insight to inform future studies involving nuclear receptor agonists.

**Disclosures:** **B. Casali:** A. Employment/Salary (full or part-time):; CWRU. **E.G. Reed-Geaghan:** A. Employment/Salary (full or part-time):; CWRU. **G.E. Landreth:** A. Employment/Salary (full or part-time):; CWRU. Other; GEL is a cofounder of ReXceptor, Inc, a biotechnology company developing RXR agonists for the treatment of neurodegenerative diseases..

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.05/L7

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

**Support:** NIH Grant R21AG048631

Swedish Research Council 36821-114015-58

**Title:** Resolution of inflammation - Relation to Alzheimer neuropathology

**Authors: C. EMRE**[1], E. HJORTH[1], A.-C. GRANHOLM[2], *M. SCHULTZBERG[1]
[1]Karolinska Institutet, Huddinge, Sweden; [2]Denver Univ., Denver, CO

**Abstract:** In Alzheimer’s disease (AD) an inflammatory response occurs as a reaction to the primary pathology, and may also be a part of it. The ensuing glial proliferation and activation contributes to the neurodegeneration by increased levels of harmful inflammatory mediators. In general, upon successful removal of the pathogenic insult inflammation is down-regulated together with restoration of the site of insult by the resolution of inflammation, which is actively induced by derivatives of omega-3 and -6 fatty acids named specialized pro-resolving mediators (SPMs). We have previously shown reduced levels of SPMs in *post mortem* brain tissue from AD patients together with alterations in proteins involved in resolution, a negative influence on resolution by the AD pathogen β-amyloid$_{42}$, and that stimulation with SPMs is neuroprotective, anti-inflammatory and increases phagocytosis of Aβ.
We are now further investigating the relationship between the pathology of AD and resolution in different brain regions including the basal forebrain, BA46, cerebellum, cingulate gyrus and

hippocampus. *Post mortem* brain tissue from AD patients and healthy controls are analysed with regard to markers for the resolution of inflammation including receptors and enzymes involved in SPM signalling and synthesis, markers of inflammation, as well as the pathology of AD using immunohistochemistry and western blotting. Data on disease stage according to the ABC scoring system are also available. Our preliminary results show that the levels of ChemR23, receptor for the SPM resolvin E1, were significantly higher in the basal forebrain and in the cingulate gyrus in AD cases compared to controls. These data correlate with neuropathology scores as well as the levels of the microglial marker HLA-DR. This suggests that the resolution of inflammation is related to the neuroinflammation in AD brains, and is supportive of a disturbance in the resolution pathway in AD. Together with our previously published data showing beneficial effects of SPMs, we suggest that stimulation of pro-resolving activities represents a potential treatment for AD and other neurodegenerative disorders.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.06/L8

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

**Support:** City University of New York (Graduate Center)

NIH Grant GM060665

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PSC-CUNY 67851-00 45

**Title:** Relationship between BRCA1 and neuroinflammation mediated by PGJ2: Relationship to Alzheimer's disease and cancer-related cognitive impairment

**Authors: *J. DINE**[1], T. JEAN-LOUIS[2], M. E. FIGUEIREDO-PEREIRA[3]
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**Abstract:** Alzheimer’s disease (AD) affects approximately 5 million people in the United States and is the leading cause of dementia in older adults. Interestingly, AD shares several genotypic and phenotypic features with cancer-related cognitive impairment (CRCI), which presents in an estimated 75% during and 35% months to years after treatment, most notably in breast and ovarian cancer survivors. Both AD and CRCI are strongly associated with aging, cognitive dysfunction, inflammation, and DNA damage response changes. Decreased expression of

BRCA1, an E3 ubiquitin ligase responsible for DNA double-strand break repair and mediating apoptotic signaling, has been identified in the neurons of individuals with mild cognitive impairment and AD. Deleterious BRCA1 variants that result in loss of functional activity are also associated with breast and ovarian cancer development, among other conditions, due to impaired DNA quality control mechanisms. Thus, BRCA1 loss may be a shared feature of AD and CRCI and warrants investigation, particularly in the context of the neurotoxic prostaglandin J2 (PGJ2). PGJ2 is an endogenous product of inflammation and is considered to be one of the most toxic prostaglandins that is produced downstream from cyclooxygenase-2 (COX-2) activation. COX-2 is highly expressed in AD brains and its activity correlates with the severity of AD. Our *in vitro* studies with human neuroblastoma SY5Y cells overexpressing APP695 (APP695-SY5Y), showed that PGJ2 recapitulates pathological events relevant to AD, including neurotoxicity, caspase-3 activation, tau aggregation, and the accumulation/aggregation of ubiquitinated proteins. We determined the effect of PGJ2 on BRCA1 expression in APP695-SY5Y cells. Upon PGJ2-treatment, the levels of BRCA1 were decreased in a concentration-dependent manner, assessed by western blot analysis. Our findings indicate that BRCA1 expression is attenuated in the presence of PGJ2. This study establishes a potential relationship between BRCA1 and PGJ2 in human neuronal cells for what we believe to be the first time. Further study is needed to investigate PGJ2, as well other the other neuroinflammatory prostaglandins PGD2 and PGE2, and BRCA1 signaling interactions. Such work may lead to the identification of potentially novel therapeutic targets for the treatment of AD and CRCI.

**Disclosures: J. Dine:** None. **T. Jean-Louis:** None. **M.E. Figueiredo-Pereira:** None.

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.07/L9

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

**Title:** Assessing the effects of various fractalkine cleavage products on microglial activation

**Authors: *D. J. FINNERAN**[1], S. KAMATH[2], D. G. MORGAN[4], K. R. NASH[3]
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**Abstract:** Fractalkine (CX3CL1; FKN) is an endogenous chemokine expressed throughout the body. It is a type 1 transmembrane protein containing the chemokine domain, a long mucin-like stalk domain, and small transmembrane and intracellular domains. FKN can be cleaved by several proteases (ADAM10, ADAM17, and cathepsin S) to signal as a soluble peptide. The putative ADAM10/17 cleavage site yields a soluble peptide consisting of the chemokine domain and the mucin-like stalk domain. It is thought that cathepsin S cleavage occurs further in the

mucin-like stalk, yielding a shorter peptide than the ADAM10/17 cleavage product. In the CNS, FKN is expressed solely by neurons and binds its unique receptor, CX3CR1, which is expressed only on microglia. FKN signaling leads to decreased expression of pro-inflammatory cytokines such as IL-1β and TNF-α. Recent studies have shown AAV-mediated overexpression of a soluble FKN in Tg4510 mice inhibited microglial activation, reduced tau pathology, and ameliorated neuron loss. However, FKN has also been implicated in neuropathic pain. Rodent spinal injury models show FKN staining near the lesion and inhibition of the lysosomal protease cathepsin S can prevent hyperalgesia. Furthermore, infusion of recombinant chemokine domain caused hyperalgesia while full-length soluble FKN did not. Our study investigates this duality of FKN signaling; full-length soluble is anti-inflammatory while the chemokine domain is implicated in neuropathic pain and microglial activation. Here, we generated conditioned media of human FKN: chemokine domain (ckFKN), full-length soluble FKN (sFKN), and a secreted mucin-like stalk of FKN (mucFKN). We assessed the ability of these constructs to reduce TNF-α secretion from both immortalized rat microglia and primary rat microglia activated by LPS treatment.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.08/L10

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

**Support:** Siriraj Grauduate Research Fund

**Title:** Anti-inflammatory effects of sea cucumber (holothuria scabra) extracts on memory deficits from vascular dementia in mice

**Authors: *S. CHOMPOOPONG**[1], F. PADUNGRAKSART[2], T. NAOWASIRI[2], N. PAKAPROT[3], T. RUNGRUANG[2], T. TAECHOWISAN[4], P. SOBHON[5]
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**Abstract:** Sea Cucumber (*Holothuria scabra*) extracts or HsE is a valuable source of several kinds of substances that can serve as natural health products, contain saponins or triterpene glycosides. Pharmacological studies indicate anti-inflammatory and anticancer properties of the sea cucumber saponins that it may enhance learning and memory and promote brain functions. To determine therapeutic potential of HsE as an alternative treatment for vascular dementia,

cerebral hypoperfusion was induced by modified common carotid artery occlusion in 60 ICR mice. After arterial occlusion, HsE was injected intraperitoneally for ten days in HsE treated group, then Morris water maze and voice-cued fear conditioning test were performed. At the end of experiment, eye balls and brains of sacrificed mice were removed for histopathological study of retinal layer, white mater (WM) damage in corpus callosum, pyramidal cell death in hippocampal CA1 and level of TNF-α and IL-1β in serum and brain. Expression of TNF-α and IL-1β in serum and brain were decreased in HsE treated group significantly when compared to occlusion group. HsE improved memory retention significantly at $p<0.05$, treated group swam up to platform with less escape latency time (14.69±2.12 sec) than occlusion group (21.54±3.22 sec). Percent thickness of outer plexiform layer and WM damage indicated by myelin index were enhanced and improved significantly by HsE. The memory retention with increased freezing behavior was also increased from 12.96% ± 4.91 to 49.53% ± 9.72%, significantly at $p<0.001$. HsE showed therapeutic effects against cerebral hypoperfusion and attenuated memory deficits. The decreased pyramidal cell death in hippocampal CA1 area, improved retina and WM damages related to decrease in TNF-α and IL-1β level in serum and brain, therefore, role of HsE may be described by its anti-inflammatory effect.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.09/M1

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

**Support:** R01NS082296

**Title:** Neurodegeneration and cognitive deficits in aged CIZ1 knock-out mice

**Authors: *M. KHAN**, J. XIAO, D. PATEL, J. TIAN, M. LEDOUX
Neurol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

**Abstract:** Preserving DNA integrity is vital for normal cellular functioning. Defective DNA repair leads to genomic instability and promotes neurodegeneration and cognitive decline. In this regards, a substantial body of work suggests that mutations in genes that encode proteins involved in cell-cycle and DNA repair have been linked to several age-related neurodegenerative diseases including Alzheimer disease (AD). CDKN1A interacting zinc finger protein 1 (CIZ1), a nuclear protein, plays an important role in DNA repairs pathway and cell-cycle progression at the G1/S checkpoint. Recently, we have shown that germline knock-out (KO) of *Ciz1* is associated with motor, and hematological abnormalities in young adult mice. However, it

remains obscure whether CIZ1 deficiency is involved in age-related neurodegenerative diseases especially in AD. To test our hypothesis that deficiency of CIZ1 has a pathogenic role in AD, we have treated primary neuronal cultures of WT and CIZ1KO mice with $A\beta_{1-42}$ (5 μM) for 24 h. We observed degenerated MAP2-positive neuronal cells along with increased DNA breaks in cultures from CIZ1KO mice compare to barely detectable degeneration in WT cells after $A\beta_{1-42}$ treatment. Interestingly, we observed that 17β-estradiol (E2) treatment confers neuroprotection against $A\beta_{1-42}$ treatment in the CIZ1KO neuronal cells, suggesting neurons derived from CIZ1KO mice were more susceptible *to* $A\beta_{1-42}$ toxicity than neurons derived from their WT littermates. Furthermore, aged CIZ1KO mice exhibited cognitive decline, increased neuronal DNA breaks, vascular dysfunction, upregulation of NF-kB and neurodegeneration in the hippocampus when compared to WT littermates. Our findings suggest that the deleterious effects of CIZ1 deficiency become more pronounced with aging and failure of CIZ1 function may contribute the cognitive decline and neuronal death in the aged brain. Moreover, Identification of the downstream signaling pathways by which CIZ1 promotes neuroprotection may open novel therapeutic avenues to treat neurodegenerative diseases and age-related cognitive deficits

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.10/M2

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

**Support:** NIH Grant NS080686 to BP-Endure

NIH Grant MD007599 to Hunter College from NIMHD

City University of New York (Graduate Center)

**Title:** Diazoxide as a protective therapeutic against the neurotoxic effects of neuroinflammation induced by the cyclooxygenase product prostaglandin J2: Relevance to Alzheimer’s disease

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**Abstract:** Chronic neuroinflammation is a major factor initiating/exacerbating neurodegeneration in Alzheimer’s disease (AD). The pro-inflammatory enzyme cyclooxygenase-2 (COX-2) is highly expressed in AD brains and its activity correlates with the severity of AD.

COX-2 catalyzes the synthesis of prostaglandins, some of which are neuroprotective while others are neurotoxic. Anti-inflammatory drugs that target COX-2 can prevent/inhibit inflammation. However, this anti-inflammatory strategy can produce adverse side effects, including renal failure, heart attack and stroke, as well as preventing the synthesis of neuroprotective prostaglandins. Therefore, to prevent the effects of neurotoxic prostaglandins without interfering with the neuroprotective ones, would offer a significant *therapeutic benefit* to the *treatment* of *AD*. Prostaglandin J2 (PGJ2) is an endogenous product of inflammation and is considered to be one of the most toxic prostaglandins that is produced downstream from COX-2 activation. Our *in vitro* studies with human neuroblastoma SY5Y cells overexpressing APP695 (APP695-SY5Y), showed that PGJ2 recapitulates pathological events relevant to AD, including neurotoxicity, caspase-3 activation, and the accumulation/aggregation of ubiquitinated proteins. We investigated the therapeutic potential of Diazoxide (DZ) against the neurotoxic effects induced by PGJ2 in APP695-SY5Y cells. DZ is a well-known potassium channel activator, is safe, is an FDA-approved drug for hypertension, and was also shown to positively modulate glutamate receptors indicating that it has potential to enhance cognition. Our *in silico* studies predicted that DZ binds to multiple kinases that are responsible for the pathology of AD. Our *in vitro* studies established that DZ significantly reduces the accumulation of ubiquitinated proteins and caspase-3 activation in the SY-APP695 cells. Our data support that DZ protects the neuronal cultures from PGJ2-induced neurodamage. The deeper study of DZ will provide insights for a therapeutic approach to overcome the neurotoxic effects of neuroinflammation and for enhancing neuronal survival in AD. NOTE: *Alec Levine and Jordy Sepulveda contributed equally to these studies.

**Disclosures:** **A. Levine:** None. **J. Sepulveda:** None. **L. Xie:** None. **P. Rockwell:** None. **M.E. Figueiredo-Pereira:** None.

**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.11/M3

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

**Support:** NIA PO1AG14449

NIA R01AG043375

NIH P30AG010161

**Title:** Frontal cortex chitinase 3-like protein 1 (CH13L1) and complement component C1q protein levels during the progression of Alzheimer’s disease

**Authors: *M. NADEEM**, S. PEREZ, E. J. MUFSON
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**Abstract:** Chitinase 3-like protein 1 (CH13L1 or YKL-40) and C1q are involved in immune regulation in several inflammatory conditions and neurodegenerative diseases, including Alzheimer's disease (AD). In brain, CH13L1 and C1q are secreted mainly by innate immune cellular elements, astrocytes and microglia, which are involved in anti-inflammatory responses. Astrocytes and microglia are increased in areas of the brain displaying amyloid beta (Aβ) and tau pathology in AD, suggesting the involvement of CH13L1 and C1q in the pathogenesis of AD. While a few studies have shown an upregulation of CH13L1 and C1q in cerebral spinal fluid and temporal cortex in AD, virtually nothing is known about the evolution of these inflammatory markers in the frontal cortex during the progression of AD. In the present study, we examined CH13L1 and C1q protein levels in frozen frontal cortex obtained from subjects who died with an antemortem clinical diagnosis of no cognitive impairment (NCI, n=14), mild cognitive impairment (MCI, n=13), mild/moderate AD (mAD, n=12) and severe AD (sAD, n=11,) from the Rush Religious Orders Study (RROS) and the Rush ADC, respectively, using quantitative immunoblotting. Levels of CH13L1 and C1q were correlated with the microglia markers triggering receptor expressed on myeloid cells (TREM2) and Iba1 values, which are known to regulate inflammation and phagocytosis. Western blot analysis revealed no significant differences in CH13L1 ($p=0.097$) and C1q ($p=0.072$) protein levels among the clinical groups. However, there was a trend towards an increase CH13L1 and C1q in MCI and mAD, which were correlated with each other in both clinical groups ($r=0.6$, $p<0.0004$). In addition, we found that C1q levels were positively associated with Iba1 values ($p=0.7$, $p<0.00009$), but not with TREM2, in MCI and mAD. Conversely, cortical CH13L1 levels did not show a correlation with Iba1 in the early stages of the disease, and only a negative correlation was observed with TREM2 in the MCI group ($r=-0.7$, $p=0.01$). These data suggest that increases in CH13L1 and C1q protein levels in the frontal cortex are indicative of an active gliosis process associated with an anti-inflammatory response early in the disease.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

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**Program#/Poster#:** 569.12/M4

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

**Support:** NIH Grant U01 AG046170

BrightFocus Fondation

Cure Alzheimer's Fund

**Title:** Microglial TYROBP deficiency modulates brain C1q phenotype in mouse models of Alzheimer's amyloidosis and tauopathy

**Authors: *M. AUDRAIN**[1], J.-V. HAURE-MIRANDE[1], S. KIM[1], T. FANUTZA[1], R. D. BLITZER[2], M. WANG[3], B. ZHANG[3], E. E. SCHADT[3], M. E. EHRLICH[4], S. E. GANDY[5]
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**Abstract:** Microglial cells are the resident phagocytes of the central nervous system (CNS) and represent a key cell type implicated in the CNS inflammatory response. Mutations and/or differential expression of microglial cell surface molecules such as TREM2, CD33 and CR3 have been associated with altered relative risk(s) for developing Alzheimer's disease (AD). TYROBP (also known as DAP12) is a microglial adaptor protein that acts as a key driver of a microglial activation network underlying AD. TYROBP binds TREM2, CD33, and/or CR3 and is increased in the brains of humans and mouse models of AD (Zhang et al., 2013; Readhead et al. 2015).
We crossed Tyrobp-deficient mice with either APP/PSEN1 or MAPT P301S mouse models in order to determine the effect of loss of TYROBP function on amyloid and/or tauopathy features of these mice. We will present the effects of Tyrobp deficiency on the microglial inflammatory response and AD pathology, and, in turn, on the behavior and electrophysiology. One unexpected feature of Tyrobp-/- x APP/PSEN1 or MAPT P301S mice was a reduction in brain levels of complement initiating protein C1q, a molecule known to modulate synaptic sculpting (Hong et al. 2016). Thus, one potential consequence of the excess TYROBP observed in the AD brain might be to play a role in complement-related synaptic pruning (and synaptic loss) by microglia.
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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.13/M5

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

**Support:** Fundació La Marató de TV3 20142210

Ministerio de Economía y Competitividad FEDER SAF2014-56546-R

**Title:** The role of IL-6 trans-signalling in the Tg2576 animal model of Alzheimer disease

**Authors: *A. ESCRIG**[1,2], A. MONTILLA[1,2], G. COMES[1,2], O. FERNÁNDEZ-GAYOL[1,2], M. GIRALT[1,2], A. MOLINERO[1,2], P. SANCHIS[1,2], S. ROSE-JOHN[3], J. HIDALGO[1,2]
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**Abstract:** Alzheimer's disease (AD) is the most common cause of dementia in the elderly. The main symptoms and signs of the AD are a progressive loss of cognitive functions and the presence of extracellular plaques of aggregated β-Amyloid and intracellular deposits of hyperphosphorylated tau protein. These features cause a prominent neuroinflammation, with interleukin-6 being one of the critical cytokines. For signalling, IL-6 binds to a membrane receptor (mIL-6R) and the complex of IL-6 and mIL-6R associates with gp130, which dimerizes and activates the intracellular signalling. The expression of mIL-6R is restricted to a few tissues. A soluble form of IL-6R (sIL-6R) exists naturally, and it is demonstrated that this form can activate gp130 in the presence of IL-6. This procedure has been called trans-signalling. Due to the ubiquitous expression of gp130, every cell in the body can be stimulated by this process. IL-6 trans-signalling is specifically inhibited by the soluble form of gp130, sgp130.
To study the role of IL-6 trans-signalling in AD, we generated a mouse model of AD, Tg2576, which expresses the human $APP_{695}$ harbouring the Swedish K670N/M671L mutation and simultaneously secretes the specific inhibitor of IL-6 trans-signalling, human sgp130-Fc, from astrocytes.
We are characterizing the model behaviourally and neuropathologically at different stages of the disease. Herewith we show body weight gain, survival and behavioural characterization before the formation of β-amyloid plaques (5-6 months of age), using Open-field, Hole-board, Novel Object Recognition Test, Elevated-plus Maze and Morris Water Maze. Additionally, preliminary neuropathological analysis (APP precursor protein, $A\beta_{40}$ and CTF-β) were carried out in animals of 10-11 months of age.
The main aim of this study is to clarify the function of IL-6 trans-signalling in the AD. At this moment, we do not observe major effects of the inhibition of the trans-signalling on the disease. Whether or not a significant effect will be observed upon amyloid plaques formation remains to be established.

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**Poster**

**569. Neuroinflammation and Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 569.14/M6

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

**Support:** R01 AG051437-01

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T32-GM95412

HHMI Gilliam Fellowship for Advanced Studies

**Title:** MicroRNAsmiR-155 and miR-146a modulate neuroinflammation in Alzheimer’s disease

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**Abstract:** Alzheimer’s Disease (AD) is a progressive age-related neurodegenerative disorder. AD is characterized by the accumulation of extracellular amyloid beta (A$\beta$) as well as CNS and systemic inflammation. Innate immune cells like CNS resident microglia and circulating monocytes use microRNAs to rapidly respond to inflammatory signals, such as A$\beta$ exposure. AD patient microRNA (miRNA) profiles are altered in tissue, circulating monocytes, and serum. miR-146a and miR-155 are two miRNAs that modulate the phasic inflammatory responses of innate immune cells. Previous studies show that miR-146a is upregulated in PS2 knock-out microglia. Deletion of miR-146a leads to an accelerated aging phenotype and multi-organ inflammatory infiltration, which is abrogated by conditional deletion of miR-155. miR-155 and miR-146a modulate transitions between inflammatory phases, however their precise roles in modulating inflammation in AD remains unknown. We hypothesize that miR-155 and miR-146a participate in AD pathology by modulating A$\beta$ equilibrium by influencing the functions of inflammatory cells. Using cultured neonatal microglia, we observed that modulation of miR-155 and miR-146a impact the internalization and degradation fibrilized A$\beta$. We generated trigenic mouse models to acutely or continuously induce CX3CR1 driven Cre-mediated deletion of floxed miR-155 or miR-146a alleles in either microglia alone, or microglia and circulating monocytes in the APP/PS1 mouse model. After *ex-vivo* percoll gradient selection of microglia, cells were isolated by FACS and miR-155 or miR-146a deletion was detected by PCR. Changes in inflammatory gene and miRNA expression in microglia 3 and 6 months post-acute and -chronic miR-155 deletion were assessed by qPCR. Cortical and hippocampal soluble and insoluble A$\beta$ levels were assessed by ELISA 3 and 6 months following miRNA-155 and miR-

146a deletion. Conditional miR-155 and mIR-146a deletion was detected in FACS isolated microglia at 3 and 6. In addition, we observed changes in inflammatory gene expression in microglia. Our results thus far support the hypothesis that acute and continuous miR-155 deletion in microglia and monocytes alters innate immune gene expression and Aβ equilibrium, further elucidating the molecular pathways regulating neuroinflammation in AD.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.01/M7

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

**Support:** National Institutes of Health Grant U01 AG024904

Department of Defense award number W81XWH-12-2-0012

**Title:** Clinical significance of functional connectome measures in Alzheimer’s Disease

**Authors: *J. E. JOSEPH**[1], D. VANDERWEYEN[1], O. BRAWMAN-MINTZER[2,5], B. DEAN[6], B. MUNSELL[7], D. CLARK[3], J. MINTZER[4,8]
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**Abstract:** Current research efforts have begun to explore functional brain network alterations in mild cognitive impairment (MCI) and Alzheimer’s Disease (AD). However, the clinical significance of different network measures is not yet clear. Here, we present a framework in which different network properties constitute different hypotheses of network configuration and information organization, which may be related to different aspects of cognitive impairment and decline in AD. The framework is tested using graph-theory measures of intrinsic connectivity from resting state fMRI (rsfMRI). Data collected as part of ADNI and ADNI-DOD included fifteen AD patients and 14 age-matched healthy controls. After data preprocessing, fully-connected and weighted connectivity matrices were constructed using partial correlation and a shrinkage operation. Clustering coefficient and eigenvector centrality were computed for each of 264 brain regions (nodes) for each subject. The 264 node measures were reduced to 12 components and factor scores served as predictors (using linear modeling) of either cognitive impairment (the MMSE score at the time of scanning, which was at least 6 months after the

baseline visit) or cognitive decline (the change in MMSE score from baseline to the time of scanning). Clustering coefficient marginally predicted MMSE score at the time of scanning (Accuracy = 41.8%, p = .062). Lower MMSE was associated with higher clustering coefficient in a number of different nodes in frontal, parietal and occipital cortex. Eigenvector centrality (EC) significantly predicted change in MMSE score from baseline (Accuracy = 66.2%; p = .023). A more restricted set of brain regions predicted change in MMSE, with greater cognitive decline associated with higher EC in the right superior occipital and right inferior frontal cortex and with lower EC in the right cuneus. The prediction of MMSE from eigenvector centrality persisted even when age and sex were added as predictors (Accuracy = 59.4%; p = .042). Although preliminary, these initial findings indicate that different graph-theory measures of functional network connectivity are associated with cognitive impairment versus cognitive decline, indicating that models of network degradation and compensation in AD should consider the different information properties of graph-theory measures.
*Data used in preparation of this abstract were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.02/M8

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** DFG Kr1879/5-1

SFB779 TPB8

DFG Kr1879/6-1

**Title:** Posttranslational-modification impacts on the mechanism by which oligomeric Aβ triggers synaptic dysfunction

**Authors: *P. YUANXIANG**, K. GROCHOWSKA, M. KREUTZ
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**Abstract:** It is generally believed that soluble oligomeric amyloid beta (Aβo) disrupts synaptic plasticity and memory at an early stage of Alzheimer's disease (AD). Various posttranslational-modified Aβo have been identified, and the most abundant types are N-terminally truncated forms. It is not clear, however, whether modified forms can induce synaptic dysfunction on their own. Here, we show that the D1/D5R agonist SKF38393 protects LTP of hippocampal CA1 synapses from the deleterious action of oligomeric amyloid beta 1-42 (Aβ 1-42). Moreover, we found that inhibition of Src-family tyrosine kinases completely abolished the protective effects of D1R/D5R stimulation. In addition, we show that a prominent isoform, pyroglutamated Aβ3(pE)-42, induces synaptic dysfunction to a similar extent like Aβ1-42 but by clearly different mechanisms. In contrast to Aβ1-42, Aβ3(pE)-42 does not directly associate with synaptic membranes or the Prion protein but is instead taken up by astrocytes and potently induces glial release of the pro-inflammatory cytokine TNFα. Moreover, Aβ3(pE)-42 induced synaptic dysfunction is not related to NMDAR signalling and Aβ3(pE)-42 induced impairment of synaptic plasticity cannot be rescued by D1R/D5R-agonists. Collectively the data point to a scenario where neuroinflammatory processes together with direct synaptotoxic effects are caused by posttranslational modification of soluble oligomeric Aβ and contribute synergistically to the onset of synaptic dysfunction in AD.

**Disclosures: P. Yuanxiang:** None. **K. Grochowska:** None. **M. Kreutz:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.03/M9

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

**Support:** Alzheimer's Drug Discovery Foundation 20130805

**Title:** Heterogeneity in local hippocampal connectivity as measured by functional magnetic resonance imaging increases with Alzheimer's disease progression

**Authors: *K. SHATTUCK**[1], S. ASHBURN[2], W. HORTON[3], J. H. HOWARD, Jr.[4], G. W. REBECK[2], R. S. TURNER[3], X. JIANG[2]
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**Abstract:** Early detection of Alzheimer's disease (AD) symptoms enables treatment strategies based on slowing disease progression. However, despite recent efforts and significant progress, reliable detection of pre-symptomatic and prodromal AD remains a major challenge. Synaptic dysfunction precedes neurodegeneration in early stages of AD, but is difficult to detect and quantify. Functional magnetic resonance imaging (fMRI), with its ability to measure neuronal

function, has the potential to non-invasively identify systems-level connectivity impairments associated with cognitive decline prior to identifiable structural changes. Using a previously validated analysis technique (Hcorr) that estimates the heterogeneity of functionally localized connectivity, we report that local hippocampal activity shows marked differences between patients diagnosed with AD or mild cognitive impairment (MCI) and healthy control subjects. Methods: Fifteen individuals with AD, 12 with MCI, 24 high-risk control subjects (with a family history of AD and/or APOE-4 genetic status), and 27 low-risk control subjects (all subjects: 56-85 years old, 43 females) underwent a T1-weighted structural MRI scan as well as six 6-minute fMRI scans. The fMRI scans comprised two runs each of a 1-back face-detection task, a modified serial reaction time task, and resting scans. Hcorr was calculated within left and right hippocampus for each MRI scan using a revised algorithm to improve robustness. ROI localization was based on a publicly available brain atlas and customized within each subject to voxels estimated via structural scan segmentation to contain at least 50% grey matter volume. Results: Mean bilateral hippocampal Hcorr for each of the three scanning tasks was greater in patients than control subjects (2-way *t*-tests, all $p < .001$) as well as greater for MCI patients than high-risk control subjects (2-way *t*-tests, all $p < .01$). Across all subjects, bilateral hippocampal Hcorr for each of the three scanning tasks correlated with performance on tests of general cognition (MMSE and ADAS) as well as scores of immediate and delayed memory (Hopkins Verbal Learning Test; all $p < 0.01$). Conclusion: Local hippocampal functional heterogeneity as measured by Hcorr is informative to disease state even between high-risk healthy individuals and MCI patients, and measurement is robust to the task performed during functional scan acquisition. Further research and technique refinement is warranted to improve applicability in clinical settings.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.04/M10

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** AβO-mediated calcium disruption connects excitotoxicity to cell cycle re-entry and subsequent neuron death in Alzheimer's disease

**Authors: *E. J. KODIS**[1], S. CHOI[2], G. S. BLOOM[3]
[1]Biol., [2]Biochem., [3]Univ. of Virginia, Charlottesville, VA

**Abstract:** Excitotoxic neuron death caused by excess calcium influx is initiated by amyloid-β oligomers (AβOs) in Alzheimer's disease (AD), but the downstream signaling that results in neuron death remains less well understood. Ectopic neuronal cell cycle re-entry (CCR) is an early symptom of neuron dysfunction, ultimately leading to neuron death, rather than replication of neurons, accounting for substantial neuron death in AD. The present study tests the idea that excitotoxicity and the resulting synaptic dysfunction, in addition to being toxic in their own right, also contribute to the CCR pathway of neuron death. To test this, we first asked whether AβO-mediated calcium influx is necessary for CCR *in vitro* using primary cortical neurons. Pharmacologically blocking N-methyl-D-aspartate receptor (NMDAR) activity with memantine or MK-801, or knocking down the NMDAR subunit, NR1, blocks CCR. Furthermore, NMDAR inhibition by these same methods prevents AβO-stimulated activation of calcium-calmodulin-dependent protein kinase II (CaMKII), which mediates excitotoxicity and is necessary for CCR. Next, we tested whether treating neurons with a canonical excitotoxic insult would induce CCR. Exposing primary neuron cultures to a 30 second shock of 10 μM NMDA induces CCR in a manner similar to AβOs. Next, we wanted to recapitulate our *in vitro* results using the Tg2576 mouse model of AD. To test the role of AβO-mediated calcium influx *in vivo*, Tg2576 mice were treated with memantine, an FDA approved drug for the treatment of AD that gates the NMDA receptor. Memantine treatment in these mice blocks CCR compared to the untreated Tg2576 mice and WT controls. Together, these data imply a functional connection between NMDAR excitotoxicity and the CCR pathway in AD.

**Disclosures: E.J. Kodis:** None. **S. Choi:** None. **G.S. Bloom:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.05/N1

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

**Support:** NIH

**Title:** Alzheimer's disease-related dementias research programs at the National Institute of Neurological Disorders and Stroke

**Authors: J. T. GLADMAN**[1], S. JEON[1], D. BABCOCK[1], M. EMR[1], A. K. GUBITZ, Ph.D.[1], C. MOY[1], P. A. SCOTT[1], B.-A. SIEBER[1], M. SUTHERLAND[1], C. TORBORG[1], W. J. KOROSHETZ[2], *R. A. CORRIVEAU[1]
[1]NINDS/NIH, Rockville, MD; [2]NINDS/NIH, Bethesda, MD

**Abstract:** Goal 1 of the National Plan to Address Alzheimer's Disease is to prevent and effectively treat Alzheimer's disease and Alzheimer's disease-related dementias (AD/ADRD) by

2025. To inform the research agenda toward achieving this goal, the National Institute of Neurological Disorders and Stroke (NINDS) hosts periodic Summits that set and refine relevant research priorities for multiple etiology dementias, Lewy body dementia, frontotemporal degeneration, vascular contributions to cognitive impairment and dementia, collectively referred to as the Alzheimer's Disease-Related Dementias (ADRD). Here we introduce and outline several major new NINDS-led ADRD research programs supported by additional funds that first became available for AD/ADRD research during fiscal year 2016. Each of these programs represents a top research priority based on scientific progress and broad stakeholder input provided at the ADRD Summit 2016. The Center without Walls for the Identification and Validation of Molecular Mechanisms Contributing to Tau Pathogenesis (RFA-NS-16-023) is made up of interdisciplinary, multi-institute research that will lead to the identification and validation of molecular mechanisms that are relevant to human biology that contribute to tau toxicity associated with Frontotemporal Degeneration. The Lewy Body Dementias Biomarker initiative (RFA-NS-16-022) supports hypothesis-driven clinical research to discover biomarkers to improve the efficiency and outcome of clinical trials. Mechanistic Basis of Diffuse White Matter Disease in Vascular Contributions to Cognitive Impairment and Dementia (VCID) research (RFA-NS-16-021) will elucidate cellular and molecular mechanisms that underlie diffuse white matter disease of vascular origin that contribute to cognitive impairment and dementia. Finally, MarkVCID (RFA-NS-16-019; RFA-NS-16-020) is a consortium of US academic medical centers whose mission is to identify and validate biomarkers for the small vessel diseases of the brain that produce VCID. As additional resources become available, the NINDS will implement other top recommendations.

**Disclosures: J.T. Gladman:** None. **S. Jeon:** None. **D. Babcock:** None. **M. Emr:** None. **A.K. Gubitz:** None. **C. Moy:** None. **P.A. Scott:** None. **B. Sieber:** None. **M. Sutherland:** None. **C. Torborg:** None. **W.J. Koroshetz:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.06/N2

**Topic:** C.01. Brain Wellness and Aging

**Support:** Arizona Alzheimer's Consortium, Arizona Dept of Health Services

McKnight Brain Institute

**Title:** The impacts of family history of Alzheimer's disease and education on white matter integrity

**Authors: *N. J. GALLEGOS**[1], A. STICKEL[1], L. RYAN[2]
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**Abstract:** A family history of Alzheimer's disease (AD) has been shown to increase risk for the disorder beyond known genetic risk factors such as e4 status. Other factors, such as higher education, may decrease risk or delay the onset of AD. Our study investigated the effects of family history on white matter microstructure. Greater deterioration of white matter with age may serve as an indicator of AD-related pathology in preclinical stages of AD. Diffusion magnetic resonance imaging scans were obtained from 28 cognitively normal older adults with a family history of AD and 29 older adults without family history. Groups were matched on age, education, gender, and apolipoprotein e4 status. Fractional anisotropy (FA) measurements were computed using DTI-tk (http://dti-tk.sourceforge.net) in the uncinate fasciculus and the inferior longitudinal fasciculus (ILF) bilaterally, two regions susceptible to damage in the early development of AD (Smith et al., 2010). General linear models controlling for age, education, and gender found no differences in FA between the two groups. However, this relationship was moderated by education. Participants were split into two groups, high education (>14 years) and moderate-to-low education (≤14 years). Controlling for age and gender, individuals with a family history and moderate-to-low education had significantly lower FA values in both the uncinate fasciculus and ILF compared to those with family history and high education and those with no family history. These results suggest that education may protect white matter against the negative impact of risk factors for AD such as family history. Future analyses will investigate whether the interaction between family history and education is protective across the entire brain or in specific tracts (e.g., the uncinate and ILF), and whether other known risk factors such as cardiovascular health may also be moderated by education.

**Disclosures: N.J. Gallegos:** None. **A. Stickel:** None. **L. Ryan:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.07/N3

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

**Support:** NIRG-14-317353

NR015452

AG053193

**Title:** Altered resting-state brain complexity in alzheimer's disease: A multiscale entropy analysis

**Authors: *P. REN**[1], F. LIN[2]
[1]Univ. of Rochester, Rochester, NY; [2]Univ. of Rochester Med. Ctr., Rochester, NY

**Abstract: Objective:** Reduced complexity in multiple physiological systems is considered an aging sign. Alzheimer's disease (AD) is a progressive neurodegenerative disorder with aging. Using multiscale entropy (MSE) analysis, which measures brain complexity in different time scales, we examined change of brain complexity in AD associated neurodegeneration. **Methods:** Resting-state fMRI data and neuropsychological assessments from 62 HC, 81 amnestic mild cognitive impairment (aMCI), and 25 AD participants from the Alzheimer's Disease Neuroimaging initiative were used. A three-group comparison of 90 ROI from the Automated Anatomical Labeling (AAL) atlas was conducted to determine the optimal parameters for calculating MSE. Group comparison on whole brain voxel-wise MSE and correlations between MSE and neuropsychological assessments were then conducted. **Results:** We found two opposite MSE patterns in small (scale=1 and 2) and large time scales (scale=3 and 4) between groups. Applying the optimal parameters of MSE analysis (m=1, r=0.35, scale=2), significant changes of entropy was found in four brain regions, including hippocampus, precentral gyrus, intraparietal lobe, and superior frontal gyrus. Post-hoc analysis showed significant lower MSE in all the four regions in AD than HC, and significant lower MSE in hippocampus and precentral gyrus in MCI than HC. In addition, MSE in the four regions were positively correlated with Montreal Cognitive Assessment (MoCA), memory and executive function. **Conclusions:** The study revealed altered patterns of brain complexity in AD compared to normal aging, which depend on different time scales.

**Disclosures: P. Ren:** None. **F. Lin:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.08/N4

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** The effects of SDC3 and FGFRL1 on neurodegeneration in AD and PD

**Authors: *J. WANG**, W. SONG
UBC, Vancouver, BC, Canada

**Abstract:** Neuritic plaque, the pathological hallmark of Alzheimer's Disease (AD), is formed by extracellular deposits of amyloid β protein (Aβ) cleaved from amyloid β precursor protein (APP). Parkinson's Disease (PD) is featured by intracellular Lewy bodies (LBs), primarily consisted of aggregated alpha-synuclein protein (αSyn), encoded by SNCA gene. Both AD and PD are characterized by extensive neuronal loss within selected brain regions exemplified by

basal forebrain cholinergic dystrophy in AD and nigrostrital dopaminergic deficient in PD. In this study, we stably overexpressed human wildtype/ mutant APP and SNCA in the cholinergic SN56 cells and dopaminergic MN9D cells. The total RNA was extracted from eight stable cell lines and performed gene expression profiling by Illumina platform. Differential gene expression was analysed in APP-related stable cells and SNCA-related stable cells, separately. The microarray results showed that SDC3 gene was differentially expressed in SN56-$APP_{SWE}$ and MN9D-$APP_{SWE}$ cells, while FGFRL1 gene was differentially expressed in SN56-$SNCA_{A53T}$ and MN9D-$SNCA_{A53T}$. By conducting qt-PCR and immunoblotting, the results were consistent with the findings observed in microarray. In the $APP_{SWE}$ knock-in mice ($APP^{NL/NL}$) developed by Saito's group, the expression of SDC3 protein was significantly increased in medium septal (MS) with comparison to substantial nigra(SN) by immunoblotting. Knockdown of SDC3 gene showed protective effects in SN56-$APP_{SWE}$ cells under hydrogen peroxide-induced oxidative stress, but not in MN9D-APPSWE cells. Meanwhile, the expression of FGFRL1 protein was upregulated the in dopaminergic neurons instead of cholinergic neurons in the Prnp-$SNCA_{A53T}$ transgenic mice, which was detected by immunofluorescent staining. By knocking down of FGFRL1 gene in the SNCA-related stable cells, it only rescued cell death in MN9D-$SNCA_{A53T}$ cells under hydrogen peroxide treatment, but not in SN56-$SNCA_{A53T}$ cells. Taken together, differential expression of SDC3 and FGFRL1 in the cholinergic and dopaminergic neuron may mediate the selective neurodegeneration in $APP_{SWE}$-associated AD and $SNCA_{A53T}$-associated PD.

**Disclosures:** **J. Wang:** None. **W. Song:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.09/N5

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

**Support:** new investigator research grant (NIRG) Alzheimer Association

Sex and Gender in Alzheimer (SAGA) Alzheimer Association

center of excellence for transnational neuroscience and therapeutics seed grant

**Title:** Effect of app and tau pathology in humanized serotonergic and nonserotonergic neurons

**Authors: *A. P. REDDY**
Intrnl. Med., ttuhsc, Lubbock, TX

**Abstract:** The purpose of our research was to understand the role of comorbidity of depression in Alzheimer's disease (AD). Neurotransmitter, serotonin plays an important role in regulating

midbrain pathology in AD. However, molecular links between serotonin regulation and AD progression are not completely understood. To achieve our objective, we stably transfected rat serotonergic (5HT) cell lines RN46A and B14, and non-serotonergic IMR32 and SHSY5Y cells with mutant APP (Swiss) and WT APP and mutant Tau (P308L) and WT Tau cDNAs. Stably transfected and un-transfected cells were studied for mitochondrial biogenergetics, apoptosis, cell viability and mRNA and protein levels of mitochondrial, AD, serotonin, and synaptic genes. Using immunocytochemistry, we studied localization of synaptic and mitochondrial proteins and mechanism of serotonin in AD pathology. Using sandwich ELISA, we measured amyloid beta (Aβ) 40 and 42, Serotonin, beta and alpha secretase levels in transfected and un-transfected cells. Using transmission electron microscopy, we also studied ultra-structural changes. Preliminary results of our study revealed that significantly increased cell apoptosis, reduced cell viability in stably transfected cells with mutant APP and mutant Tau cDNAs relative to un-transfected cells. Serotonin neurons have never been studied under the effect of APP/Tau pathology so this will be first studies in *in vitro* mid brain neurons. Based on these observations, we conclude that RN46A, B14 and non-serotonergic cell lines IMR32 and SHSY5Y are unique cell models to study mechanisms involving the serotoninergic system in AD pathology. These stable cell lines will be useful for drug screening in AD.

**Disclosures: A.P. Reddy:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.10/N6

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

**Support:** NIH NS085770

NIH DC008552

NIH AG13854

**Title:** Loss of basal forebrain cholinergic neurons in primary progressive aphasia with Alzheimer pathology

**Authors:** N. LALEHZARI[1], ***D. T. OHM**[2], F. RAHMANI[1], G. KIM[3], S. WEINTRAUB[4], E. BIGIO[1], M.-M. MESULAM[5], C. GEULA[6]
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Alzheimer's Dis. Ctr., Chicago, IL; [6]Cogn Neurol & Alzhei Dis Cent, Northwestern Univ. Med. Sch., Chicago, IL

**Abstract:** Primary progressive aphasia (PPA) is a neurological disorder that typically presents with a progressive loss of language capabilities. Approximately 30-40% of PPA cases present with Alzheimer's disease pathology (PPA-AD), with an asymmetric distribution favoring the cortical language network in the dominant hemisphere (usually left). The typical amnestic variant of AD is characterized by significant loss of basal forebrain cholinergic neurons (BFCN). However, the status of BFCN in PPA is unknown. The purpose of this study was to determine the presence, extent and hemispheric asymmetry of BFCN loss in PPA-AD. Tissue from five PPA-AD and three normal control participants were used in this experiment. Sections from basal forebrain containing BFCNs were stained immunohistochemically for low affinity neurotrophin receptor (p75), which is specifically enhanced in BFCN. Unbiased stereological counting was used to estimate changes in the number of p75-positive BFCN in PPA-AD. The number of BFCN in the language-dominant hemisphere of PPA-AD participants was 35% lower when compared with the right hemisphere ($p<0.008$). The left hemisphere of the PPA-AD brains also contained 73% fewer BFCN than the left hemisphere of control brains ($p<0.02$). Examination of Nissl stained material corroborated loss of magnocellular, hyperchromic BFCN in PPA-AD participants. Qualitatively, BFCNs in PPA-AD participants contained substantial densities of tangles and pre-tangles as revealed by thioflavin-S staining and abnormally phosphorylated tau (AT8) immunostaining. These findings indicate that, like typical amnestic AD, PPA-AD is characterized by substantial degeneration of basal forebrain cholinergic neurons. They also suggest that cholinesterase inhibitors used as therapeutic agents in AD are likely to also be effective in PPA-AD.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.11/N7

**Topic:** C.01. Brain Wellness and Aging

**Support:** University of Hartford College of Art and Sciences

**Title:** Can probiotics alter the progression of Alzheimer's disease?

**Authors: *K. MCMURRY**[1], M. STANLEY[1], A. SILVER[1], P. SACCHETTI[2]
[1]Biol., [2]Biol. Sci., Univ. of Hartford, West Hartford, CT

**Abstract:** The gut microbiome consists of trillions of microorganisms that include bacteria, archaea, viruses and eukaryotes. Previous studies have shown that this diverse community communicates with the central nervous system (CNS) via a bidirectional pathway termed the gut-brain axis. Recent research concerning the gut-brain axis has implicated microbiome dysregulation in a variety of pathological states, including neurodegenerative disorders such as Alzheimer's disease (AD). So far, research has shown characteristic pathological dysbiosis (microbial imbalance), but probiotic treatment as a therapy has not been thoroughly explored. In our study, we sought to correct the dysbiosis with a probiotic diet in an AD mouse model, with the hope of exploring the potential benefits of an easily accessible therapeutic. Transgenic APPSWE-Tau301 mice were fed a probiotic diet (*Lactobacillus plantarum KY1032* and *Lactobacillus curvatus HY7601*) for 12 weeks, during which cognitive functions were evaluated via the Barnes Maze during the final 5 weeks of the study. Our data show that AD mice on probiotics performed comparably to wild-type controls, while AD mice on a regular diet showed deficiencies. To determine if the improved cognitive functions were reflective of decreased tissue damage, brains were collected from all animals at the end of the probiotic treatment, and several CNS cell populations were analyzed using immunohistochemistry. Number of total cells ($DAPI^+$), neurons ($NeuN^+$) and astrocytes ($GFAP^+$) were assessed first in the cortex. Preliminary data show that AD mice on control diet have an approximate 50% decrease in total cell numbers and an increased percentage of $GFAP^+$ cells. These changes are mitigated in the AD probiotic treatment group, returning cell numbers close to wild-type controls' level. Ongoing studies are assessing these cell populations in other brain regions, as well as determining the CNS inflammatory state in these animals via analysis of astrocytic and microglial reactivity. Our results suggest that an alteration of the microbiome could be effective in delaying or mitigating neurodegeneration and improving cognitive abilities in this AD animal model. It is vital to explore new possibilities for palliative care of Alzheimer's disease, and a supplementary probiotic diet could provide an inexpensive and easily implemented clinical treatment.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.12/N8

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** CONACYT -CIENCIA BASICA 241009

**Title:** Arsenic exposure aggravates neurobehavioral deficits, amyloid and tau pathology in a triple transgenic model of Alzheimer´s disease

**Authors: *S. A. ESQUIVEL NIÑO**[1], E. CHI-AHUMADA[2], A.-R. AGUILAR-VÁZQUEZ[3], M. MARTEL-GALLEGOS[1], R. SALGADO-DELGADO[4], S. DIAZ-CINTRA[3], M. JIMENEZ-CAPDEVILLE[2], S. ZARAZUA[1]
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**Abstract:** Worldwide, every year there is an increase in the number of people exposed to inorganic arsenic (iAs) via drinking water. Human populations present impaired cognitive function as a result of prenatal and childhood iAs exposure, while studies in animal models in similar conditions demonstrate neurobehavioral deficits accompanied by protein and enzyme alterations associated to Alzheimer´s disease (AD). In primary cultures of Tg2576 mouse brain, iAs promotes the production of βA and decreases the activity of α -secretase. In order to determine whether arsenic promotes the pathophysiological progress of AD, we used the 3xTg-AD mouse model since it mimics the development of amyloid plaques, neurofibrillary tangles and behavioral dysfunction. Male and female 3xTg-AD mice (25-30 g) were divided into 2 groups (n=7): 1) control without arsenic; and 2) exposed to 3 ppm sodium arsenite in drinking water. Animals received the treatment from gestation until 6 months. We investigated the behavior phenotype on a test battery including locomotor behavior, Morris water maze (MWM) and contextual fear conditioning. Immunohystochemical studies were performed to detect AD markers. 3xTg-AD mice exposed to iAs presented alterations in their circadian rhythm, specifically, males showed higher locomotor activity during the day and shorter free running periods prior to the onset of AD-pathology. Females had a slightly decrease in activity levels during their typical active phase compared to controls. Longer escape latencies and higher number of failures to reach the platform were found in the iAs group than in the control group in the MWM. Short term memory was assessed by means of contextual fear conditioning. Exposed animals exhibited a significant difference in freezing time compared to the control group. Immunopositivity to amyloid and tau markers in sections of hippocampus and frontal cortex resulted in meaningful effects of the treatment in both regions. Significantly higher inmunoreactivity to amyloid isoforms particularly in the cortex and CA3, as well as desestabilized microtubules in CA1-hippocampus at the 6$^{th}$ month of age were found in the iAs group as compared to the control group. In summary, iAs prenatal and life-long exposure results in contextual and spatial memory decline. Furthermore, arsenic enhances the development of amyloid pathology. With less significant effect on tau pathology. These results are consistent with classical pathway of amyloid cascade hypothesis. These findings confirm that iAs exposure induces cognitive deficits and degenerative markers in the central nervous system that are characteristic of AD.

**Disclosures: S.A. Esquivel Niño:** 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.; 241009. **E. Chi-Ahumada:** None. **A. Aguilar-Vázquez:** None. **M. Martel-**

**Gallegos:** None. **R. Salgado-delgado:** None. **S. Diaz-Cintra:** None. **M. Jimenez-capdeville:** None. **S. Zarazua:** 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.; 241009.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.13/N9

**Topic:** C.01. Brain Wellness and Aging

**Title:** *In vivo* calcium imaging of hippocampal neuronal network activity associated with memory behavior deficits in the Alzheimer's disease mouse model

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**Abstract:** Alzheimer’s disease (AD) causes progressive deficits in memory and cognitive function, and it has emerged as a major health and socio-economic concern in the US and worldwide. In order to develop more effective therapeutic treatments, we need to further understand neural mechanisms underlying Alzheimer’s disease. Toward this goal, we study large-scale hippocampal CA1 neuronal network activation and memory behavior deficits in a well-established model for AD, the 3xTg-AD mouse model which presents amyloid plaques and neurofibrillary pathology. Stereotaxic injection of AAV-Camk2a-GCaMP6f is used to selectively express GCaMP6 in CA1 excitatory neurons in AD and control mice. A new object-location based task to assess learning and memory updating is coupled with simultaneous *in vivo* GCaMP6-based calcium imaging of hippocampal CA1 neuronal populations using miniature microscopes placed on freely moving mice. Compared with control mice, our preliminary data analysis indicates that AD mice of 4 months and older appear to have major deficits in memory updating, as reflected by discrimination measurements. We are able to track the activation of more than one hundred of the same CA1 neurons in each mouse across days of behavior training and testing using *in vivo* microscopic Ca2+ imaging. In marked difference from control mice, CA1 neuronal responses in the AD mice are far more heterogeneous in calcium response dynamics and signal pattern. Based on these initial results, we conclude that differential neuronal activity patterns in AD and control mice are associated with their memory behavior outcomes, and that hippocampal CA1 networks in AD mice have alterations in organizing spatially correlated activities likely due to AD histopathology.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.14/N10

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH Grant 1R01AG042890

**Title:** Oxidative stress and antioxidant response in frontal cortex of demented and non-demented individuals with Alzheimer's neuropathology

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**Abstract:** Alzheimer’s disease (AD) is the most common form of dementia characterized by progressive neurodegeneration affecting specific brain areas, particularly frontal cortex. Histopathological features include extracellular senile plaques, composed of amyloid β (Aβ) polymers, and intracellular neurofibrillary tangles, formed by hyperphosphorylated Tau protein. Several mechanisms have been put forward to explain the pathogenic events occurring in AD, among which the so-called amyloid cascade, involving a critical role of Aβ peptide. However, the accepted correlation between Aβ and dementia has recently been challenged by the emergence of a group of individuals, referred to as "Non-Demented with Alzheimer’s Neuropathology" (NDAN), showing severe neuropathological signs, though remaining cognitively intact. The existence of these individuals suggests that some unknown mechanisms are triggered to resist the detrimental events, normally leading to cognitive impairment in AD. Such mechanisms would not impede Aβ overproduction or aggregation, but possibly prevent neurotoxic effects of the peptide. Noteworthy, Aβ accumulation affects mitochondrial redox balance increasing oxidative stress status, which has long been recognized as a major trait of the disease and been proposed as a primary culprit in AD pathogenesis. To clarify the relationship linking Aβ, oxidative stress and cognitive impairment, we performed a comparative immunofluorescence study on AD, NDAN and normally aged human *post-mortem* frontal cortices. To assess the precise localization of oxidative damage at a cellular level, we evaluated the occurrence of 8-oxo-dG marker in neuronal and astroglial cells. We also investigated the expression of SOD2, PGC1α and PPARα as key factors in antioxidant response and energy metabolism. This study allowed us to confirm the crucial role of redox imbalance in AD pathogenesis, at least due to an impairment of antioxidant defences. By the contrast, the analysis

of NDAN individuals proved especially enlightening, in clarifying the role of regulators of antioxidant response pathways. Indeed, in NDAN subjects, the low oxidative damage, correlated with the high content of scavenging systems, suggests the ability to activate an efficient PGC1α-dependent "safety mechanism" to cope with oxidative imbalance, thus preventing Aβ-mediated detrimental effects. The analyses conducted in a comparative manner in neurons and astrocytes further highlighted cell-specific mechanisms to counteract redox imbalance. These emerging concepts may help envisioning neuroprotective therapies aimed at ameliorating defects in antioxidant response in AD patients.

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**Poster**

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Göran Gustafsson Foundation, Stockholm, Sweden (HSS),

India-EU Co-operation Program (RP/AS/HSS)

IT 794/13 (JVL), Government of Basque Country

**Title:** Traumatic brain injury induced amyloid beta peptide and tau is aggravated at high environmental temperature. Nanowired delivery of anti-tau antibodies and cerebrolysin induces marked neuroprotection

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**Abstract:** Military personnel are often exposed to high environmental temperature in desert areas during combat operations. Since traumatic brain injury (TBI) is quite common in combat operation, a possibility exists that hot environment (HE) could affect pathophysiology of TBI. However, effects of HE on TBI are not well known. Previous reports show that hyperthermia duirng TBI could worsen the pathological outcome. However, TBI induced pathophysiological outcome following HE still require further investigation. TBI induces increase in amyloid beta peptide (AbP) and tau protein in the brain and in the cerebrospinal fluid (CSF). AbP and tau are responsible for neuronal, glial and microvascular damages leading to breakdown of the blood-brain barrier (BBB) and vasogenic edema formation. However, effects of HE on AbP and tau are unknown. In present investigation we examined whether TBI could induce an increased production of AbP and tau in HE. Furthermore to understand the role of tau in neuropathology we infused tau antibodies [Anti-Tau antibody [E178, ab32057) together with cerebrolysin (a known neuroprotective agent) in the CSF after TBI with HE. TBI was inflicted by dropping a weight of 114.6 g from 20 cm height on the exposed parietal skull bone in Equithesin anaesthetized rats acclimatized at 21±1°C (room temperature) or exposed to 34°C for 4 h per day for 2 weeks in biological oxygen demand incubator (BOD, relative humidity 45-47 %, wind speed 20-25 cm/sec). Exposure to HE alone did not result in BBB breakdown, edema formation or changes in AbP or tau levels. However, TBI in HE animals resulted in about 2-to 3-fold higher breakdown of the BBB to Evans blue albumin and radioiodine ($^{[131]}$-I) and neuronal, glial and axonal damage as compared to TBI in rats at room temperature after 24 trauma. The AbP and tau levels in TBI group after heat exposure increased by e- to 6-fold in the CSF (control AbP ng/ml 0.23±0.04; TBI 0.82±0.05; TBI+HS 2.34±0.12; Control tau pg/ml 20±2; TBI 34±6; TBI+HS 76±8). Treatment with H-290/51 (100 mg/kg, i.p.) together with 50 µl 1:20 tau antibodies i.c.v. 4 h after injury resulted in reduction of tau and AbP levels and brain pathology in TBI but failed to induce neuroprotection in HE group. However, when TiO2-nanowried H-290/51 was co-administered with nanowired tau antibodies in identical conditions pronounced neuroprotection was observed in HE group with TBI. Also the tau and AbP levels were considerably reduced (AbP ng/ml 0.43±0.08; tau pg/ml 28±7) in the CSF of HE rats after TBI. This indicates that both AbP and tau levels are somehow responsible for enhanced brain pathology in TBI following HE, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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**Title:** Alzheimer's disease neuropathology is exacerbated following traumatic brain injury. Neuroprotection by co-administration of nanowired mesenchymal stem cells and cerebrolysin

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**Abstract:** Military personnel are often prone to traumatic brain injury (TBI) that enhances the possibility of Alzheimer’s disease (AD) at a later stage. Since TBI leads to breakdown of the blood-brain barrier (BBB) and extravasation of serum proteins into the brain fluid compartment, it is quite likely that plasma amyloid beta protein (AbP) may enter into the brain after TBI leading to development of AD. Thus, there is a need to understand the role of TBI in AD. AD like brain pathology was induced by intracerebroventricular (i.c.v.) administration of soluble form of AbP 200 ng/30 µl per day into the left lateral ventricle for 4 weeks in a rat model. This treatment resulted in AD like pathology e.g., deposit of AbP in the brain as well as BBB breakdown, edema formation and neuronal, glial and axonal injuries. In order to find out role of TBI in AD development, rats were subjected to mild concussive head injury (CHI) by dropping a weight of 114.6 g over the exposed parietal skull bone from a 20 cm height through a guide tube. This adjustment resulted in an impact of 0.224 N over the skull surface. In these CHI inflicted animals AbP was infused in identical conditions starting from 1 week after injury for 4 weeks.

Our observations show that AbP infusion in CHI rats exacerbated BBB breakdown to serum proteins by 2-4 fold, edema formation by 1.5 to 2 fold and marked increase in neuronal, glial or axonal injuries as compared to AbP treatment in normal animals. Immunohistochemical examination revealed enhanced deposits of AbP in the brain in CHI group after AbP infusion. The glial reactions and myelin damages were also much more aggravated. Extravasation fo albumin was also increased in several brain regions in CHI group after AbP infusion as compared to normal animals. Administration of mesenchymal stem cells (MSCs, ca. 1 million, i.c.v.) 1 week after AbP infusion resulted in marked neuroprotection as seen by reduced BBB leakage, AbP deposits and brain pathology in normal animals. Likewise i.c.v. administration of 50 µl cerebrolysin daily for 3 weeks starting from 1 week after AbP infusion was neuroprotective in normal animals. However, in CHI group these treatments either alone or in combination were ineffective. Interestingly when TiO2 nanowired MSCs and cerebrolysin administered together in identical conations, significant neuroprotection was achieved in AD cases in CHI group. Taken together, our observations are the first to point out that co-administration of MSCs and cerebrolysin using nanowired delivery has far more superior neuroprotective effects in AD model in CHI, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.17/O1

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Hypusinated eIF5A governs TDP-43 accumulation in the cytoplasm and stress granules

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**Abstract:** TDP-43 pathology is involved in impairment of motor neuron function and cognitive dysfunction in a spectrum of disorders. The hallmark of TDP-43 proteinopathy is loss of nuclear function and accumulation as cytoplasmic inclusions. Recent evidence suggests for unique accumulation of TDP-43 in stress granules as disease progress. Hypusination of eIF5A (eIF5AhypK50) denotes its activation and cytoplasmic localization where it also binds various RNA binding proteins. Its overall cellular function is translational elongation but translation inhibition in stress granules (SG) might also occur. The localization of eIF5AhypK50 in SG lead to further accumulation of TDP-43 and translation of aberrant truncated forms causing TDP-43

seeding and aggregation once SGs are dissolved. Our data demonstrate increases in the eIF5AhypK50 levels and expression levels of enzymes involved in hypusination pathway in AD brain tissue as well as in TDP-43 animal models, suggesting implication to the disease progression. We show that both pharmacological inhibition and siRNA approaches targeting hypusination pathway reduce cytoplasmic accumulation of total TDP-43 and its phosphorylation in various cellular models. The same inhibitor effectively reduces TDP-43 accumulation in SG as demonstrated by immunocytochemical assays. We hypothesize that eIF5AhypK50 orchestrates the fate of TDP-43 and determines the biological signature of SGs. As the exact mechanism by which eIF5AhypK50 interacts with TDP-43 is unknown our data strongly suggests for protein-protein binding properties between both proteins, hence promoting TDP-43 cytoplasmic accumulation. However, eIF5AhypK50 could regulate TDP-43 fate via several other potential mechanisms, including translational regulation. Utilizing SUnSET assay (surface sensing of translation), we demonstrate that eIF5A inactivation reduces the overall protein synthesis rate in cells, whether this has a direct impact on TDP-43 proteinopathy remains to be investigated. Overall, we demonstrate that inhibition of hypusination either by reducing eIF5A levels or specific enzymes reduce TDP-43 burden and provide the foundation for detailed understanding of eIF5A role in neurological diseases and viable therapeutic approaches.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Ministry of Science & Technology, Govt. of India (HSS/AS),

**Title:** Gold nanoparticles (AuNPs) exacerbate spinal cord injury induced amyloid beta peptide and tau production, cord pathology and functional disturbances. Neuroprotection by nanodelivery of cerebrolysin

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**Abstract:** Military personnel in combat operation often inflicted with brain and/or spinal cord injury (SCI) causing lifetime disability. Thus, exploration of novel treatment strategies and factors affecting the functional or pathological outcome after SCI require further investigations. Recently, gold nanoparticles (AuNPs) are used for the treatment of neurodegenerative diseases. However, AuNPs induced neurotoxicity is not well known. Our previous studies show that AuNPs depending on the size and route of administration induces neurotoxicity in normal rats. Thus, AuNPs for therapeutic strategies needs further investigation in vivo models. Several reports suggest that trauma to the brain results in upregulation of amyloid beta peptide (AbP) and tau proetins responsible for neuronal, glial and axonal injuries. However, increased AbP and tau following SCI is not well known. Few reports indicate that AuNPs therapy reduce AbP and tau protein accumulation in Alzheimer's disease (AD) however, the matter is still controversial. We examined SCI induced changes in cerebrospinal fluid (CSF) concentrations of AbP and tau and their modification with AuNPs in a rat model. In addition, we also investigated the influence of nanodelivery of cerebrolysin (CBL)- a multimodal neuroprotective drug in SCI. SCI was inflicted in Equithesin anaesthetized rats by making a longitudinal lesion on the right dorsal horn of the T10-11 segments (2 mm deep and 4 mm long) in normal animals or in rats intoxicated with AuNPs (50-60 nm, 50 mg/kg, i.p. once daily for 7 days). Laminectomized animals served as controls. A sample of CSF (30 µl) was withdrawn in all groups after cisterna magna puncture 24 h after injury and analyzed for AbP and tau using commercial ELISA protocol. Blood-spinal cord barrier (BSCB) breakdown to Evans blue albumin and neuronal injuries were assessed in T9 and T12 segments. SCI in AuNPs intoxicated group exhibited about 180 to 230 % increase in AbP and 220 to 256 % elevation of tau as compared to saline treated SCI rats. The BSCB breakdown and cord pathology was also 2-to 3-fold increased in AuNPs group from saline treated injured group. Intrathecal administration of CBL (50 µl) 4 and 8 h after SCI significantly reduced AbP and tau increase and cord pathology in saline treated group. However, TiO2-nanowired delivery of CBL is needed to reduce AbP, tau and cell injuries in AuNPs treated injured group. These observations are the first to show that AuNPs aggravate AbP and tau production after SCI and cord pathology. TiO2-nanowired delivery of CBL is capable to induce neuroprotection by reducing these peptides following SCI, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

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The University Grants Commission, New Delhi, India (HSS/AS),

**Title:** Methamphetamine exacerbates traumatic brain injury at high altitude. Neuroprotective effects of TiO2 nanodelivery of antioxidant compound H-290/51

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**Abstract:** Military personnel are often exposed to high altitude (HA) for combat operations. Staying at HA alone causes neurological dysfunctions. We showed that traumatic brain injury (TBI) at HA (ca. 4500 to 5000 m) aggravates brain pathology. People living at HA often consume methamphetamine (METH) or cocaine to cope HA stress. METH alone at HA induces neuronal, glial and axonal damages. Thus, METH exposure could aggravate TBI induced brain pathology. We explored the effects of METH exposure on TBI at HA on brain pathology in a rat

model. Animals exposed to HA in a hypobaric chamber equivalent to 5000 m (53.8 kPa) for 1 week. TBI was inflicted under Equithesin anesthesia by dropping a weight of 114.6 g from 20 cm height on right parietal skull (impact of 0.224 N). Control animals received identical TBI at sea level (200m). Another group of rats received 9 mg/kg, s.c. METH and TBI was inflicted 4 h after either at HA or at sea level. After 8, 12 and 24 h after TBI in METH or saline group, blood-brain barrier (BBB) breakdown, edema formation and neuronal, glial and myelin injuries were examined. Our results showed that TBI at HA in METH group induced about 2- to 3-fold higher BBB breakdown to Evans blue albumin (EBA) and radioiodine ([131]-I) as compared to identical TBI in saline treated animals. Interestingly, TBI at sea level induced only 0.5-to 1.5-fold greater BBB damage in METH group as compared to saline controls. This indicates that METH exposure at HA is more dangerous for TBI than at sea level. Reductions in cerebral blood flow (CBF) were 70 to 90 % greater in METH exposed group after TBI at HA as compared to saline controls after TBI. Brain edema formation was 4 to 6 % higher in METH exposed HA rats after TBI than the saline treated traumatized animals. On the other hand, METH exposed TBI group at sea level showed only 40 to 60 % higher reduction in the CBF and 2 to 3 % greater brain edema formation as compared to saline treated TBI group. Neuronal, glial and myelin damages were also exacerbated in HA rats in METH exposed group after TBI as compared to identical TBI in METH exposed rats at sea level. In general, the magnitude and intensity of TBI in METH or saline treated rats at HA or sea levels were progressive in nature. Treatment with antioxidant H-290/51 (100 to 150 mg/kg, i.p.) was able to thwart brain pathology in TBI either at HA or sea levels. However, TiO2-nanowired delivery of H-290/51 (150 mg/kg, i.p.) is needed to reduce brain pathology in METH exposed rats after TBI at HA or sea level. Taken together our observations are the first to point out that METH exposure in TBI is exacerbated at HA probably due to greater production of oxidative stress as compared to sea level, not reported earlier.

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**Poster**

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IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain

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**Title:** Traumatic brain injury exacerbates Parkinson's disease neuropathology. Neuroprotective effects of co-administration of TiO2 nanowired mesenchymal stem cells and cerebrolysin

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**Abstract:** Military personnel are often subjected to traumatic brain injury (TBI) during combat operations. There are evidences that TBI is one of the predisposing factors in causing development of Parkinson's disease (PD) at a later stage. This is largely due to the fact that mild or moderate TBI induces rapid production of tau protein and alpha synuclein (ASNC) that could be seen in various brain areas as well as in the cerebrospinal fluid (CSF). Enhanced tau-phosphorylation and associated ASNC leads to profound cellular stress and alter molecular machinery of the brain leading to brain pathology. Recent evidences show that upregulation of constitutive isoform of hemeoxygenase (HO-2) is elevated in patients of PD that correlates well with the brain pathology. Thus, it would be interesting to examine whether TBI could exacerbate PD pathology in relation to tau, ASNC and HO-2 expression. Since trophic factors and stem cells are known to reduce brain pathology in stroke and trauma, we examined the role of mesenchymal stem cells (MSCs) and cerebrolysin (CBL), a well-balanced composition of several neurotrophic factors and active peptide fragments on brain pathology in PD model after TBI. PD like symptoms were produced in mice by administration of 1-metyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg, i.p.) daily within 2-h intervals for 5 in normal animals or following TBI. Mild TBI was induced by dropping a weight of 28.6 g from 20 cm height on the right parietal skull bone (impact 0.056 N). On the $8^{th}$ day after MPTP administration brain pathology was examined. Our observations showed a 2-4-fold greater increase in the blood-brain barrier (BBB) breakdown to Evans blue albumin and radioiodine ([131]-I) in several brain areas in PD group after TBI. Immunohistochemical studies showed marked higher upregulation of HO-2 in the PD brain after TBI in areas exhibiting neuronal and glial cell injuries than PD group alone. Biochemical measurement of tau protein and ASNC also showed greater accumulation in the brain and CSF samples of TBI cases in PD group. Treatment with MSCs ($10^6$ cells and CBL 2.5 ml/kg, i.v.) 5 days after MPTP administration resulted in marked neuroprotection in normal

PD group and reduced HO-2 expression, tau protein and ASNC levels. However, TiO2-nanowired delivery of MSCs and CBL in identical doses is needed to reduce brain pathology and biochemical changes in PD group after TBI. These observations are the first to show that TBI exacerbates PD pathology and nanowired delivery of co-administration of MSCs and CBL has superior neuroprotective effects as compared to either agent alone, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.21/O5

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** CIHR Grant MOP123448

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GRSC

**Title:** Astrocyte-produced lipoxins protect retinal neurons from acute and chronic stress

**Authors: *I. LIVNE-BAR**[1], J. WEI[4], H.-H. LIU[5], S. ALQAWLAQ[2], K. GRONERT[4], J. G. FLANAGAN[6], J. M. SIVAK[3]
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**Abstract:** Astrocytes and related Müller glia play critical homeostatic roles in the inner retina and become activated upon retinal disease or injury. However, astrocytes' role in protecting retinal neurons under stress remains unclear. We previously published that resting retinal astrocytes promote retinal ganglion cells (RGC) survival following acute injury *in vivo*, and that inducing stress in retinal astrocytes curtails RGC survival. Here, we report that intravitreal injection of astrocyte conditioned media (ACM), but not control media, promoted RGC survival following acute insult. Size fractionation of the ACM indicated that a small-molecule component contained prominent protective activity. We analyzed the ACM by LS-MS/MS based lipidomics and detected high enrichment of the lipoxins $LXA_4$ and $LXB_4$. Lipoxins are part of a family of potent specialized pro-resolving lipid mediators (SPMs). The protective activity of these lipoxins was tested by intravitreal injection prior to induction of retinal insult. RGC survival was quantified by immunofluorescence microscopy. We found that lipoxin injection reduced RGC loss from acute injury with $LXB_4$ being more protective than $LXA_4$. Furthermore, inhibition of

the key enzyme for lipoxin biosynthesis compromised RGC survival while an antagonist against the $LXA_4$ receptor also reduced survival. Finally, therapeutic $LXB_4$ treatment in a chronic RGC-degeneration model significantly rescued RGC function and promoted their survival. In summary, we have established that astrocyte produced lipoxins protect retinal neurons from acute and chronic injury.

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**Poster**

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**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Göran Gustafsson Foundation, Stockholm, Sweden (HSS),

Ministry of Science & Technology, Govt. of India (HSS/AS),

**Title:** Intraspinal administration of TiO2-nanowired mesenchymal stem cells and cerebrolysin improved functional outcome and induces neuroprotection following spinal cord injury

**Authors: *L. FENG**[1], A. SHARMA[2], D. F. MURESANU[3], J. V. LAFUENTE[4], A. OZKIZILCIK[5], R. TIAN[6], R. PATNAIK[7], H. MOESSLER[8], A. TRIPATHI[9], H. S. SHARMA[2]
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**Abstract:** Military personnel are often victims of spinal cord injury (SCI) for which there is no suitable treatment available. Thus, exploring new therapeutic avenues using select combination

of novel neuroprotective agents is the need of the hour to improve the quality of life of SCI patients. Previous experiments from our laboratory show that a focal SCI inflicted in rat by making an incision of the right dorsal horn of the T10-11 segment resulted in pronounced functional disability on Tarlov Scale, inclined plane angle test and walking on a mesh grid in a progressive manner after 12 and 24 h trauma. This behavioral dysfunction correlated well with breakdown of the blood-spinal cord barrier (BSCB), edema formation and cell injuries seen in both the rostral and caudal segments after SCI in a progressive manner. Recording of spinal cord evoked potentials using epidural electrodes at rostral and caudal to lesion segments exhibited significant increase in latency and amplitude changes indicating loss of spinal cord conduction. Several lines of evidences suggest that intraspinal administration of mesenchymal stem cells (MSCs) improves functional outcome and enhance spinal cord conduction. However, MSCs induced restoration of BSCB and reduction in edema formation is not well known. In present investigation we examined the role of MSCs alone or in combination with cerebrolysin (CBL)- a multimodal drug on SCI induced restoration of cellular and behavioural functions in a rat model. SCI was inflicted in Equithesin anesthetized rats over the right dorsal horn of the T10-11 segments (2 mm deep and 4 mm long) and the animals were allowed to survive 12 or 24 h after trauma. In separate groups of SCI rats MSCs ($10^6$ cells) and CBL (50 µl) were administered into the rostral and caudal spinal cord around the lesion site after 3 h injury using a 100 µl Hamilton syringe connected to a constant infusion pump (10 µl/min). Since nanodelivery of MSCs or CBL has superior neuroprotective effects in CNS injury, we also examined TiO2 nanodelivery of MSCs and CBL in SCI. Our observations showed that co-administration of MSCs and CBL significantly reduced BSCB breakdown, edema formation and cell injuries at 12 h but not at 24 h after SCI. In this group significant improvement on behavioral function was seen after 12 h SCI. However, when TiO2-nanodelivery of MSCs and CBL was done in identical conditions, these treatment strategies significantly attenuated cord pathology and improved behavioral dysfunctions after 24 SCI. These observations are the first to show that intraspinal administration of nanowired MSCs and CBL have superior neuroprotective effects in SCI, not reported earlier.

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**Poster**

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Swedish Medical Research Council (Nr 2710-HSS),

Swedish Strategic Foundation, Stockholm, Sweden (HSS/AS)

Ministry of Science and Technology of the People's Republic of China

Göran Gustafsson Foundation, Stockholm, Sweden (HSS),

**Title:** Melanocortin receptors modulate spinal cord pathophysiology. An experimental study in the rat using co-administration of melanocortin receptor 4 agonists and an antioxidant compound H-290/51 for superior neuroprotection

**Authors: *A. K. PANDEY**[1], A. SHARMA[2], R. PATNAIK[3], D. F. MURESANU[4], J. V. LAFUENTE[5], A. NOZARI[6], A. OZKIZILCIK[7], R. TIAN[8], P.-O. SJOQUIST[9], L. FENG[10], H. S. SHARMA[2]

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**Abstract:** Previous reports from our laboratory showed neuroprotective effects of melanocortin (MC) receptor 4 agonist on the pathophysiology of spinal cord cell and tissue injury as well as on the functional outcome. Topical application of MC compounds 1 µg to 10 µg over the traumatized cord after 5 to 10 min results in significant improvement in Tarlov scale up to 3 h. Nanodelivery of these compounds with or without dexamethasone applied topically did not induce any further functional improvement beyond 3 h. High doses of dexamethasone (100 µg) together with MC-4 agonist (20 µg) shows some late beneficial effects on Tarlov scale at 10 to 12 h SCI. This suggests that MC receptor agonists are neuroprotective and potentiate the ability of dexamethasone. In present investigation we applied MC-4 agonist or antagonist or MC1 receptor antagonist with partial agonist of MC3 and MC5 receptors in SCI on cord pathology and functional outcome. In addition we also co-administered TiO2 nanowired H-290/51 systemically (50 mg/kg, i.p.) and examined any potentiation of MC compounds on the outcome following SCI. SCI was inflicted in Equithesin anesthetized rats by making a longitudinal incision of the right dorsal horn of the T10-11 segments. Melanocortin $MC_4$ receptor antagonist ML 00253764 or potent and selective $MC_4$ receptor agonists PG931 or THIQ or potent $MC_1$ receptor antagonist with $MC_3$ and $MC_5$ partial agonist MSG 606 were used in this study. MC compounds were applied 5 or 10 µg over the traumatized cord 5 min after injury with or without H-290/61 (50 mg/kg, i.p.) 30 min after SCI. MC 4 receptor agonists and MC 3 and MC 5 receptor agonist with

MC1 antagonist were the most effective in neuroprotection and improved Tarlov scale up to 5 h afire SCI. When given in combination with H-290/51 functional improvement was extended beyond 8 h and neuroprotection was seen up to 12 h after injury. However TiO2 nanowired delivery of PG931 or THIQ and MSG606 together with H-290/51 in SCI resulted in functional improvement up to 12 h and reduced cord pathology at 24 h. MC4 receptor antagonist did not induce neuroprotection or improved functional parameters either given alone or together with H-290/51 in SCI. These observations provide new evidences on MC 4 receptor agonists and MC3 and 5-receptor agonist with MC1 antagonist are powerful in inducing neuroprotection in SCI that was further potentiated by antioxidant H-290/51. Moreover TiO2-nanodelivery of these MC 4 agonist compounds together with H-290/51 has prolonged superior effects on functional outcome and neuroprotective capability in SCI, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.24/O8

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH P50

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**Title:** Stabilizing neuronal cytoskeleton facilitates neurite development and repair

**Authors: *Y. SONG**[1,2,3], T. J. MITCHISON, 02115[1]
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**Abstract:** Microtubules (MTs) are structural components vital for important neuronal functions such as neurite outgrowth and maintenance, as well as for axonal trafficking and synaptic remodeling. Neuronal MTs are particularly stable compared with those in other cell types and often exist for years before they get degraded and recycled. They are mostly synthesized in the somatodendritic compartment and have to be transported along axons as much as a meter or more in humans to reach the terminals where synaptic proteins are delivered and synaptic

transmission occurs. A significant portion of neuronal MTs stay polymerized when challenged with cold temperature, Ca2+ and antimitotic drugs (e.g. Nocodazole), all of which destabilize MTs effectively in test tubes and various non-neuronal cell lines, suggesting that baseline MT stability is high in neurons. Such stability increases during development and maturation, but decreases with axonal injury and neurodegeneration. This leads to an intriguing question: can stabilizing MTs facilitate neurite outgrowth during early development or restore axonal integrity and function upon injury? To address these questions, we have characterized a group of MT stabilizing drugs regarding their binding affinity to MTs in vitro using biochemical assays and fluorescent live imaging; and evaluated their effects on neurite growth during normal differentiation and regrowth after axotomy. Dose and time dependent drug treatments were performed on ReNcell VM cells (an immortalized human neural progenitor cell line), and differentiated VM cells that exhibit neuronal morphology and electrophysiology, followed by high-content live-cell imaging and automated imaging analysis. We found that these drugs showed bimodal effects on initial neurite extension and regeneration after injury. To understand the molecular mechanisms underlying regulation of MT stability by these drugs and their biological effects, we have used multiplex proteomics to study dose-dependent changes in signaling pathway components and MAPs over time. These results may provide useful information for understanding not only neuronal MT dynamics and stability in health and disease, but also for determining the therapeutic value of MT stabilizers in axonal injury and neurodegeneration where loss of neuronal MT integrity may exacerbate disease pathology.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.25/O9

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Neuroprotective and behavioral effects of weak static magnetic fields in the rat

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**Abstract:** Magnetic field’s (MF's) beneficial effect was documented over 4000 years ago, especially for analgesia. In the modern era, the use of MF as a medical treatment gained momentum in the mid-eighties with the development of transcranial magnetic stimulation (TMS), which is an accepted treatment for major depression. Accordingly many studies address MF’s effect on biological tissues as a non-invasive and innovative medical treatment for different disease conditions.
In a previous study from our lab, (Ben-Yakir Blumkin et al. 2014), it was found that exposure of

primary cortical neurons to static magnetic field (SMF) causes a reduction in etoposide-induced apoptosis. Accordingly we explored SMF’s neuroprotective effect on the neurodegenerative effect of 6-hydroxydopamine (6OHDA) in vivo, by implanting a small (3mm X 0.5mm) titanium-coated magnetic disc on the cranium above bregma. In addition, since TMS is commonly used for the treatment of depression, and alterations in memory and increased anxiety are observed in ageing, we studied SMF's age related and time course related, effects on rat behavior, using young (2 month) , middle age (12 month) and aged rats (24 month). Using proteomic analysis (mass spectrometry) we studied SMF's effect on protein expression in the rats' hippocampus.
Our study demonstrated that 1 week of SMF exposure, (but not 3 or 5 weeks), caused an anxiolytic effect in elderly rats, and improved recognition and spatial memory in young rats. We also found that exposure to SMF protected the dopaminergic cells in substantia nigra pars compacta (SNc) from 6OHDA-induced neurodegeneration preserving cell number at normal levels. In the mechanistic study, we found that SMF exposure affected proteins related to cell energy and metabolism, calcium channels and myelination processes.
The results indicate that 1 week of SMF exposure exerts age dependent effects on anxiety and memory related parameters, and a neuroprotective effect on dopaminergic neurons of SNc. Alterations in cell metabolism, myelin related and calcium channel proteins observed in SMF-exposed brains can partly explain SMF’s mechanism of action. In general, the study indicates a possibility of using SMF for treatment of neurodegeneration and anxiety in humans.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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IT 794/13 (JVL), Government of Basque Country

**Title:** Diabetes exacerbates brain pathology following focal blast brain injury. New roles of a multimodal drug cerebrolysin and nanomedicine

**Authors: *D. F. MURESANU**[1], A. SHARMA[2], J. V. LAFUENTE[3], A. NOZARI[4], R. PATNAIK[5], A. OZKIZILCIK[6], R. TIAN[7], H. MOESSLER[8], H. S. SHARMA[2]
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**Abstract:** Blast brain injury (bBI), a combination of pressure, rotation, penetration of sharp objects and chemical exposure is prevalent in military personnel during combat operations causing laceration, perforation and tissue loss in the brain. Since combat stress could induce wide variety of cardiovascular and endocrine deregulation, it is unclear whether bBI could exacerbate brain pathology in diabetes. Thus, in present investigation influence of diabetes on bBI was investigated in a rat model. bBI was produced using a shock tube blast devise in which compressed air-and compressed helium-driven membrane rupture induces pressure waves to simulate some aspects of bBI. The rats were anesthetized with Equithesin (3 ml/kg, i.p.) and their head was exposed to overpressure blast (100, 150 or 200 kPa) in the shock-tube with a shockwave velocity of ca. 400 to 450 m/sec). After the bBI the animals were allowed to survive 8 and 12 h after trauma. Identical procedures were applied for bBI in diabetic rats that were produced by streptozotocine (50 mg/kg, i.p.) administration once daily for 3 days. The animals develop clinical diabetes after 4 to 6 weeks (blood glucose level 220-25 mM/L, control 5-6 mM/L). In both normal and diabetic rats blood-brain barrier breakdown to Evans blue albumin and radioiodine ($^{[131]}$-I), brain edema formation and neuronal, glial and axonal injuries were evaluated. In addition, regional cerebral blood flow (rCBF) was also examined using standard procedures.
A progressive increase in the BBB permeability to EBA and radioiodine in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem was seen that correlates well with the blast overpressure strength. In these brain areas rCBF was reduced by -30 to -58 %. Brain edema formation as measured using water content exhibited a 2- to 4 % increase (ca. 8 to 16 % volume swelling). Expansion of neuropil, sponginess and neuronal, glial and myelin damages are quite frequent. These pathophysiological changes were 2-to 5-fold exacerbated in diabetic animals after bBI. Treatment with cerebrolysin (a multimodal drug comprising neurotrophic factors and active peptide fragments) 30 min to 1 h after bBI (5 to 10 ml/kg, i.v.) significantly reduced brain pathology in normal animals. However, TiO2 nanodelivery of cerebrolysin (5 ml/kg, i.v.) is needed to induce neuroprotection in bBI in diabetic animals. These observations are the first to show that (i) bBI is exacerbated in diabetes, (ii) cerebrolysin has the potential to reduce brain pathology in bBI in healthy animals, whereas, TiO2-nanowired

cerebrolysin is needed to induce neuroprotection in diabetic animals after bBI, not reported earlier.

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**Poster**

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**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

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IT 794/13 (JVL), Government of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain

UFI 11/32 (JVL) University of Basque Country, Spain

**Title:** Brain injury exacerbates neuropathology of sleep deprivation. Superior neuroprotection by co-administration of TiO2-nanowired alpha-MSH and cerebrolysin

**Authors: *A. SHARMA**[1], D. F. MURESANU[3], J. V. LAFUENTE[4], A. OZKIZILCIK[5], R. TIAN[6], A. NOZARI[7], R. PATNAIK[8], H. MOESSLER[9], H. S. SHARMA[2]
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**Abstract:** Sleep deprivation (SD) is common in military personnel engaged in combat operations. Our previous reports show that 12 h SD alone induces brain pathology and continued until 72 h in a progressive manner. However, these military personnel with SD are also prone to traumatic brain injury (TBI). Thus, a possibility exists that TBI could further exacerbate SD induced brain pathology. Several lines of evidences suggest that both in TBI and in SD a decrease in alpha-melanocyte stimulating hormone (MSH) and brain derived neurotrophic factor (BDNF) levels occur in plasma as well as in the brain. This could be one of the leading causes of brain pathology in SD or in TBI. Thus, exogenous supplement of alpha-MSH and/or BDNF could induce neuroprotection in SD or TBI. In present investigation effect of concussive head injury (CHI) in SD induced brain pathology and effects of alpha-MSH and neurotrophic factors treatment leading to neuroprotection in a rat model was examined. SD was induced in rats using the inverted flowerpot methods surrounded by water level 1 cm below the platform (6.5 cm in diameter) that allow free movement but continuously sleep leads to fell down in water disturbing the sleep process. SD was induced in healthy rats as well as in rats that were subjected to CHI by dropping a weight of 114.6 g over the skull causing an impact of 0.224 N on the brain without skull fracture. Rats subjected to 48 h SD in CHI (24 h after insult) exhibited greater brain pathology e.g., higher leakage of Evans blue albumin and radioiodine ([131]-I) by 3-to 4 fold as compared to naïve rats subjected to identical SD. Neuronal, glial and axonal damages using histopathological techniques were also exacerbated by several fold in CHI after SD. Plasma alpha-MSH and BDNF level shows significant reduction (alpha-MSH 8.34±0.23 vs. Control 20.34±0.12 pg/ml; BDNF 8.23±0.11 vs. control 22.34±0.21 pg/ml) in SD group after CHI as compared to SD group alone (alpha-MSH 15.13±0.12 pg/ml; BDNF 14.23±0.08 pg/ml). Intravenous administration of alpha-MSH (100 µg/kg) together with cerebrolysin (a balanced composition of several neurotrophic factors and active peptide fragments 5 ml/kg) significantly induced neuroprotection in CHI or SD groups alone. However, TiO2 nanowired delivery of alpha-MSH and cerebrolysin is needed to induce neuroprotection in SD rats after CHI. The levels of alpha-MSH and BDNF were also retired by this treatment in SD rats after CHI (alpha MSH 22.34±0.12 pg/ml; BDNF 23.34±0.17 pg/ml). Taken together our results are the first to point out that TiO2 nanowired administration of alpha-MSH and cerebrolysin induces superior neuroprotective effects following SD in CHI, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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India-EU Co-operation Program (RP/AS/HSS)

**Title:** Repeated emotional stress exacerbates amyloid-beta peptide induced Alzheimer's disease brain pathology. Neuroprotective effects of PLGA NPs-loaded cerebrolysin

**Authors: *G. TOSI**[1], A. SHARMA[2], D. F. MURESANU[4], J. V. LAFUENTE[5], B. RUOZI[6], F. FORNI[6], M. A. VANDELLI[6], F. PEDERZOLI[6], J. T. DUSKEY[6], N. ODDONE[6], H. MOESSLER[7], R. PATNAIK[8], R. J. CASTELLANI[9], H. S. SHARMA[3]
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**Abstract:** Military personnel lead very stressful life during combat situations. Stress alone induces breakdown of the blood-brain barrier (BBB) and neuronal damages. Long-term stress is related to the development of Alzheimer’s disease (AD) as compared to their stress free counterparts. In present investigation we explored whether AD induced brain pathology caused by amyloid beta peptide (AbP) infusion is exacerbated in rats after repeated emotional stress enough to open the BBB to large molecules e.g., proetins.
AD like brain pathology was induced by administered intraventricularly (i.c.v.) of AbP (1-40) in the left lateral ventricle (250 ng/10 µl) of rats (250-300 g body weight) once daily for 4 weeks in naïve animals as well as in rats that were subjected to repeated immobilization stress 2 h daily for 1 week. Control rats received identical dose of saline instead of AbP. In these control and AbP infused rats BBB breakdown, edema formation, neuronal, glial injuries and AbP deposits in the brain was examined in a blinded fashion by at least 3 independent workers.
Our observations showed that repeated 2 h stress for 1 week induced marked BBB breakdown to Evans blue albumin and radioiodine tracers in the cerebrla cortex (55 to 78 %), hippocampus (89 to 98 %), thalamus (34 to 46 %), hypothalamus (35 to 50 %), caudate nucleus (78 to 97 %), cerebellum (80 to 134 %) and brainstem (23 to 35 %) from the naive rats. Infusion of AbP in

these stressed rats further enhanced the BBB breakdown to protein tracers by 3- to 5-fold and resulted in pronounced neuronal damages (+400 %), astrocytic activation (+350 %) and brain swelling (+10 %) compared to identical AbP infusion in naive rats. The number of AbP positive cells increased by 3- to 6-fold in these brain areas in stressed group as compared to naive rats. Administration of PLGA nanoparticles (NPs) loaded cerebrolysin (2.5 ml/kg, i.v. /day from 2nd week of AbP infusion for 2 weeks) induced profound neuroprotection in stressed rats, whereas 5 ml normal cerebrolysin is needed to attenuate brain pathology after AbP infusion in these rats. Taken together our observations are the first to demonstrate that repeated stress exacerbates AD pathology in brain and nanodelivery of cerebrolysin has superior neuroprotective effects in AD.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.29/P3

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065;

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**Title:** Histamine H3 and H4 receptors modulate Parkinson's disease induced brain pathology. An experimental study using BF-2639 and clobenpropit in association with anti-histamine-antibody therapy for neuroprotection

**Authors: *R. PATNAIK**[1], A. SHARMA[2], D. F. MURESANU[3], J. V. LAFUENTE[4], A. OZKIZILCIK[5], R. TIAN[6], A. NOZARI[7], H. S. SHARMA[2]
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**Abstract:** Military personnel deployed to combat operations are highly susceptible to develop Parkinson's disease (PD) or Alzheimer's disease (AD) particularly after sustaining traumatic brain injury (TBI). TBI causes increased amyloid beta peptide, alpha synuclein (ASNC) or tau protein in the brain or cerebrospinal fluid (CSF) leading to neuropathology. Although, PD involves dopaminergic pathways, recent evidences suggest that histaminergic system is also involved in the pathophysiology of PD. Increased histaminergic nerve fibers in substantia niagra pars Compacta (SNpc), striatum (STr) and caudate putamen (CP) occurs in human brain postmortem study associated with increase in Histamine H3 receptors and decrease in H4 receptors in these areas. This indicates involvement of histaminergic transmission in PD. Thus, a possibility exists that modulation of histamine H3 and H4 receptors as well as histaminergic transmission could reduce PD brain pathology. Previously, we showed that a potent histaminergic H3 receptor inverse agonist BF-2549 and clobenpropit a partial histamine H4 agonist with H3 receptor antagonistic activity induced profound neuroprotection in amyloid beta peptide infusion induced AD in a rat model. In present investigation we examined the role of histamine H3 and H4 receptor in a mouse model of PD. In addition we also evaluated intracerebroventricular (i.c.v.) administration of monoclonal ant-histamine-antibodies on PD induced brain pathology with or without histaminergic drug treatments. PD like symptoms was produced by administration of 1-metyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP, 20 mg/kg, i.p.) daily within 2-h intervals for 5 days in mice. On the 8$^{th}$ day, marked decrease in the number of tyrosine hydroxylase (TH) positive cells in the SNpc and STr as well as decrease in dopamine (DA) and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) occurred associated with increased ASNC and tau proetins in the CSF. Mice treated with BF 2649 (1 mg/kg, i.p.) or Clobenpropit (1 mg/kg, i.p.) once daily for 1 week accompanied with normal or TiO2 nanowired histamine antibodies (25 µl, MAB0021, Clone HYHIS1, Abnova, USA) resulted in significant reduction in ASNC and tau protein in the CSF as well as restoration of DA and DOPAC in SNpc, STr. These neuroprotective effects were most marked when histamine antibodies were administered through nanowired technique in combination with clobenpropit as compared to BF-2649. These observations strongly indicate an involvement of histamine and opens up new avenues in drug development for PD using modulation of histamine H3 and H4, not reported earlier.

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**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.30/P4

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Title:** Which factor has a stronger effect on cerebral $^{18}$F-FDG distribution in cognitively normal older subjects, plasma glucose level or insulin resistance?

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**Abstract: Background:** Increasing plasma glucose level and insulin resistance can alter the distribution pattern of fluorine-18-labeled fluorodeoxyglucose ($^{18}$F-FDG) in the brain and relatively reduce $^{18}$F-FDG uptake in Alzheimer's disease (AD)-related hypometabolic regions, leading to the appearance of an AD-like pattern (1,2). However, of the two parameters, it is unclear which factor is more responsible for the distributional change of $^{18}$F-FDG followed by the appearance of the AD-like pattern. **Methods:** Fifty-nine cognitively normal older subjects (age = 75.7 ± 6.4 years) who were free of diabetes underwent $^{18}$F-FDG positron emission tomography along with measurement of plasma glucose and insulin levels. As an index of insulin resistance, the Homeostasis model assessment of Insulin Resistance (HOMA-IR) was calculated. To normalize regional $^{18}$F-FDG uptake in the brain, the global brain was used as a reference region. **Results:** Whole-brain voxelwise analysis showed a negative correlation of $^{18}$F-FDG uptake with plasma glucose level in the precuneus and lateral parietotemporal regions (cluster-corrected $p < 0.05$), and no correlation with HOMA-IR. In the significant cluster, $^{18}$F-FDG uptake decreased by approximately 5-6% when plasma glucose level increased by 20 mg/dL. In the precuneus region, volume-of-interest analysis confirmed a negative correlation of $^{18}$F-FDG uptake with plasma glucose level ($r = -0.603$, $p < 0.001$), and no correlation with HOMA-IR. **Conclusion:** This study showed that plasma glucose level has a negative correlation with $^{18}$F-FDG uptake in the precuneus and lateral parietotemporal regions which are the main component of AD-related hypometabolic areas, and confirmed that increasing plasma glucose level can induce the appearance of the AD-like pattern in $^{18}$F-FDG images. However, there was no significant negative correlation of $^{18}$F-FDG uptake with insulin resistance. Thus, increasing plasma glucose level has a stronger effect on the appearance of the AD-like pattern in $^{18}$F-FDG images than increasing insulin resistance does. **References:** 1) Ishibashi K, et al. Relationship between Alzheimer disease-like pattern of $^{18}$F-FDG and fasting plasma glucose levels in cognitively normal volunteers. J Nucl Med. 2015. 56:229-33. 2) Baker LD, et al. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch Neurol. 2011. 68:51-7.

**Disclosures:** **K. Ishibashi:** None. **A. Onishi:** None. **Y. Fujiwara:** None. **K. Ishiwata:** None. **K. Ishii:** None.

**Poster**

**570. Mechanisms of Alzheimer's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 570.31/P5

**Topic:** C.09. Brain Injury and Trauma

**Support:** VA Grant 1I01RX000511

**Title:** Memantine and simvastatin improve memory in humanized Amyloid-Beta mice after CCI injury: A dual intervention study

**Authors: E. E. ABRAHAMSON**[1,3], Z. MI[1,3], S. CULVER[2], X. MA[2], C. DIXON[2,3], *M. D. IKONOMOVIC[1,3]
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**Abstract:** Altered glutamatergic neurotransmission after traumatic brain injury (TBI) results in excitotoxic cell damage and death, and may contribute to an increased risk for developing Alzheimer's disease (AD) pathology. Suppression of such changes in the aftermath of TBI is a desirable therapy goal. Memantine (3,5-dimethyl-1-adamantanamine) is a voltage-dependent open channel blocking NMDA receptor antagonist. A previous study reported neuroprotective effects of memantine in rats evaluated acutely after severe controlled cortical impact (CCI) injury. Our pilot study showed that 3-week long daily treatment of humanized Amyloid-Beta (hAβ) mice with memantine (5mg/kg, intraperitoneal, i.p.) lowered injury-induced elevations in brain Aβ peptides concentration and blunted behavioral deficits at 21 days after CCI. The current chronic (3-months) study explored further the effects of daily administration of memantine (5mg/kg daily i.p.), or vehicle, on behavioral outcomes in adult hAβ mice exposed to severe CCI injury (depth=1.6 mm; velocity=5.82 m/s), sham surgery (craniotomy), or no surgery (naïve). Additional groups received simvastatin (daily, 3mg/kg by oral gavage) or a combination of memantine and simvastatin. Mice were evaluated for vestibulomotor (VM) function (days 1-5 and 71-75) and memory acquisition/retention (Morris water maze, MWM, days 14-21 and 83-90). On VM tests, all CCI groups performed worse than non-CCI groups, but did not differ from one another (both time points). MWM test results also separated CCI and non-CCI groups and, additionally, CCI groups treated with simvastatin, memantine, or the two drugs combined performed better than the vehicle-treated group at the first MWM testing trial (days 14-21). Remotely after injury (days 83-90) MWM testing showed persistent effects of CCI injury in the CCI/vehicle group, but not in CCI/treatment groups. In summary, daily memantine treatment improved behavioral recovery (spatial memory acquisition/retention) at acute and chronic time

points after severe CCI injury in hAβ mice. Simvastatin also improved behavioral outcomes and there was evidence of beneficial effects of combined treatment. We are currently assessing brain Aβ concentrations and histological outcomes to relate to these behavioral results. These preclinical data warrant further investigations of the effects of memantine and simvastatin in other brain injury models, such as blast and repetitive mild TBI.

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**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.01/P6

**Topic:** C.03. Parkinson's Disease

**Title:** A novel immunregulatory action of alpha-synuclein on human natural killer cells

**Authors: *R. H. EARLS**[1], J. CHUNG[2], J.-K. LEE[2]
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**Abstract:** Alpha-synuclein (α-Syn) species (monomer, oligomer and fibril) are present in human plasma and cerebrospinal fluid in Parkinson's disease (PD) patients. In PD patients, natural killer (NK) cell numbers are increased in blood and their activity level is associated with disease severity. NK cells as part of the innate immune system exhibit the highest cytotoxic capacity as a first-line of defense and modulate adaptive immune response. Despite of their potential involvement in PD pathogenesis, its role in PD has hardly been explored. We aim to investigate if NK cell functions are modulated by monomer and pathologic forms (oligomers or fibrils) of α-Syn. Our study, for the first time, demonstrated that pathologic forms of α-Syn significantly attenuate NK cell cytotoxicity against K562 leukemia target cells while monomeric α-Syn has no effect on cytotoxicity. In addition, both monomeric and pathologic forms of α-Syn attenuate the production of interferon-γ (IFN-γ), a major cytokine produced by NK cells, which promotes Th1 differentiation and myeloid cell activation. Based on our findings, using flow cytometry analysis, we are in the process of testing whether monomer and/or pathologic forms (oligomers or fibrils) of α-Syn change NK cell receptor expressions including NKG2D (activating) and NKG2A (inhibitory). Both of these receptors are reported as having altered expressions on NK cells in PD patients. We are also conducting *in vivo* study to investigate the involvement of NK cells in PD pathogenesis. Collectively, our studies will provide us critical, novel information about the immunoregulatory role of α-Syn on NK cells and the role of NK cells in the context of PD.

**Disclosures: R.H. Earls:** None. **J. Chung:** None. **J. Lee:** None.

**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

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**Program#/Poster#:** 571.02/P7

**Topic:** C.03. Parkinson's Disease

**Support:** NIH/NINDS R21NS09963201 (FPM)

**Title:** Loss of alpha synuclein function within nigrostriatal neurons initiates a toxic, neuronally-mediated neuroinflammatory cascade

**Authors: *M. J. BENSKEY**[1,2], R. C. SELLNOW[2], I. M. SANDOVAL[2,3], C. E. SORTWELL[2,3], J. W. LIPTON[2,3], F. P. MANFREDSSON[2,3]
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**Abstract:** Human studies and preclinical models of Parkinson's disease (PD) implicate the involvement of both the innate and adaptive immune systems in disease progression. PD affected brain regions show high levels of activated microglia, increased pro-inflammatory cytokine expression, and infiltration of peripheral leukocytes. Additionally, these pro-inflammatory markers are highly enriched near neurons containing pathological forms of alpha synuclein (α-syn), and α-syn overexpression recapitulates these neuroinflammatory changes in models of PD. These data suggest that α-syn may initiate a pathological inflammatory response. However, the mechanism by which α-syn initiates neuroinflammation is poorly understood. Silencing endogenous α-syn results in a similar pattern of nigral degeneration observed following α-syn overexpression. Here we aimed to test the hypothesis that loss of α-syn function within mature nigrostriatal neurons results in neuronal dysfunction, which subsequently stimulates neuroinflammation. Adeno-associated virus (AAV) expressing an shRNA targeting endogenous rat α-syn was unilaterally injected into the substantia nigra pars compacta (SNc) of adult rats, after which nigrostriatal pathology and indices of neuroinflammation were examined. Silencing endogenous α-syn below 50% of basal levels within nigrostriatal neurons resulted in a rapid (10 days), cell autonomous up-regulation of the major histocompatibility complex class 1 (MHC-1) within transduced nigral neurons. Nigral MHC-1 expression occurred prior to any overt cell death and resulted in the recruitment of microglia and peripheral T-cells to affected neurons. Recruited microglia were in a reactive state and made physical contact with MHC-1 expressing neurons, indicative of microglial phagocytosis of live neurons. Concomitantly, α-syn knockdown resulted in a progressive loss of tyrosine hydroxylase expression prior to the occurrence of overt neuronal death. Within 3 weeks of injection, α-syn knockdown resulted in a 50% loss of nigrostriatal soma in the SNc and a corresponding loss of nigrostriatal terminals and dopamine concentrations within the striatum. Silencing α-syn within glutamatergic neurons of the

cerebellum did not elicit inflammation or cell death, suggesting that inflammation and toxicity initiated by α-syn silencing is specific to dopamine neurons. These data provide compelling evidence that loss of normal α-syn function within nigrostriatal neurons initiates a neuronal-mediated neuroinflammatory cascade, involving both the innate and adaptive immune systems, which ultimately results in the death of affected neurons.

**Disclosures: M.J. Benskey:** None. **R.C. Sellnow:** None. **I.M. Sandoval:** None. **C.E. Sortwell:** None. **J.W. Lipton:** None. **F.P. Manfredsson:** None.

**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.03/P8

**Topic:** C.03. Parkinson's Disease

**Title:** Characterization of a novel conditional alpha-synuclein knockout mouse model using somatic brain transgenesis

**Authors: *K. MILLER**[1], S. C. KELLY[1], M. J. BENSKEY[2], C. E. SORTWELL[1], I. M. SANDOVAL[4], F. P. MANFREDSSON[3]
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**Abstract:** The protein alpha-synuclein (α-syn) is implicated as causative in both sporadic and familial forms of Parkinson's disease (PD). Consequently, targeting α-syn has been proposed as a therapeutic strategy for PD. However, α-syn is one the most abundant proteins in the nervous system, and its endogenous function is not fully understood. Indeed, removal of α-syn from mature dopamine (DA) neurons of mice, rats, and nonhuman primates result in dosedependent and progressive neurodegeneration. In contrast, non-DAergic brain regions are resistant to α-syn silencing. These results demonstrate that α-syn is essential for the survival of mature DA neurons. Moreover, removing α-syn from non-DA neurons offers the opportunity to study the effect of α-syn removal without the complication of neurotoxicity. In this study we aimed to determine the consequences of postnatal α-syn silencing in areas such as the hippocampus and cerebellum; areas which express high levels of α-syn, but which do not display the selective vulnerability observed in PD. To better study the consequences following α-syn removal from mature neurons *in vivo* we generated a conditional knockout mouse engineered to have exons 1-2 of the α-syn gene flanked by loxP sites. Adeno-associated virus (AAV) expressing CRE recombinase (iCRE) was bilaterally injected into the lateral ventricles of neonate α-syn-floxed mice. This novel approach allows us to efficiently target these areas with one injection so that we can assess changes in neuronal physiology at the behavioral and cellular level. With this method

we achieved robust, widespread, and persistent transduction throughout the brain. Expression of iCRE successfully excised the floxed genomic region, eliminating α-syn gene and protein expression. Monthly behavioral tests are being performed to assess motor, balance, gait, and memory phenotypes. Animals will be sacrificed 3 months post surgery and histological analyses will be performed to determine physiological changes that are occurring. Once validated, this new model will provide a foundation for investigations into the physiological function of α-syn without the potential confounds that may occur due to removal of α-syn prior to neuronal development (e.g. germline compensation) or toxicity. This model will also be valuable when investigating the role of α-syn in PD etiology and its role in dopaminergic neurodegeneration.

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**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.03. Parkinson's Disease

**Support:** NIH Grant R01-NS088322

NIH Grant T32-AG000255

**Title:** α-Synuclein expression levels determine neuronal subpopulation vulnerability to Lewy-like pathology induced neurodegeneration

**Authors: *E. LUNA**[1], A. CAPUTO[2], B. ZHANG[3], Y. LIANG[4], D. M. RIDDLE[4], S. C. DECKER[4], V. M. LEE[5], K. C. LUK[6]
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**Abstract:** Cytoplasmic inclusions of the pre-synaptic protein α-synuclein (α-syn) known as Lewy pathology and neurodegeneration of select neuronal subpopulations define the synucleinopathies, a group of disorders that include Parkinson's disease (PD), Multiple System Atrophy, and Dementia with Lewy Bodies. It is increasingly appreciated that α-syn aggregates spread in a trans-neuronal fashion between vulnerable brain regions. Although point mutations, multiplications, and polymorphisms in the α-syn gene either cause or influence the risk of developing PD, determining the relationship between α-syn and neurodegeneration has proven

difficult as few model systems concurrently recapitulate key aspects of PD, including α-syn pathology, selective neuron loss, and trans-neuronal spread of pathology. We explored the relationship between α-syn and selective neurodegeneration using pre-formed α-syn fibrils (PFFs) to induce the conversion of endogenous α-syn into Lewy-like inclusions both in primary hippocampal cultures and *in vivo*. Using immunostaining for neuronal markers (NeuN and Neurofilament light chain) to determine neuronal viability after PFF-treatment, we found that PFFs cause toxicity in dose and time dependent manner. Toxicity is also pathology dependent since more pathogenic PFFs show increased toxicity whereas toxicity is not observed in PFF-treated $Snca^{-/-}$ neurons. Interestingly, we observed that toxicity was limited to only a subset of neurons, even after extensive periods of PFF treatment. In particular, glutamatergic Math2-positive neurons derived from the CA region were vulnerable to PFF-induced toxicity. In contrast Prox1-expressing glutamatergic neurons derived from the dentate gyrus (DG) and GABAergic neurons were relatively resistant both *in vitro* and *in vivo*. Vulnerability correlated with relative α-syn expression as $Math2^{+}$ neurons expressed higher levels compared to $Prox1^{+}$ neurons. Neuron loss was partially rescued by the antioxidant N-acetyl cysteine indicating that toxicity is oxidative stress-dependent. Our ongoing experiments will seek to determine the mechanisms linking α-syn aggregation to toxicity.

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**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.05/P10

**Topic:** C.03. Parkinson's Disease

**Title:** Characterization of systemic metabolic abnormalities in alpha-synuclein mutant mice

**Authors: *S. ZHANG**[1], S. CAMANDOLA[1], K. W. FISHBEIN[1], R. G. SPENCER[1], M. C. MARING[1], M. PETR[1,2], J. F. O'CONNELL[1], M. P. MATTSON[1]
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**Abstract:** Accumulating evidence suggests that many individuals with Parkinson's disease (PD) exhibit abnormalities in systemic energy metabolism prior to motor symptom onset, but the relationships between such abnormalities and the characteristic alpha-synuclein pathology are unclear. We previously reported that a high fat diet exacerbates, and alternate day intermittent fasting (IF) reverses, autonomic nervous system dysfunction in presymptomatic mice overexpressing a familial PD alpha-synuclein mutation (A53T) in neurons (Griffioen et al., 2013), and that this same PD mouse model exhibits a marked reduction in adipose tissue (Rothman et al., 2014). We further interrogated the metabolic status of cohorts of wild type (WT)

and PD mice maintained on control or alternate day IF diets for two months beginning at 10 weeks of age. MRI analysis data revealed that the PD mice have less adipose tissue, especially visceral fat, compared with WT mice when maintained on a standard ad libitum diet, and that IF accentuates fat loss. Measurements of metabolic parameters indicated reduced energy expenditure (EE) in PD mice compared to WT mice fed ad libitum, but not when maintained on IF. WT and PD mice exhibited increased spontaneous activity when on the IF diet compared to the control diet. As expected, the respiratory exchange ratio was reduced on fasting days in both the WT and PD mice. PD mice consumed less oxygen than WT mice when on the ad libitum diet, but not when on IF. Motor performance was significantly impaired in PD mice on both diets compared to WT mice, and mortality was hastened in PD mice on IF compared to those fed ad libitum, suggesting the contribution of a presymptomatic "wasting" phenotype to disease progression and death in this PD mouse model. This research was supported by the Intramural Research Program of the National Institute on Aging.

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**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.06/Q1

**Topic:** C.03. Parkinson's Disease

**Support:** The Michael & Elizabeth Gilbert PhD Scholarship

**Title:** α-Synuclein mediates neuronal cholesterol efflux

**Authors: *T. HSIAO**[1,2,3], G. M. HALLIDAY[4], W. S. KIM[2]
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**Abstract:** α-Synuclein is a 17-kDa protein that is highly expressed in neurons. Although the exact function of α-synuclein is unclear, it is thought to play a role in vesicle transport. α-Synuclein is at the center of focus in understanding a group of neurodegenerative diseases called α-synucleinopathies, including Parkinson's disease and multiple system atrophy, where it is proposed to be passed between cells with advancing disease. Structurally, α-synuclein is similar to lipid-binding apolipoproteins, which are known extracellular lipid acceptors. The function of extracellular α-synuclein as a lipid acceptor is unknown, and therefore we asked the question whether extracellular α-synuclein mediates cholesterol efflux for neurons. We treated cholesterol-loaded SK-N-SH neuronal cells with extracellular α-synuclein and performed

cholesterol efflux assays. Extracellular α-synuclein potently stimulated cholesterol efflux from neurons to a similar extent as the positive control apolipoprotein E. The process was dose dependent and was saturated at an α-synuclein concentration of 1.0 μg/ml. The process was also time dependent. Here, we established, for the first time, a function for extracellular α-synuclein as a mediator of cholesterol efflux from neurons. This novel data provides a new facet to the role of α-synuclein in the brain and reveals new leads to understanding the pathomechanisms of α-synucleinopathies.

**Disclosures: T. Hsiao:** None. **G.M. Halliday:** None. **W.S. Kim:** None.

**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.07/Q2

**Topic:** C.03. Parkinson's Disease

**Support:** 5R21NS088923

Michael J. Fox target validation

**Title:** Pum2-dependent translational regulation of alpha-synuclein mRNA on mitochondrial outer surface

**Authors: *Y.-S. KIM**, S. GUHATHAKURTA, S. BASU, G. JE
Biomed. Sci., Burnett Sch. of Biomed. Sci., Orlando, FL

**Abstract:** Alpha-synuclein (α-SYN) is a central molecule in the pathogenesis of Parkinson's disease (PD). α-SYN and mitochondrial functions are intimately entwined but the molecular mechanisms are incompletely understood. Here, we show that α-SYN mRNA is associated with the outer surface of mitochondria, where mitochondrial reactive oxygen species (ROS) caused by inhibition of respiratory complex I triggers local translation of α-SYN protein. The 3'-UTR of α-SYN mRNA is indispensable in the regulation of α-SYN translation on the surface of mitochondria. Next, we identified that the RNA-binding protein, Pum2, binds to its cognate binding site in the α-SYN 3'-UTR that is highly conserved across species. While knockdown of Pum2 significantly increased mitochondrial α-SYN, Pum2 overexpression lowered α-SYN levels, indicating that α-SYN translation is repressed by Pum2 until mitochondrial ROS derepress it. To investigate the role of Pum2-mediated translational regulation of α-SYN in mitochondrial functions, mutagenesis of a Pum2-binding motif in the 3'-UTR was achieved using CRISPR/Cas9-based genome editing in the mammalian cells. Homozygous mutation of a Pum2 binding motif resulted in changes in mitochondrial morphology, and reductions in cellular oxygen consumption and ATP synthesis. Altogether, we report a novel mechanism that Pum2-

dependent translational regulation of a pool of α-SYN mRNA pre-associated with mitochondrial surface contributes to the fine-tuning of mitochondrial respiratory functions. The results suggest that dysregulation of these mechanisms could contribute to mitochondrial impairment, leading to PD pathogenesis.

**Disclosures: Y. Kim:** None. **S. Guhathakurta:** None. **S. Basu:** None. **G. Je:** None.

**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.08/Q3

**Topic:** C.03. Parkinson's Disease

**Title:** Endogenous alpha-synuclein expression patterns revealed using a novel mouse model

**Authors: *A. CAPUTO**[1], Y. LIANG[2], V. M. KEHM[2], E. LUNA[2], S. C. DECKER[2], B. ZHANG[2], K. C. LUK[2]
[1]Pathology and Lab. Med., Perelman Sch. of Med. at UPenn, Philadelphia, PA; [2]Pathology and Lab. Med., Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Alpha synuclein (aSyn) is involved in synaptic vesicle trafficking and synaptic transmission, but is also strongly linked to Parkinson's disease (PD) and other neurodegenerative disorders. In diseases where aSyn aggregates, or synucleinopathies, it accumulates in and spreads to different brain areas and peripheral organs. Although increased aSyn levels likely underlie familial PD with SNCA mutations thus implicating the protein in disease initiation, most synucleinopathies arise sporadically indicating that the expression of normal levels of wild type aSyn is sufficient for the development of disease. In spite of our increasing knowledge in the field, the physiological function of aSyn and its precise role in disease remain enigmatic urging the development of new tools for further investigations. Here, we report the development and characterization of a new mouse model expressing a GFP-aSyn fusion protein under the control of the endogenous Snca promoter. We describe the expression pattern of the fusion protein in the brain and peripheral organs and characterized its subcellular localization and trafficking in the brain. Primary neurons expressing GFP-aSyn were also successfully derived from this line. In addition, intracerebral injection of aSyn pre-formed fibrils induced formation of GFP-positive inclusions with a similar distribution pattern to that observed in wild type mice. We anticipate that this new mouse model will facilitate in vitro and in vivo studies that incorporate live imaging and detection of endogenous alpha synuclein, therefore providing new insights into aSyn function in health and disease.

**Disclosures: A. Caputo:** None. **Y. Liang:** None. **V.M. Kehm:** None. **E. Luna:** None. **S.C. Decker:** None. **B. Zhang:** None. **K.C. Luk:** None.

**Poster**

**571. Alpha-Synuclein Normal Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 571.09/Q4

**Topic:** C.03. Parkinson's Disease

**Support:** ERC Advanced Grant 340527

CiberNed CB06/05/006

Fundacio La Marato TV3 20141331

**Title:** Glucocerebrosidase expression patterns in the non-human primate brain

**Authors: *J. L. LANCIEGO**[1,2], D. SUCUNZA[1,2], A. J. RICO[1,2], D. PIGNATARO[1,2], D. MARIN-RAMOS[1,2], E. RODA[1,2], I. G. DOPESO-REYES[1,2]
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**Abstract:** Glucocerebrosidase (GCase) is a lysosomal enzyme encoded by the GBA1 gene. Mutations in GBA1 gene lead to Gaucher's disease (GD), the most prevalent lysosomal storage disorder. GBA1 mutations reduce GCase activity, therefore promoting the aggregation of alpha-synuclein, a common neuropathological finding underlying Parkinson's disease (PD) and dementia with Lewy bodies. Bearing in mind that a number of strategies increasing GCase expression for the treatment of PD are currently under development, here we sought to analyze the baseline expression of GCase in the brain of *Macaca fascicularis*, which has often been considered as the gold-standard animal model of PD. Although as with other lysosomal enzymes, GCase is expected to be ubiquitously expressed, here a number of regional variations have been consistently found, together with several specific neurochemical phenotypes expressing very high levels of GCase. In this regard, the most enriched expression of GCase was constantly found in cholinergic neurons from the nucleus basalis of Meynert, dopaminergic cells in the substantia nigra pars compacta, serotoninergic neurons from the raphe nuclei, as well as in noradrenergic neurons located in the locus ceruleus. Moreover, it is also worth noting that high levels of expression were also found in a number of areas within the paleocortex and archicortex, such as the entorhinal cortex and the hippocampal formation, respectively.

**Disclosures: J.L. Lanciego:** None. **D. Sucunza:** None. **A.J. Rico:** None. **D. Pignataro:** None. **D. Marin-Ramos:** None. **E. Roda:** None. **I.G. Dopeso-Reyes:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.01/Q5

**Topic:** C.03. Parkinson’s Disease

**Support:** BK21 plus

NRF

**Title:** Neuroprotective effects of novel osmotin against MPTP/$MPP^+$-induced neurodegenerative disease model

**Authors:** *M.-G. JO, ***M.-G. JO**, M.-H. JO, M. IKRAM, M. S. KHAN, M.-O. KIM
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**Abstract:** Parkinson’s disease (PD) is the second most frequent progressive neurodegenerative disorder of aging characterized by apoptosis and neuro-inflammation, which subsequently leads to the deterioration of nigrostriatal dopaminergic (DA) neurons. This study was designed to investigate the neuroprotective effects of osmotin against MPTP/$MPP^+$-induced in mice and SH-SY5Y human neuroblastoma cell lines respectively. Osmotin is a tobacco plant-derived protein associated to pathogenesis-related proteins of family 5. Being a structural and functional homolog of the mammalian adiponectin, osmotin is believed to have similar biological and physiological effects as adiponectin, and so has been implicated as anti-inflammatory and anti-apoptotic agent in different experimental settings. Osmotin administration to MPTP-induced models of PD led to improvement of motor impairment. Furthermore, Osmotin was able to enhance the expression of tyrosine hydroxylase (TH) protein and increase TH-positive neurons in SNpc and Striatum. Osmotin attenuated MPTP/$MPP^+$-induced DA neuronal cell death in SNpc through regulation of anti/pro-apoptotic markers as Bcl-2, Bax, caspase-3 and Cytochrome c. In addition, our data demonstrated that osmotin suppressed JNK phosphorylation and promoted ERK activation in PD model. To the best of our knowledge, this research was the very first investigation confirmed that novel osmotin is the best candidate which can ameliorate neurological deteriorations associated with PD.

**Disclosures:** **M. Jo:** None. **M. Jo:** None. **M. Ikram:** None. **M.S. Khan:** None. **M. Kim:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.02/Q6

**Topic:** C.03. Parkinson's Disease

**Support:** Fundação Amazônia de Amparo a Estudos e Pesquisas do Pará (FAPESPA)

**Title:** Copaifera reticulata Ducke oil-resin reduces apomorphine-induced rotations in the striatal 6-OHDA mouse model of Parkinson's disease

**Authors: *A. VALENTE**[1], R. D. M. GOMES[1], S. M. G. SERRÃO[1], E. T. COSTA[1], D. C. F. LOPES[1], V. S. L. CARDOSO[1], W. GOMES-LEAL[2], E. S. YAMADA[1]
[1]Exptl. Neuropathology Lab., Univ. Hosp. João De Barros Barreto, UFPA, Belém, Brazil; [2]Lab. of Exptl. Neuroprotection and Neuroregeneration, Inst. of Biol. Sciences, UFPA, Belém, Brazil

**Abstract:** Parkinson's disease (PD) is traditionally classified as a motor disorder characterized by resting tremor, muscular rigity, postural instability and bradykinesia, which result from dopaminergic neuronal death in the substantia nigra pars compacta (SNpc) and consequent depletion of dopamine in the striatum. The exact trigger of the neuronal cell death in PD remains unknown, but oxidative stress and neuroinflammation are involved in the mechanism underlying the dopaminergic neurodegeneration. The 6-OHDA mouse models of PD are useful tools for the study of the biological processes involved in the pathophysiology of PD, as well as to investigate neuroprotection strategies. In this way, the Amazonian biome represents a great source of natural resources for compounds with neuroprotective potential. *Copaifera reticulata* Ducke (Copaíba) is among the most used plants in folk medicine and its oil-resin has several sesquiterpenes with anti-inflammatory and antioxidant properties. Thus, the aim of this study was to evaluate a neuroprotective effect of the Copaíba oil-resin in the striatal 6-OHDA mouse model of PD. Forty-Eight male Swiss mice were used, divided in 4 groups: Vehicle/Vehicle (N = 8), Vehicle/Copaíba (N = 8), 6-OHDA/Vehicle (N = 15) and 6-OHDA/Copaíba (N = 17). Stereotaxic surgeries were performed in all animals; vehicle or 10 µg 6-OHDA solution was infused into the striatum. Copaíba oil-resin treatment (50 mg/kg) was performed for 7 days after stereotaxic surgeries and the survival time was 28 days. Open field test, apomorphine-induced rotational test and immunostaining for tyrosine-hydroxylase (TH) were performed. The analyses from the open field test showed that 6-OHDA/Vehicle and 6-OHDA/Copaíba was reduced on the $14^{th}$ (59%) and $28^{th}$ day (56%) in relation to day 0, suggesting that Copaíba oil-resin treatment did not improve the motor performance at this test. Additionally, 6-OHDA/Vehicle and 6-OHDA/Copaíba groups remained less time in the peripheral zone in open field test in relation to Vehicle/Vehicle and Vehicle/Copaíba groups, which did not display thigmotaxis behavior. The apomorphine-induced rotational test on the $14^{th}$ day showed that 6-OHDA/Vehicle group

presented significant decrease of 54% in relation to 6-OHDA/Vehicle group, but this difference did not remain on the 28th day. Histological analyses showed that the Copaíba oil-resin treatment was associated with less degeneration of TH+ neurons in the nigrostriatal pathway. Hence, the Copaíba oil-resin treatment has a potential neuroprotective effect in this mouse model of PD, which deserves to be better characterized for translational application.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 572.03/Q7

**Topic:** C.03. Parkinson's Disease

**Support:** Fondecyt 1150200 (to MEA)

PCI-BMBF 21050065 (to PZ)

Conicyt PhD Fellowship 21150971

**Title:** Cerebral dopamine neurotrophic factor (CDNF) attenuates endoplasmic reticulum stress-induced apoptosis

**Authors: *D. A. ARANCIBIA**[1], P. ZAMORANO[2], M. E. ANDRES[3]
[1]Pontificia Univ. Católica De Chile, Santiago, Chile; [2]Univ. de Antofagasta, Antofagasta, Chile; [3]Pontificia Univ. Catolica De Chile, Santiago, Chile

**Abstract:** The endoplasmic reticulum (ER) is an organelle that maintains the equilibrium between synthesis, folding and degradation of proteins. Many stressors can induce failing the adaptive capacity of ER producing the accumulation of misfolded proteins in the ER lumen and triggering the unfolded protein response (UPR). Failure to maintain the proteostasis at the ER in neurons has been associated with many neurodegenerative diseases, such as Parkinson, Alzheimer and Huntington's disease among others. CDNF is a non-conventional neurotrophic factor that has been reported to exert a neuroprotective and neurorestorative effect in animal models of Parkinson disease. Although the molecular mechanisms of the protective role of CDNF are still unclear, a participation of the UPR has been observed, suggesting a modulating role in ER stress. CDNF is constitutively secreted to the extracellular medium, but is also an ER-resident protein. In this study, we investigated whether CDNF expressed intracellularly inhibited ER stress and apoptosis in HEK-293 cells upon exposure to thapsigargin (TG), an ER stress inductor, to determine its effects on cell viability and the UPR response. Our results show that

intracellular overexpression of CDNF improved cell viability in cells exposed to TG. In addition, CDNF increased GRP78 and decreased ATF6 and XBP-1 protein levels. Furthermore, the ER stress-induced apoptosis response proteins CHOP, pJNK and cleaved caspase-3 are decreased with CDNF overexpression. Collectively, our results suggest that protective effect of CDNF are related to the inhibition of ER stress-induced apoptosis by increasing GRP78 expression, a principal regulator of UPR. Thus, here we show a role of CDNF in ER proteostasis and suggest a role for CDNF in the context of neuroprotection.

**Disclosures: D.A. Arancibia:** None. **P. Zamorano:** None. **M.E. Andres:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.04/Q8

**Topic:** C.03. Parkinson’s Disease

**Support:** CNPq

CAPES

FAPERGS

IPA

**Title:** Pakinson's disease provokes oxidative stress and movement disorders: Neuroprotective potential of purple grape juice

**Authors: *C. S. FUNCHAL**[1], M. ROCHA FRUSCIANTE[1], A. SOUTO FERREIRA[1], J. PEREIRA MARINHO[1,2], A. QUINCOZES DOS SANTOS[2], D. POCHMANN[1], C. DANI[1]
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**Abstract:** Parkinson’s Disease (PD) is a chronic degenerative disease of the central nervous system. The aim of this study was to evaluate the effect of purple grape juice on the movement disorders and oxidative parameters in hippocampus of reserpine-treated rat model of PD. Adult Wistar rats were treated with water or purple grape juice (gavage) for 14 days (7 μL/g) and on the 15$^{th}$ day the animals received a subcutaneous single dose of reserpine (1.0 mg/mL/kg) for induction of DP. After 1, 6 and 24 h induction of PD model some motor and behavioral parameters were observed by a trained evaluator: head, jaw and vibrissae movements, and changes in curvature and tail rigidity. After 24 h of PD induction, the hippocampus was dissected for analysis of the parameters of oxidative stress: lipid peroxidation (TBARS), carbonyl, sulfhydryl (SH), reduced glutathione (GSH) and the activity of the antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (CEUA IPA-15/2015).

Rats treated with reserpine presented changes in curvature and tail stiffness, repeated jaw, head and vibrissae movements after1, 6 and 24 h of PD induction. Purple grape juice prevented most of these changes. Reserpine increased the levels of carbonyl, reduced GSH and the activity of CAT and SOD in hippocampus. Purple grape juice was effective in preventing all damages. The levels of TBARS and GH were not modified. Therefore, purple grape juice revealed a possible antioxidant and neuroprotective effect and might be used as a therapeutic adjuvant in PD patients.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.05/Q9

**Topic:** C.03. Parkinson’s Disease

**Support:** Department of Biotechnology, New Delhi, India

Council of Scientific and Industrial Reserch, New Delhi, India

**Title:** Silymarin corrects chaperon-mediated autophagy and contributes to neuroprotection in MPTP-induced Parkinsonism

**Authors: *M. K. TRIPATHI**, M. S. U. RASHEED, A. K. MISHRA, M. P. SINGH
TOXICOGENOMICS AND PREDICTIVE TOXICOLOGY DIVISION, CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH, LUCKNOW, India

**Abstract:** 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induces α-synuclein aggregation and impairs lysosomal function. Abnormal α-synuclein is eliminated from the nigrostriatal dopaminergic neurons primarily via the chaperon-mediated autophagy (CMA). Impaired CMA leads to an accumulation of aggregated α-synuclein leading to Parkinson’s disease in humans and Parkinsonism in intoxicated experimental animals. Silymarin, an antioxidant, anti-inflammatory and anti-apoptotic agent, is found to encounter MPTP-induced oxidative stress and neuronal apoptosis. The present study aimed to investigate the effect of silymarin on MPTP-induced changes in CMA and Parkinsonism. Male Swiss albino mice (20-25 g) were treated intraperitoneally with silymarin (40 mg/kg, daily) for 15 days along with vehicle controls. Subsets of animals were also treated with MPTP on the day 7 (20 mg/kg intraperitoneally; 4 times at 2 h interval). Mice were sacrificed, brain was dissected out and western blot analysis of α-synuclein, heat shock cognate-70 (Hsc-70) and lysosome-associated

membrane protein-2A (LAMP-2A) was performed. Besides, immunofluroscence employing lysotracker and dansylcadaverine dye was also performed to ensure the autophagosome formation and lysosomal quality. Silymarin significantly encountered MPTP-induced changes in the expression of LAMP- 2A and Hsc-70 proteins in the nigrostriatal tissue. Silymarin attenuated the level of aggregated α-synuclein accumulation and increased the autophagosome formation in the substantia nigra of MPTP-treated mice. The results obtained thus suggest that silymarin corrects CMA and contributes to neuroprotection in MPTP-induced Parkinsonism. **Keywords:** Parkinson's disease; Antioxidants; Autophagy

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 572.06/Q10

**Topic:** C.03. Parkinson's Disease

**Support:** Ratchadapiseksomphot Endowment fund (CU-072-AS), Chulalongkorn University.

**Title:** Neuroprotective effects of the standardized extract of Centella asiatica ECa233 in rotenone-induced parkinsonism rats

**Authors: *N. TEERAPATTARAKAN**[1], H. BENYA-APHIKUL[1], R. TANSAWAT[2], O. WANAKHACHORNKRAI[3], M. TANTISIRA[4], R. RODSIRI[1]
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**Abstract:** *Centella asiatica* extract exhibits various pharmacological activities *in vitro* and *in vivo*. In this study, the neuroprotective effects and underlying mechanisms of the standardized extract of *Centella asiatica* ECa233 were investigated in rotenone model of Parkinson's disease. Male Wistar rats were divided into three experimental groups (n=10/group) which were control, PD and PD+ECa233. Rats in control and PD groups received 0.5% CMC (1 mL/kg p.o.) while rats in PD+ECa233 received ECa233 (30mg/kg p.o.) for 14 days. On day 15-20, rats in the control group received 2% DMSO (1 mL/kg i.p.) with 0.5% CMC while PD and PD+ECa233 rats received rotenone (2.5 mg/kg i.p.) with 0.5% CMC and ECa233 respectively . Rats were performed locomotor activity test on day 1, 14 and 20. Brains were collected and determined tyrosine hydroxylase (TH)-positive cells and malondialdehyde (MDA) levels. The results showed that locomotor activity of rats in PD+ECa233 group was significantly higher than that of

rats in the PD group on day 20 ($p<0.05$). In addition, ECa233 protected against rotenone-induced dopaminergic neuronal loss in the substantia nigra as the TH-positive cells of rats in PD+ECa233 group were significantly higher than that of PD rats ($p<0.05$). ECa233 tended to decrease brain MDA levels in rotenone-treated rats, but this effect did not significantly different from PD rats. The present study showed that ECa233 can protect against rotenone-induced neurotoxicity via ameliorating lipid peroxidation. As rotenone is a mitochondria complex I inhibitor, the mitochondria protection effect of ECa233 should be further investigated.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.07/Q11

**Topic:** C.03. Parkinson’s Disease

**Support:** NIH Grant RO1

**Title:** NADPH oxidase (NOX1) mediates testosterone-induced neurodegeneration

**Authors: *M. A. TENKORANG**[1], R. L. CUNNINGHAM[2]
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**Abstract:** One of the primary characteristics of Parkinson’s disease (PD) is oxidative stress (OS). Men have a higher risk for PD than women. Testosterone, a primary male sex hormone has been implicated in PD, and is a known oxidative stressor. Previous studies in our lab have shown that testosterone exacerbates OS damage in dopaminergic neurons. However, the mechanism by which testosterone increases OS is unknown. We hypothesize that in dopaminergic cells, testosterone increases OS by activating NOX 1, a major OS generator in cells. To test our hypothesis, we used a dopaminergic cell line (N27 cells). For an oxidative stressor, we used tert-butyl-hydrogen peroxide ($H_2O_2$) to induce 20% cell loss prior to testosterone (100nm) administration. NOX1 inhibitors (Apocynin, Diphenyleneiodonium-DPI) were administered before $H_2O_2$ exposure. Cell viability and Oxidative stress were quantified using the MTT and Reduced Thiols assays respectively.
Testosterone is only damaging in the presence of OS. DPI, alone, was damaging to N27 cells, hence this was no longer used as a NOX1 inhbitor. Unlike DPI, Apocynin had no effect on cell viability and oxidative stress. Further, Apocynin alone, did not alter $H_2O_2$-induced cell loss, indicating that $H_2O_2$ increases OS via a non-NOX1 mechanism. However, Apocynin blocked testosterone’s induced cell loss and oxidative stress generation. Testosterone-induced cell loss is

mediated by NOX1, indicating that NOX1 is involved in testosterone induced OS generation. By understanding testosterone's mechanism of action, potential therapeutic targets for Parkinson's disease can be explored.

**Disclosures: M.A. Tenkorang:** None. **R.L. Cunningham:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Topic:** C.03. Parkinson's Disease

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Fundación Séneca (19540/PI/14)

"Prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development" -European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative Grant Agreement No115009

**Title:** Physical activity alone is not enough in Parkinsonism; but significantly protective when combined with an antioxidant

**Authors: *A.-L. GIL-MARTÍNEZ**, L. CUENCA, C. ESTRADA, E. FERNÁNDEZ, M. HERRERO
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**Abstract:** Neuroprotection is becoming relevant to slow down dopaminergic cell death and inflammatory processes related to progressive degeneration in Parkinson's Disease (PD). Interestingly, among others, physical exercise and anti-oxidant treatments (such as *N*-acetyl-L-cysteine, NAC) are common therapeutic strategies. Therefore, this study aims to analyze the synergistic effect of physical activity and NAC treatment on inflammatory activation and dopaminergic degeneration in a Parkinsonism -induced 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model. To ascertain this possibility, 48 eight-weeks-old mice (C57BL/6 strain) were used. 24 of them were individually placed in cages where voluntary physical exercise was monitored during four weeks and divided into groups: i) control; ii) NAC treatment; iii) MPTP and iv) MPTP+NAC. The other 24 mice were divided into the same four groups, described above, without physical exercise. Post-mortem studies with midbrain cells (substantia nigra, SNpc) and striatum show a significant decrease of the cell death in all treated groups compare with MPTP group but especially significant in NAC+exercise group. Moreover, it was observed a significant decrease of GFAP and Iba-1 expression in NAC groups and a very

significant in NAC+exercise groups. These results suggest that physical exercise, on its own, does not have an improvement effect on neuronal death and inflammation activity, it is necessary to combine it with an anti-oxidant (as NAC) so that these effects are significant. In conclusion, the combination of physical exercise with an anti-oxidant drug has a synergistic effect improving motor condition as well as reducing inflammatory processes that protects dopaminergic neurons against neurodegeneration.
Research work of the Authors was supported by the Spanish Ministry of Science and Innovation (FIS PI13 01293), Fundación Séneca (19540/PI/14) and "Prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development" (European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative under Grant Agreement No 115009) to MTH.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.03. Parkinson's Disease

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"Prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development"European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative (Grant Agreement No115009)

**Title:** Is combined treatment neuroprotective in old parkinsonian mice? Story of NAC and HA-1077

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**Abstract:** Experimental and clinical evidence suggests that inflammatory factors, controlled by glial cells may play an important role in the development of neurodegenerative processes associated with Parkinson's disease. Even though PD is an aging-related disorder, only few studies have been performed in old animals. Otherwise, it has been demonstrated that c-Jun N-terminal kinase (JNK), an important kinase member of the MAPK family, is implicated in 1-

methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxic mechanisms. Therefore, the inhibition of JNK could prevent or delay the dopaminergic injury of MPTP intoxication and the secondary inflammatory response. In this study, we analyzed two different agents which mechanisms of action are related to JNK pathway: i) the *N*-acetyl-L-cysteine (NAC), a glutathione precursor, and ii) HA-1077, a ROCKinase inhibitor and microglia polarizer. Additionally, we evaluated the combination of both treatments (NAC+HA-1077). 75 twenty-weeks-old C57BL/6 mice were used in this study; 33 of them were control groups and the other 42 animals were acutely intoxicated with MPTP and divided into 4 groups: i) MPTP; ii) MPTP+NAC; iii) MPTP+HA1077; iv) MPTP+NAC+HA1077. Postmortem quantitative analysis showed a significant decrease of TH expression in the SNpc and in the striatum as well as significant increased Iba-1 and GFAP levels in all MPTP-animals comparing with control groups. However, old-Parkinsonian mice treated with NAC had TH+ cells and fibers, GFAP and Iba-1 expression similar to control animals. Surprisingly, microglial and astroglial cells were significantly incremented in MPTP-intoxicated animals treated with both drugs (NAC+HA1077) compared with all the other MPTP groups. Unfortunately, these unexpected results discard the use of the combined treatment (HA1077+NAC) in old mice. However, NAC treatment does have neuroprotective effects in old Parkinsonian mice probably due to its anti-oxidant properties (associated to JNK inhibition).
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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.10/R2

**Topic:** C.03. Parkinson’s Disease

**Support:** NRF Grant 2017R1A2B4008456

NRF Grant 2011-0030049

**Title:** Neuroprotection induced by HDAC inhibitor in Parkinson's disease model

**Authors: *S. SONG**, T. KIM, J. KIM, H. NOH, S. KANG, H. SEO
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**Abstract:** Parkinson’s disease (PD) is one of the progressive neurodegenerative diseases with dopaminergic neuronal loss and Lewy body deposit in substantia nigra pars compacta. We hypothesized that histone hypoacetylation is associated with progressive pathology of PD. In this study, we aimed to understand target of HDAC inhibition for PD. We determined the effects of pan HDAC inhibitor as well as selective HDAC inhibitor such as valproic acid (VPA), sodium butyrate (SB), MS-275, and tubacin, in dopaminergic SHSY-5Y cells. We also administered VPA in PD model LRRK2 R1441G mice. VPA significantly decreased cytokine expression levels and PD pathological markers in midbrain region of LRRK2 R1441G mice. VPA also significantly increased the number of TH positive neurons in substantia nigra of LRRK2R1441G mice. VPA increased latency to fall in rota-rod test and decreased number of rearing counts in open-field test in LRRK2R1441G mice. These data suggest that histone deacetylation may be a promising therapeutic target for PD.
*S.S. and T.K. equally contributed to this work.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.11/R3

**Topic:** C.03. Parkinson’s Disease

**Title:** Neuroprotection using a novel PAAN1 inhibitor in model of Parkinson's disease

**Authors: *H. PARK**[1], T.-I. KAM[1], H. PENG[2], J. LIU[2], T. M. DAWSON[1], V. L. DAWSON[1]
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**Abstract:** Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta and the accumulation and aggregation α-synuclein (α-syn). Misfolded fibrillar forms of α-syn are thought to spread via cell-to-cell communication contributing to the progression and neurodegeneration in PD. However, the underlying molecular mechanisms by which α-syn causes cell death and neurodegeneration in PD is not known. Here, we show that the PAAN mediates α-syn-induced degeneration in A53T transgenic mice. Conditional expression of the familial associated A53T α-syn mutant in DA neurons of transgenic mice leads to loss of DA neurons and behavioral deficits that is significantly reduced in the absence of the PAAN. Further, we developed an endonuclease assay and screened a novel hybrid macrocyclic rapafucin library (~11,248 compoinds) to find PAAN inhibitors. Novel PAAN antagonists can inhibit DNA cleavage and

prevent α-syn induced degeneration. Thus, targeting PAAN activity may provide an important therapeutic opportunity in Parkinson's disease.

**Disclosures: H. Park:** None. **T. Kam:** None. **H. Peng:** None. **J. Liu:** None. **T.M. Dawson:** None. **V.L. Dawson:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 572.12/R4

**Topic:** C.03. Parkinson's Disease

**Title:** Block of A1 astrocyte conversion is neuroprotective in models of Parkinson's disease

**Authors: *T.-I. KAM**[1], S. YUN[1], N. PANIKER[1], Y. OH[2], J.-S. PARK[2], Y. PARK[2], S.-H. KWON[1], S. S. KARUPPAGOUNDER[1], H. PARK[1], S. KIM[1], S. LEE[1], S. BRAHMACHARI[1], D. KIM[1], D. NA[4], Z. MARI[3], V. L. DAWSON[1], S. LEE[2], T. M. DAWSON[1], H. KO[1]
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**Abstract:** Activation of microglia leads to the conversion of resting astrocytes to toxic A1 type astrocytes in a variety of neurodegenerative diseases. Development of agents that could inhibit the formation of A1 type astrocytes have profound therapeutic potential since they could be used to treat a variety of neurologic disorders for which there currently are no disease modifying therapies. The glucagon-like peptide-1 receptor (GLP1-R) agonist have shown some efficacy in providing a reduction in inflammation, augmentation in mitochondrial function and neuroprotection, however, the mechanism of how GLP1-R agonism elicits these actions, especially in Parkinson's disease (PD), is not known. Here we show that a potent, brain penetrant pegylated long acting GLP1-R agonist NLY01, has neuroprotective effect in PD models via direct actions on microglial mediated conversion to neurotoxic A1 astrocytes. NLY01 reduced pathological phosphorylation of α-synuclein, dopaminergic (DA) neuronal loss, and behavioral deficits in the α-synuclein preformed fibril (α-syn PFF) model of sporadic PD. In addition, NLY01 significantly prolongs the lifespan and reduces neuropathology of human A53T α-syn (hA53T) transgenic mice, a model of familial α-synucleinopathy. NLY01 protective effects are independent of its potential action on neurons, but appear to primarily involve inhibition of microglia. We show that α-syn PFF induced microglial activation is blocked by NLY01 both in vitro and in vivo. The inhibitory effect of NLY01 on microglial activation also prevents the conversion of resting astrocytes into toxic A1 astrocytes, which contributes to the demise of DA neurons in the α-syn PFF mouse model and in human DA neuronal cultures. Taken together these findings strongly suggest that NLY01 has broad neuroprotective properties in a variety of

neurodegenerative disorders including PD characterized and involving A1 astrocyte activation. In addition, due to its significantly increased half-life and bioavailability it offers greatly improved therapeutic opportunities for the treatment of chronic neurologic diseases.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Topic:** C.03. Parkinson’s Disease

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**Title:** Neuroprotective effect of MHY908, a PPAR α/γ dual agonist, on the MPTP-induced Parkinson’s disease model

**Authors:** ***Y. LEE**, S. LEE, W. LEE, J. LEE
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**Abstract:** Parkinson’s disease (PD) is a progressive neurodegenerative disease, and induced by a selective loss of dopaminergic neurons in the nigrostriatal pathway. Previous studies demonstrated that several peroxisome proliferator-activated receptor (PPAR) agonists were protective against various neurodegenerative diseases including PD. MHY908, developed as PPAR α/γ dual agonist, has been shown to have multiple effects, such as anti-diabetic, anti-inflammatory, and anti-melanogenic effects. In the present study, we evaluated neuroprotective effects of MHY908 on the PD mouse model. Pretreatment of MHY908 attenuated MPTP-induced dopaminergic neuronal loss and motor deficit. MPTP-induced glial activations were alleviated by MHY908 in nigrostriatal pathway. MHY908 was also effective to prevent $MPP^+$-induced neuronal cell death and ROS production in SH-SY5Y cells. Further study revealed that MHY908 inhibited $MPP^+$-induced astroglial activation via suppressing NF-κB signaling in primary astrocytes. Taken together, the present study suggests that PPAR α/γ dual agonist could be useful agent for therapeutic candidates for PD and other neurodegenerative diseases associated with neuroinflammation.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Program#/Poster#:** 572.14/R6

**Topic:** C.03. Parkinson’s Disease

**Support:** Council of Scientific and Industrial Research, New Delhi, India

**Title:** Melatonin protects from aberrant mitochondrial dynamics in cypermethrin model of Parkinson’s disease

**Authors: *A. K. MISHRA**, M. K. TRIPATHI, M. P. SINGH
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**Abstract:** The study aimed to investigate the effect of melatonin on the mitochondrial dynamics in a cypermethrin model of Parkinsonism. Male pups were treated with cypermethrin during the postnatal days 5-19 (1.5 mg/kg; twice a week) along with vehicles. Animals were re-challenged with cypermethrin during adulthood for 12 weeks (15 mg/kg, twice a week). Subsets of animals were also treated with melatonin (10 mg/kg, daily), during adulthood, 2 h prior to cypermethrin. Indicators of the mitochondrial dynamics were measured in the nigrostriatal tissue of control and cypermethrin-treated rats. Animals were sacrificed and nigrostriatal tissue was dissected out from the decapitated brain. Cypermethrin increased the expression of dynamin-related protein-1 (Drp-1) and Parkin as compared with control. Cypermethrin also increased the ubiquitination of the mitochondrial proteins. Melatonin reduced the expression of the mitochondrial Drp-1 and Parkin and ameliorated the ubiquitination of the mitochondrial proteins in cypermethrin-treated rats. Moreover, cypermethrin induced the translocation of optic atrophy-1 protein from the mitochondria to the cytosol, which was considerably normalized in melatonin co-treated rats. The results of the study indicate that melatonin resists the impairment of the mitochondrial dynamics in cypermethrin-induced Parkinsonism.
**Keywords:** Parkinsonism; Melatonin; Mitochondria

**Disclosures:** **A.K. Mishra:** None. **M.K. Tripathi:** None. **M.P. Singh:** None.

**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.15/R7

**Topic:** C.03. Parkinson’s Disease

**Title:** Plasticidade Promovida pelo Exercício Físico no Córtex Motor e no Comportamento Motor de Ratos na Fase Inicial do Modelo da Doença de Parkinson

**Authors: *K. H. BINDA**[1], P. C. GARCIA[1], C. D. CARNEIRO[2], D. D. FARIA[2], C. A. BUCHPIGUEL[2], L. R. G. BRITTO[1], C. C. REAL[1]
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**Abstract:** Parkinson's disease (PD) is common among the elderly. Physical exercise, in turn, is described by its beneficial effects. The purpose of this study was to analyze treadmill exercise, may promote plasticity in the motor cortex, area with altered excitability in PD. Wistar rats were submitted to the unilateral PD model induced by striatal 6-OHDA, and a treadmill exercise protocol (3x / week for 40 minutes). Groups control received saline injection only. The animals were divided into 4 groups (n = 5 animals / group). Before induction of the model and 10 days later, the animals were submitted for 5 minutes to the cylinder test for motor behavior analysis. The plasticity in the motor cortex was determined by the expression of synaptic proteins (synapsin; synaptophysin) and structural (neurofilaments; MAP-2) 10 days after the induction of the model. In addition, immunohistochemical data were compared with data obtained on positron emission tomography labeled with [18F] FDG radiopharmaceutical. The results showed a neuroprotection of the dopaminergic neurons in the pars compacta in the exercised+6-OHDA animals compared to the sedentary animals+6-OHDA (27%, $p < 0.05$, SED + 6-OHDA 72 ± 5, EX + 6 -OHDA 99 ± 3). In addition, dopaminergic depletion in the striatum is also reduced in the exercised animals (15%, $p < 0.05$, SED + 6-OHDA 0.73 ± 0.05, EX + 6-OHDA 0, 88 ± 0.04). In the cylinder test, the SED + 6-OHDA group revealed asymmetry in the use of the paws, which was not observed in the exercised group (16%, $p < 0.05$, SED + 6-OHDA 69 ± 7.4, EX + 6 -OHDA 53 ± 0.8, EX 50 ± 0.7), suggesting an improvement in motor behavior. Besides that, there was an increase in MAP-2 expression in the motor cortex (primary motor cortex 133%, $p < 0.0001$, SED + 6-OHDA 1.82 ± 0.15, EX + 6-OHDA 0.50 ± 0 , EX 1.08 ± 0.02, secondary motor cortex 78%, $p < 0.0001$, SED + 6-OHDA 1.77 ± 0.41 EX + 6-OHDA 1.85 ± 0.85 EX 0 , 94 ± 0.06), and decreased neurofilaments in the primary motor cortex of the SED + 6-OHDA group (ca. 50%, $p < 0.05$, SED + 6-OHDA 0.55 ± 0.17, EX + 6-OHDA 1.10 ± 0.197, EX 1.20 ± 0.125). The expression of synaptophysin showed an increase in the primary motor cortex of SED + 6-OHDA animals (36%, $p < 0.01$, SED + 6-OHDA 1.36 ± 0.24, EX + 6-OHDA 1.03 ± 0.04 ; EX 0.99 ± 0.03). Therefore, we can suggest that this model promotes alteration of cortical

excitability, which seems to be reversed by the exercise protocol. Data from [18F] FDG in the striatum corroborate immunohistochemical data with decreased uptake (10%, $p < 0.001$) in SED + 6-OHDA animals, suggesting lower dopaminergic activity. Thus, this study suggests that this exercise protocol may be able to normalize cortical excitability and improve motor behavior that occurs in the early stage of the disease.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Topic:** C.03. Parkinson's Disease

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**Title:** Neuroprotective effect of the C-Terminal domain of the heavy chain of tetanus toxin on dyskinesia caused by Levodopa in 6-hydroxydopamine-lesioned rats

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**Abstract:** Recombinant Hc-TeTx fragment has been successfully assayed as a neuroprotector in Parkinson's disease (PD) models. While levodopa has been the gold standard in the therapeutic treatment of PD, unfortunately almost all patients develop dyskinesia or abnormal involuntary movements (AIMs). The aim of this study was to evaluate the effect of post-lesion or pre-lesion treatment with Hc-TeTx on levodopa-induced dyskinesia in rats. AIMs were induced with low, mid and high levodopa doses (6, 10 and 25 mg/kg respectively) and examined for 22 days. Also all rats were monitored the motor activity in closed-field box or cylinder test. Finally, tyrosine hydroxylase-positive (TH) neurons in the *substantia nigra pars compacta* (SNpc) and FosB/ΔFosB expression in the striatum were examined. Hc-TeTx (20 µg/kg i.m.) co-administrated with levodopa (6 or 10 mg/kg) did not reduce the severity of the AIMs, while the

group treated with Hc-TeTx plus levodopa (25 mg/kg) presented a temporary attenuation of dyskinetic limb and orolingual movements. However the restorative effects of Hc-TeTx on motor behavior and dopaminergic neuronal death in the SNpc were not observed. There was a significant post-treatment increase in FosB/ΔFosB expression in the dorsolateral and ventral striatum of animals treated with Hc-TeTx. When Hc-TeTx was administrated prior to dopaminergic lesion and levodopa administration (10 mg/kg), there was a slight recovery of motor asymmetry, one week post-lesion. Moreover, while the Hc-TeTx slightly reduced the AIMs score in the first days of levodopa treatment, it was not effective by the end of the experiment. Also, the loss of TH neurons in the SNpc revealed that Hc-TeTx did not induce effective neuroprotection. It is worth noting that a reduction of FosB/ΔFosB expression in the dorsolateral striatum was observed. In summary, Hc-TeTx treatment did not effectively reduce the induction of AIMs by levodopa in hemiparkinsonian rats, although the pre-treatment administration of Hc-TeTx causes minor FosB expression in the dorsolateral striatum.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 572.17/R9

**Topic:** C.03. Parkinson’s Disease

**Title:** Neuroprotective effects of spinal cord stimulation on Parkinson's disease model of rats

**Authors: *K. KUWAHARA**, T. SASAKI, Y. TOMITA, M. UMAKOSHI, I. KIN, K. KIN, J. MORIMOTO, M. OKAZAKI, A. SHINKO, M. KAMEDA, T. YASUHARA, N. TAJIRI, I. DATE
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**Abstract:** Object
In clinical practice, deep brain stimulation (DBS) is effective for the treatment of motor symptoms in Parkinson’s disease (PD). However, the mechanisms have not been understood completely. There are some reports that electrical stimulation exerts neuroprotective effects on the central nervous system disorders including cerebral ischemia, head trauma, epilepsy and PD. Meanwhile, there are only few reports on neuroprotective effects of spinal cord stimulation (SCS). In the present study, we investigated neuroprotective effects of SCS on PD model of rats.
Methods
Adult female Sprague-Dawley rats received hour-long SCS (2, 50 or 200Hz) with an epidural electrode at C1-2 level for 16 consecutive days. At 2 days after initial SCS, 6-hydroxydopamine (6-OHDA), a neurotoxin of dopaminergic neurons, was injected into the right striatum of rats.

Behavioral evaluations of PD symptoms, including cylinder test and amphetamine-induced rotation test, were performed at 1-2 weeks after 6-OHDA injection. Animals were subsequently euthanized for immunohistochemical investigations. In order to explore neurotrophic and growth factor upregulation induced by SCS, another cohort of rats that received 50Hz SCS was euthanized at 1 and 2 weeks after lesion for protein assays.
Results and Discussion
Behavioral tests revealed that the number of amphetamine-induced rotations decreased in SCS groups. Immunohistochemically, tyrosine hydroxylase (TH)-positive fibers in the striatum were significantly preserved in SCS groups. TH-positive neurons in the substantia nigra pars compacta were significantly preserved in 50Hz SCS group. The level of vascular endothelial growth factor (VEGF) was upregulated by SCS at 1 week after the lesion. These results suggest that SCS exerts neuroprotection in PD model of rats, at least partially by upregulation of VEGF. SCS is supposed to suppress or delay Parkinson's disease progression and might become a less invasive therapeutic option for PD patients. Although neuroprotective effect was observed by hour-long SCS paradigm for 16 consecutive days in the present study, longer continuous stimulation might exert greater effect. We are currently investigating this possibility.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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**Topic:** C.03. Parkinson's Disease

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**Title:** Neuroprotective effect of chronic spinal cord stimulation (SCS) in a alpha-synuclein animal model of Parkinson's disease

**Authors: A. PARRA PEÑA**[1,3,4], R. VIDAL[5,3], *R. A. FUENTES[2,3,4]
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**Abstract:** Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the presence of inclusions in dopaminergic neurons from substantia nigra, known as Lewy bodies, and the progressive loss of these neurons. The protein α-Synuclein (α-syn) constitutes one of the main components of the Lewy bodies in sporadic cases of PD. Animal models overexpressing α-

syn exhibit neuronal degeneration and abundant α-syn-positive inclusions, which recapitulates essential neuropathological features of PD. On the other hand, chronic Spinal Cord Stimulation (SCS), a neuromodulation technique consisting in the epidural delivery of electrical pulses in the dorsal portion of the spinal cord, has emerged as a potential treatment of PD. SCS showed to be very effective alleviatingparkinsonian motor symptoms both in animal models and patients. Evidence collected in both the classical 6-OHDA neurotoxin and the α-syn animal models of PD, suggest that SCS could have long-term effects associated to neuroprotection. Neurotrophic factors, i.e. the Vascular Endothelial Growth Factor (VEGF), have been shown to support the survival of many neuronal populations both in culture and pre-clinical models of PD. Our research focuses in the study of the neuroprotective mechanisms that could be involved in the improvement of the motor performance of an α-syn animal model of PD submitted to chronic spinal cord stimulation. We used an α-syn animal model corresponding to Sprague Dawley male rats injected unilaterally in the substantia nigra with adenovirus AAV6-α-syn. Four weeks after the viral injection, rats were treated with high-frequency (300 Hz) SCS during five weeks, two sessions/week. Assays of immunohistochemistry showed that chronic SCS modulates the expression of vascular endothelial growth factor (VEGF) in the nigro-striatal pathway. This suggests that chronic SCS might exert neuroprotective effects through the regulation of VEGF, which is known for angiogenic and pro-survival functions.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

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VCRGE, University of Wisconsin – Madison

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**Title:** Post mortem evaluation of sympathetic neurodegeneration and neuroprotection in a nonhuman primate model of cardiac dysautonomia

**Authors:** ***J. SHULTZ**[1,2], R. FLEDDERMANN[1], H. MATSOFF[1], G. WACHOWSKI[1], V. BONDARENKO[1], H. SIMMONS[1], A. KAPOOR[1], T. ZIEGLER[1], C. MOORE[3], M. E.

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**Abstract:** Cardiac dysautonomia is a common nonmotor symptom of Parkinson's disease associated with loss of sympathetic innervation to the heart and decreased plasma catecholamines. Disease-modifying strategies are not available, and biomarkers are lacking. Systemic administration of the catecholaminergic neurotoxin 6-hydroxydopmaine (6-OHDA) recapitulates this loss of cardiac sympathetic innervation and circulating catecholamines. We recently used positron emission tomography (PET) imaging in 10 6-OHDA-treated (50mg/kg; iv) adult, male rhesus to visualize and quantify cardiac sympathetic neurodegeneration and increased inflammation and oxidative stress following neurotoxin, which were attenuated in pioglitazone treated rhesus (n=5; 5mg/kg) compared to placebo (n=5). Here we report post mortem characterization of heart and adrenal tissue in these animals compared to age and gender matched normal controls (n=5). Tissues were collected following PFA perfusion, blocked in paraffin, sectioned at 5µm, immunostained, and immunoreactivity (-ir) quantified as area above threshold (%AAT) in NIH ImageJ. In the heart, immunohistochemistry was performed for sympathetic innervation (tyrosine hydroxylase; TH) and inflammation (HLA-DR) in 3 base to apex layers and 4 regions (septal, anterior, lateral, inferior) of the left ventricle. Average TH-ir in all left ventricle nerve fascicles was significantly lower in pioglitazone (48.2%) and placebo (46.9%) groups than in healthy controls (65.6%; ANOVA $p<0.001$). Placebo vs pioglitazone groups showed a difference in combination with cardiac layer and region (ANOVA $p=0.039$). No group differences were observed for HLA-DR. Adrenal tissue was analyzed in the medulla for catecholamine production capability by TH-ir and aromatic amino acid decarboxylase (AADC)-ir. TH-ir revealed significant 6-OHDA associated loss (control 90.5%; placebo 53.6%; ANOVA $p<0.0001$), and preservation in the pioglitazone group compared to placebo (pioglitazone 78.9%; $p<0.01$). AADC-ir confirmed these findings, with a significant difference between control (86.7%) and placebo (50.0%) animals (ANOVA $p<0.01$), but not between control and pioglitazone (69.0%). HPLC for plasma norepinephrine (NE) showed dramatic %loss from baseline to 1wk post-6-OHDA in placebo (69.1%) and pioglitazone (57.4%) groups, which was significantly higher in placebo animals compared to pioglitazone (Mann Whitney $p=0.016$). Overall, these results validate in vivo findings of 6-OHDA associated cardiac sympathetic denervation and demonstrate the ability of pioglitazone to preserve enzymes critical for catecholamine production in the adrenal medulla.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.20/S2

**Topic:** C.03. Parkinson’s Disease

**Support:** MJFF Foundation Validation Mechanism

Graduate and Postdoctoral Training in Environmental Health Science and Toxicology NIH Fellowship 2T32ES012870-11

**Title:** Modulation of peripheral and central inflammation via cannabinoid type 2 receptors to protect against Asyn-induced PD-like pathologies

**Authors: *V. JOERS**[1], B. MURRAY[1], D. OLIVER[1], S. KELLY[1], F. P. MANFREDSSON[2], B. M. MOORE, III[3], M. G. TANSEY[1]
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**Abstract:** Numerous studies have demonstrated that neuroinflammation accompanies and may promote progression of alpha-synuclein (Asyn)-induced nigral dopaminergic degeneration as found in Parkinson’s disease (PD). During degenerative processes, peripheral immune cells cross the BBB and can exert actions on the brain, yet it remains unclear how their brain activity affects PD progression. Modulating the activation state of microglia or infiltrating cells can largely alter the local environment via cytokine signaling, and in turn impact neuronal survival. The cannabionoid type 2 receptor (CB2) is highly expressed on activated microglia and circulating monocytes, upregulated in the nigra of PD patients and when modulated protects against rotenone-induced nigral degeneration. Studies conducted by collaborator Bob Moore using a novel CB2 inverse agonist SMM-189 demonstrated immunomodulatory effects that improve acute neuronal injury and behavioral outcomes potentially explained by a mechanism that suppresses pro-inflammatory markers and increases anti-inflammatory effects. Here we report the peripheral and central immunomodulatory effect of SMM-189 treatment in an AAV2/5-hAsyn rat model of PD. We hypothesize that targeting CB2 will benefit the local central inflammatory environment by inhibiting activation of central and peripheral immune cells and promoting phagocytosis and clearance of Asyn to protect against PD-like pathology. Spraque-Dawley rats (n=28) were unilaterally injected in the nigra with AAV2/5-hAsyn and followed for 8 weeks. One week later, animals were randomly selected to receive daily systemic SMM-189 (6 mg/kg, n=14) or vehicle (n=14). At sacrifice, peripheral blood mononuclear cells (PBMCs) were isolated, animals were transcardially perfused, and midbrain post-fixated for immunohistochemistry. Our preliminary results indicate, rats treated with SMM-189 demonstrate

a decreased size of IBA1+ cells ($p<0.0001$) compared to vehicle-treated, suggesting that targeting CB2 ameliorates persistent microgliosis from Asyn overexpression. Additionally, SMM-189 decreased gene expression of pro-inflammatory marker TNF ($p=0.0084$) while promoting anti-inflammatory behavior with increased TGFβ ($p=0.0204$) in PBMCs, suggesting that SMM-189 skews the peripheral immune phenotype towards an alternatively activated state. We are currently completing analysis of MHCII to establish the microglial activation state, toxic Asyn to determine the effect on Asyn aggregate clearance, and PBMC frequencies and cell surface markers by flow cytometry to elucidate changes in circulating immune populations following SMM-189 treatment.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.03. Parkinson’s Disease

**Support:** Support for this study was provided by the Neuroscience program, the College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at CMU

**Title:** Transplantation of partial differentiated dopaminergic like cell into in the 6-ohda rat model of Parkinson’s disease

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**Abstract:** Parkinson’s disease (PD) is a progressive and continuous neurodegenerative disorder. Transplantation of human embryonic dopaminergic progenitors within the striata of PD patients has given the field encouraging results, but ethical concerns and tissue availability limit this approach. The use of mesenchymal stem cells (MSCs) as an alternative cell source for transplantation circumvents the ethical issues, and provides a readily available source of cells, as they are derived from adult tissue. In this study, we transplanted MSCs that were semi-differentiated into dopamine like cells (DA). MSCs were cultured with a DA gene expressing adenovirus, to initiate differentiation. We transplanted these semi-differentiated DA-like cells

into the striatum of rats who were given unilateral 6-hydroxydopamine (6-OHDA) lesions. The unilateral 6-OHDA lesion was assessed utilizing the cylinder test and by assessing the asymmetry of amphetamine-induced rotations. The induced DA-like cells were transplanted into the dorsal striatum of rats at 4 weeks, following verification of the 6-OHDA lesion. After transplantation, rats were divided into two groups, with one group sacrificed at 8 weeks post transplantation and the second group at 24 weeks post transplantation. There was a significant improvement in the cylinder test observed for both groups. In addition to a significant improvement in amphetamine-induced rotational asymmetry was observed for both groups. These results are suggestive of a potential clinical utility for this method.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.22/S4

**Topic:** C.03. Parkinson’s Disease

**Support:** Michael J Fox Foundation

**Title:** ANAVEX 2-73, a clinical Alzheimer drug candidate, induces neurorestoration in experimental parkinsonism

**Authors: *V. FRANCARDO**[1], F. BEZ[1], J. S. SPROUSE[2], C. MISSLING[2], A. CENCI[1]
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**Abstract:** Background: Sigma-1 receptor (Sig-1R) is an endoplasmic reticulum-chaperone protein promoting mechanisms that protect cells under stress. We have previously shown that a selective agonist of the Sig-1R promotes recovery of motor functions and activates neuroplasticity mechanisms in a mouse model of nigrostriatal dopaminergic degeneration (Francardo et al Brain 2014).
Aim: Using the same animal model as in our previous study, we set out to evaluate the potential neurorestorative effects of ANAVEX 2-73 (ANA 2-73), a ligand at both Sig-1 and muscarinic receptors currently being tested in Alzheimer´s disease patients.
Methods: Mice sustained intrastriatal 6-OHDA lesions and were treated with ANA 2-73 (doses 0.3, 1 and 3 mg/kg/day) for 5 weeks, starting on the day of the lesion. Tests of forelimb hypokinesia and spontaneous rotation were carried out at the end of each week. After this period, brains were processed for tyrosine hydroxylase (TH) immunohistochemistry.

Results: After 5 weeks of treatment, the two lower doses of ANA 2-73 induced a significant behavioral improvement in all the behavioral tests. The highest dose of ANA 2-73 (3 mg/kg) significantly improved animals' forelimb use in the cylinder test (dependent on a dopaminergic activity in the dorsolateral striatum) but did not have effects on the spontaneous rotations (depending mostly on dopamine stimulation in the substantia nigra, SN). Immunohistochemical analysis of TH immunoreactivity revealed a massive TH+ fibers innervation in the dorsolateral striatum of mice treated with ANA 2-73, regardless of the dose. These fibers were positive for GAP43, a marker of axonal regeneration. In the SN, only the two lower doses of ANA 2-73 significantly protected nigral dopaminergic neurons, whereas the dose of 3 mg/kg did not have neuroprotective effects.
Conclusion: Our results reveal that ANA 2-73 ameliorates motor deficits in tests assessing spontaneous rotational activity and forelimb use asymmetry, and exerts noticeable neurorestorative effects on the damaged nigrostriatal dopamine system. Further experiments are now ongoing to elucidate the mechanisms underlying these effects.

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**Poster**

**572. Parkinson's Disease: Neuroprotective Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 572.23/T1

**Topic:** C.03. Parkinson's Disease

**Title:** Doxycycline protective role upon dopaminergic neuron

**Authors: *E. DEL BEL**[1], M. BORTOLANZA[2], M. DOS-SANTOS-PEREIRA[3], G. CRIVELARO-DO-NASCIMENTO[3], K. BARIOTTO-DOS-SANTOS[3], S. MARTIN[4], M. LAZZARINI[5], R. RAISMAN-VOZARI[6], W. STUEHMER[5]
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**Abstract:** The tetracycline-derivative doxycycline (α-6 deoxy-5-hydroxytetracycline) has been shown to be neuroprotective in *in vitro* and *in vivo* models of neurodegenerative diseases. The proven reliability and safety of the medication suggests its potential as an effective and inexpensive treatment to protect or at least mitigate the central nervous system from neurodegenerative diseases such as Parkinson's disease. It has been suggested that the

modulation of astrocyte and microglial activation could prevent neuronal demise and thus the progression of neurodegeneration. We hypothesize that doxycline could exert a neuroprotective effect by suppressing astrocyte and microglial activation induced by the neurotoxin 6-hydroxydopamine (6-OHDA), a preparation with similarities to Parkinson's disease. **Methods:** We investigated in striatal 6-OHDA lesioned mice the effects of a doxycycline given chronically either orally or subcutaneously at sub-antibiotic concentrations. To assess the protective mechanism conveyed by doxycycline we quantified using immunoreactive labeling tyrosine hydroxylase (neurons), glial fibrillary acid protein for astrocytes and cell surface marker macrophage antigen complex-1 for microglial cells. Brain regions containing cell bodies and fibers of dopamine in the nigrostriatal pathway were evaluated. Neuroinflammation indicators were sampled by Western blot analysis of metalloproteinase-3, cycloxygenase-2 and caspase-3 from the striatum. **Results:** Chronic treatment with doxycycline, either administered orally or injected subcutaneously at sub-antibiotic concentrations, mitigates the loss of dopaminergic neurons in the substantia nigra compacta and nerve terminals in the striatum. This protective effect was associated with: (1) a reduction of microglia in normal mice as a result of doxycycline administration *per se*; (2) a decrease in the astrocyte and microglia response to the neurotoxin 6-OHDA in the globus pallidus and substantia nigra compacta, and (3) decrease of the astrocyte reaction in the striatum. **Conclusion**: Our results suggest that doxycycline blocks 6-OHDA neurotoxicity *in vivo* by inhibiting microglial and astrocyte expression. This action of doxycycline in nigrostriatal dopaminergic neuron protection is consistent with a role of glial cells in Parkinson's disease neurodegeneration.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.01/T2

**Topic:** C.03. Parkinson's Disease

**Support:** National Natural Science Foundation of China Grant 31471308

**Title:** Ectonucleotidase CD73-mediated adenosine signaling regulates neuroinflammation in a Parkinson's disease model

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**Abstract:** The ecto-5' -nucleotidase (CD73) is one of the major ectonucleotidases that dephosphorylate extracellular AMP into adenosine in the brain. Recent studies have shown that CD73 is abundantly expressed in the striatum and physically associated with adenosine $A_{2A}$ receptors ($A_{2A}$Rs). Interestingly, suppression of $A_{2A}$Rs signaling has been shown to be beneficial for Parkinson's disease (PD). While studies have focused on the adenosine-mediated downstream signaling, little is known how extracellular adenosine production is regulated. Using enzyme histochemistry and HPLC analysis, we found that CD73 was the prominent ectonucleotides that generates adenosine in the striatum. Removal of CD73 significantly reduced the rate of microglia process retraction and enhanced acute injury- triggered striatal microglial movement. Moreover, CD73 deletion suppressed pro-inflammatory responses in LPS-primed microglia via suppressed $A_{2A}$R signaling pathway. Microglial activation and inflammation were attenuated in $CD73^{-/-}$ PD model (Mice were injected with 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride, namely MPTP, 25 mg/kg/day, i.p., for 5 days.). Deficiency of CD73 protected MPTP-induced neurotoxicity of dopaminergic neurons in the SNc (Substantia Nigra pars compacta) and behavioural impairment.
Together, our results reveal the neuroprotective role of blocking adenosine signaling in neuroinflammation, which may provide a new perspective to neurological disorders.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.02/T3

**Topic:** C.03. Parkinson's Disease

**Title:** The catalyzed assembly hypothesis: Novel drug-like small molecules that modulate assembly and toxicity of alpha-synuclein in a cell culture model of Parkinson´s disease

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**Abstract: Background**
The toxic effects of α-synuclein (αSYN) pre-fibrillar aggregates on dopaminergic neurons are believed to be central to the pathogenesis of Parkinson´s Disease (PD). So far, therapeutics targeting these toxic αSYN forms have not been successful. PD patients generally receive L-DOPA substitution therapy, although there is evidence that L-DOPA actually promotes degeneration of dopaminergic neurons and may accelerate PD progression. According to the catalyzed assembly hypothesis, protein complexes don't form spontaneously. Rather, their

formation is catalyzed by transient multi-protein complexes termed assembly machines. We have successfully identified cellular assembly machines for virus capsid formation and have generated a library of compounds with anti-viral activity by virtue of targeting allosteric sites governing composition of assembly machines essential for viruses.

**Objectives**

To screen for efficacy against αSYN aggregation our "Hit-finder" collection of 300 drug-like chemotypes active against aberrant forms of host assembly machines essential for viruses. This approach has been successfully used for ALS and Alzheimer's Disease.

**Methods**

We generated cellular models for robust αSYN aggregation in αSYN-transfected SH-SY5Y and performed toxicity assays in differentiated dopaminergic LUHMES cells. We applied the "Hit-finder" collection to these cells and monitored effects of assembly machine modulation on αSYN aggregation and toxicity.

**Results**

Two different assembly subcellular states of αSYN were observed, manifesting either as small aggregates or ring-like structures. These rings were αSYN associated with lipid droplets (LDs). The "Hit-finder" library contained subsets of chemotypes that were active in enhancing either the formation or dissolution of αSYN aggregates vs rings in a dose-dependent manner. Interestingly, the most potent ring-inducing compound showed the least degree of aggregated αSYN and was protective against rotenone and dopamine induced toxicity in differentiated LUHMES cells. Western Blots showed that the total αSYN load was slightly reduced. Early structure-activity relationship (SAR) optimization of a ring-inducing chemotype generated a lead compound with an $EC_{50}$ below 200 nM.

**Conclusion**

The compounds identified here prevent αSYN-mediated cell death of dopaminergic neurons via a novel molecular mechanism, namely, the increased association of toxic αSYN aggregates with LDs, thereby facilitating degradation of αSYN. With respect to L-DOPA substitution therapy, these compounds may be able to reduce neurotoxic side effects to allow enhanced, safer, and prolonged use of L-DOPA.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.03/T4

**Topic:** C.03. Parkinson’s Disease

**Support:** Jerry T. and Glenda G. Jackson Fellowship for Parkinson's Disease

NSCS Summer Research Program

Undergraduate Biology Research Program

**Title:** AAV2/1-hVEGF-B overexpression improves motor function in PINK1 gene knockout rats and prevents dopamine loss

**Authors: *M. J. BARTLETT**[1,2], D. C. Y. MULLER[1], B. D. SILASHKI[1], D. C. FARRELL[4], K. L. PARENT[4], K. P. DOYLE[1,3], M. L. HEIEN[4], S. J. SHERMAN[1], T. FALK[1,2]
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**Abstract:** Current Parkinson’s disease (PD) therapies, including the gold-standard, L-DOPA, treat patient’s symptoms but do not provide protection against the loss of dopaminergic cells in the substantia nigra (SN). One potential neuroprotective agent of interest is vascular endothelial growth factor B (VEGF-B). We have previously shown that the PD-inducing toxin, rotenone, upregulates endogenous VEGF-B in the dopaminergic cells of a rat midbrain culture model. In addition, exogenous VEGF-B provides neuroprotective support *in vitro.* VEGF-B is also neuroprotective *in vivo*. In the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD, an injection of VEGF-B into the striatum reduces the loss of motor function and reduces both the loss of dopaminergic cells in the SN and terminals in the striatum. Here we investigate the neuroprotective effects of VEGF-B in a PTEN-induced putative kinase 1 (PINK1) knockout rat model, a genetic model of PD. The PINK1 gene is thought to play a role in protecting cells from stress-induced mitochondrial dysfunction. PINK1 mutations are also linked to some cases of human familial PD.
In a pilot study, male, PINK1 KO rats, at 5-months of age were injected unilaterally with an adeno-associated virus expressing human VEGF-B (AAV2/1-hVEGF-B). AAV2/1-hVEGF-B overexpression is driven by a CAG promoter. The AAV2/1-hVEGF-B vector was injected at two sites in the striatum (AP +1.0, ML +3.0, DV -5.0; AP -0.6, ML +3.5, DV -5.0) and a single site in the SN (AP -5.0, ML +2.0, DV -7.2). AAV2/1-hVEGF-B treated PINK1 KO, PINK1 KO and wild type (WT) rats were tested monthly, up to 12-months of age, for changes in motor function with the tapered balance beam test (TBB). Unilateral AAV2/1-hVEGF-B treatment resulted in a significant improvement, as compared to untreated PINK1 KO rats, in the number of cumulative

(5-11 months) foot slip errors during TBB (One-way ANOVAs, Tukey post-hoc tests; n=5-6). These effects were seen bilaterally in both the forelimbs (ipsilateral, *p<0.05; contralateral, **p<0.01), and hind limbs (ipsilateral, **p<0.01; contralateral, *p<0.05).
Following behavioral testing, striatal tissue was analyzed via HPLC-EC to quantify changes in dopamine (DA). Striatal DA content was reduced by 25-30% in the PINK1 KO rats compared to WT, and restored to wild-type levels in the hemisphere of PINK1 KO rats injected with AAV2/1-hVEGFB (One-way ANOVAs; p<0.05; n=3-5). To delineate other potential mechanisms, we harvested striatal protein, and in a pilot analysis (n=2-4) found an increase in pigment epithelium derived factor (ELISA) and fatty acid transport protein 4 (semi-quantitative western blot) on the injected side.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.03. Parkinson’s Disease

**Support:** This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. NRF-2014R1A5A2009936)

**Title:** Proteomic change by Korean Red Ginseng in the striatum of a Parkinson's disease mouse model

**Authors: *S.-T. KIM**[1], D. KIM[1], S. KWON[2], H. JEON[1]
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**Abstract:** Recent studies have shown that Korean Red Ginseng (KRG) suppresses dopaminergic neuronal death in the brain of a Parkinson’s disease (PD) mouse model, but the mechanism is still elusive. Using a 2-dimensional electrophoresis technique, we investigated whether KRG can restore the changes in protein expressions in the striatum (ST) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-injected mice. Male C57BL/6 mice (9 weeks old) were injected with 20 mg/kg MPTP intraperitoneally four times at 2-h intervals. KRG (100 mg/kg) was orally administered once a day for 3 days from one hour after the first MPTP injection. Two hours after the third KRG administration a pole test was performed to evaluate motor function, after which the brains were immediately harvested. Survival of dopaminergic neurons in the nigrostriatal pathway and protein expression in the ST were measured by immunohistochemistry and 2-

dimensional electrophoresis. KRG suppressed MPTP-induced behavioral dysfunction and neuronal death in the nigrostriatal pathway. Moreover, 30 proteins changed by MPTP and KRG in the ST were identified and shown to be related to glycolysis/gluconeogenesis and neurodegenerative diseases including Alzheimer's disease and PD. KRG has neuroprotective effects against MPTP toxicity and alleviates protein expression profiles related to enhancing energy metabolism in the ST of MPTP-treated mice.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.05/T6

**Topic:** C.03. Parkinson's Disease

**Title:** PK-PD analysis identifies similar high amantadine plasma concentrations needed to reduce L-DOPA-induced dyskinesia across multiple species

**Authors: *B. BRIGHAM**[1], T. H. JOHNSTON[2], C. BROWN[1], J. D. S. HOLT[1], S. H. FOX[3], M. P. HILL[2], P. A. HOWSON[2], J. BROTCHIE[2], J. T. NGUYEN[1]
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**Abstract:** Dopamine replacement therapy with the dopamine precursor, L-DOPA, remains the most effective symptomatic treatment for Parkinson's disease. However, long term treatment invariably leads to the development of motor complications that include L-DOPA-induced dyskinesia (LID). There are currently no approved pharmacological therapies for LID in Parkinson's disease. Amantadine, an uncompetitive NMDA receptor antagonist, has been shown to reduce LID in rodent and non-human primate (NHP) models of LID, and in small clinical studies. While animal studies have consistently demonstrated that higher doses of amantadine resulted in greater efficacy, none of the studies determined the plasma concentrations associated with therapeutic effects.
Our objective was to evaluate the pharmacokinetic-pharmacodynamic (PK-PD) relationship between amantadine plasma concentrations and anti-dyskinetic efficacy across species to define optimal therapeutic dosing.
The single-dose pharmacokinetics of amantadine was determined in mice, rats, and cynomolgus monkeys, and PK modeling was conducted to estimate the plasma concentration at different doses. Efficacy data was generated from the 6-OHDA rat model and the MPTP NHP model of LID. These data, along with published anti-dyskinetic efficacy data in mice, rats, and NHPs, were used to establish the PK-PD relationship using a direct effect $E_{max}$ model. The model was then used to determine the effective plasma concentrations ($EC_{50}$) required for anti-dyskinetic

efficacy across animal species.
The PK-PD model demonstrated that the plasma concentrations required for the reduction of LID was highly consistent across species, with the $EC_{50}$ ranging from approximately 1100 ng/mL to 1600 ng/mL (mean ~1400 ng/mL). These results are consistent with a clinical study conducted with ADS-5102 (amantadine) extended release capsules, in which doses that provided reduction in LID were associated with mean plasma concentrations approximating 1400 ng/mL (Pahwa 2015).
In conclusion, amantadine plasma concentrations of ~1400 ng/mL are required to reduce LID across species from rodents to primates. These results provide target plasma concentrations for the reduction of LID in PD patients, which were confirmed in a clinical study with ADS-5102.
**References:** Pahwa R, Tanner CM, Hauser RA, Sethi K, Isaacson S, Truong D, Struck L, Ruby AE, McClure NL, Went GT, Stempien MJ. Amantadine extended release for levodopa-induced dyskinesia in Parkinson's disease (EASED Study). *Mov Disord.* 2015: 30(6): 788-795

**Disclosures:** **B. Brigham:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals. **T.H. Johnston:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka Inc.. F. Consulting Fees (e.g., advisory boards); Atuka Inc. **C. Brown:** F. Consulting Fees (e.g., advisory boards); Adamas Pharmaceuticals. **J.D.S. Holt:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals. **S.H. Fox:** None. **M.P. Hill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka Inc. F. Consulting Fees (e.g., advisory boards); Atuka Inc. **P.A. Howson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka Inc. F. Consulting Fees (e.g., advisory boards); Atuka Inc. **J. Brotchie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka Inc. F. Consulting Fees (e.g., advisory boards); Atuka Inc. **J.T. Nguyen:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.06/T7

**Topic:** C.03. Parkinson’s Disease

**Title:** Antiparkinsonian-like effects of Nigella sativa-oil and latent targets for microglia regulation

**Authors: *T. MALIK**[1,2]
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**Abstract:** The symptoms of Parkinsonism and oral dyskinesia are associated in the chronic inflammation in the basal ganglia which mediated by microglial activation and dopaminergic neuronal mortality. Pathogenic analogy has been observed while neuroleptics induce generation of *Extrapyramidal symptoms*. In this study, we investigate whether *Nigella sativa*-oil (NS) (black cumin seeds) - a traditional medicine used for the seizure treatment in eastern country- may reduce the haloperidol (HAL) - induced extrapyramidal symptoms (EPS) - like behavior in rats. After combine treatment with HAL (1mg/kg) on NS (0.2ml/rat), rats displayed a significant decreased EPS-like behavior including movement disorders and oral dyskinesia as compared to controls. Immuno-histochemical analysis indicates that NS reduced astrogliosis in caudate and accumbens nuclei. Activation of microglia together with reactive changes in astroglial morphology combating movement deficits. These results suggest that NS may consider as an adjunct to antipsychotics to reduce the EPS-like side effect. The prospective lab concerns are to understand how NS reduces pro-inflammatory responses, what are the latent therapeutic targets of microglial cells, and crosstalk of astrocytes and microglia, worth investigating to recognize the effective treatments for Parkinsonism and other inflammatory diseases of the CNS.

**Disclosures: T. Malik:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.07/T8

**Topic:** C.03. Parkinson’s Disease

**Support:** NRF-2017R1A2A1A05001351

DGIST 2017010095

**Title:** Role of REV-ERBα on dopaminergic neuronal death in the 6-OHDA-induced mouse model of Parkinson's disease

**Authors: *J. KIM**[1,2], S. JANG[1], M. CHOI[1], D. KIM[1,3], I. PARK[1], S. CHUNG[4], G. SON[5], H. CHOE[1], K. KIM[1,6]
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**Abstract:** Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) leading to motor dysfunctions. REV-ERBα is a transcription factor that functions not only as a repressor in the molecular clockwork but also as a repressor of tyrosine hydroxylase (*TH*) gene transcription in midbrain DAergic neurons. However, the role of REV-ERBα on DAergic neuronal cell death according to the pathophysiological process of PD is still unknown. Here, we addressed the question of whether *Rev-erbα* may play a role in the progression of DAergic neuronal cell death and pathological phenotypes in the mouse model of PD. We unilaterally injected the neurotoxin 6-hydroxydoapmine (6-OHDA) to left striatum to induce PD mouse model in both wild type (WT) and *Rev-erbα* knockout (KO) mice. We found that *Rev-erbα* KO mice began to die at 7 days after 6-OHDA injection accompanied with a sudden decline of body weight. Furthermore, REV-ERBα deficiency induced more severe motor impairment after 6-OHDA injection, suggesting accelerated PD progress in *Rev-erbα* KO. To explain the behavioral severity of 6-OHDA-treated *Rev-erbα* KO mice, we observed the progressive DAergic neuronal cell death, as a main pathological hallmark of PD, in the SNpc and the ventral tegmental area (VTA) after 6-OHDA injection. Histological results showed that DAergic neuronal loss in *Rev-erbα* KO mice was exacerbated and accelerated in both the SNpc and VTA at 2 weeks after 6-OHDA treatment compared with WT mice. To elucidate the pathogenetic mechanisms of exacerbated neurodegeneration induced by absence of REV-ERBα in PD, we analyzed the ventral midbrain transcriptome of WT and KO following 6-OHDA treatment. Notably, expression levels of genes encoding mitochondrial respiratory chain and mitochondrial transcription factors were lower in 6-OHDA-injected *Rev-erbα* KO mice than 6-OHDA-injected WT mice, indicating mitochondrial dysfunction in exacerbated neurodegeneration induced by REV-ERBα deficiency. Therefore, these findings suggest that REV-ERBα may play a neuroprotective role to pathological process of PD.

**Disclosures: J. Kim:** None. **S. Jang:** None. **M. Choi:** None. **D. Kim:** None. **I. Park:** None. **S. Chung:** None. **G. Son:** None. **H. Choe:** None. **K. Kim:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.08/T9

**Topic:** C.03. Parkinson's Disease

**Support:** Michael J Fox Foundation Repositioning Grant

**Title:** MLR1019 reduces dyskinesias and subthalamic neuron bursting in rat 6OHDA Parkinsons disease model

**Authors: *J. A. GRUNER**, J. R. CIALLELLA, A. G. REAUME, J. WANG, E. ZUVICH, K. JONES
Melior Discovery, Exton, PA

**Abstract:** Treatment of Parkinson's disease (PD) is currently limited to surgery or dopaminergic agonist medications such as L-DOPA. Drug treatments have significant limitations including reduced efficacy and appearance of side effects such as dyskinesias after several years. In order to better regulate brain levels of dopamine (DA), dopamine transport (DAT) inhibitors have been evaluated in combination with DA agonists, but with little success. MLR-1019, a repositioned molecule under development by Melior Discovery, is a uniquely potent DAT inhibitor having negligible serotonin and norepinephrine transport activity. In the rat unilateral 6-hydroxydopamine (6-OHDA) lesion model, chronic L-DOPA treatment (12 mg/kg) induced abnormal involuntary movements (AIMs), the rodent equivalent to Parkinsonian levodopa-induced dyskinesia (LID). Co-administration of L-DOPA with MLR-1019 significantly attenuated AIMs and enhanced the effects of L-DOPA on motor deficits, as measured by the forelimb adjusting step (FAS) test. MLR-1019 was also examined for effects on burst-firing in subthalamic nucleus (STN) neurons in unilateral 6-OHDA-lesioned rats. STN bursting is a well-established consequence of loss of dopaminergic innervation seen both clinically and in animal models. Single-unit recordings were obtained from STN neurons before and 1 – 3 h after administration of MLR-1019 (10 mg/kg IP). Data from 91 neurons recorded 5 min each in 12 rats showed no effect on firing frequency, interspike interval, or spike waveform shape. In contrast, the number of bursts, percentage of spikes in bursts, and number of spikes within bursts were reduced by 58%, 61%, and 9% respectively ($P < 0.001$, ANOVA). These results indicate that MLR-1019 could represent a new PD therapeutic that significantly reduces the side effects and enhances the antiparkinsonian activity of L-DOPA treatment, as well as reduces STN bursting in lesioned brain. Efforts are underway to initiate a clinical trial to test MLR-1019 in PD patients.

**Disclosures:** **J.A. Gruner:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc. **J.R. Ciallella:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc. **A.G. Reaume:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc. **J. Wang:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc. **E. Zuvich:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc. **K. Jones:** A. Employment/Salary (full or part-time):; Melior Discovery, Inc..

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.09/T10

**Topic:** C.03. Parkinson's Disease

**Support:** Parkinson Disease Foundation

NINDS NS090107

**Title:** Aav-mediated silencing of striatal cav1.3 channels provides long-term prevention of dyskinesias

**Authors: *K. A. STEECE-COLLIER**[1,2], L. R. BEGG[3], T. J. COLLIER[4,2], J. A. STANCATI[4], I. M. SANDAVOL[4,2], C. E. SORTWELL[4,2], C. J. KEMP[4], B. F. DALEY[4], N. J. COLLIER[4], N. KUHN[4], F. P. MANFREDSSON[4,2]
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**Abstract:** The L-type Ca2+ voltage (CaV) gated channel antagonist, isradipine (israd) administered continuously via subcutaneous pellets can dose-dependently dampen the severity of dyskinetic behaviors in parkinsonian rats in response to subchronic (2wks), low dose (6 mg/kg) levodopa (LD) (Schuster et al., 2009, Biol Psych 65:518). However, two questions related to the potential clinical utility of CaV (i.e.: CaV1.3) channel antagonism for LD-induced dyskinesias (LIDs) remain: 1) Can the same antidyskinetic effect, albeit modest (~25-40%), be achieved with non-continuous israd mimicking dosing in PD patients? 2) Is the modest degree of benefit linked to the pharmacological limitations of israd or the CaV1.3 target *per se*? To determine whether 2x daily israd has utility in preventing LIDs, male Sprague Dawley rats were unilaterally lesioned with 6OHDA. 24 hrs post-lesion, rats began 2x daily injections of vehicle or israd (0.2mg/kg) for remainder of the study. This israd dose is analogous to dosing tested in neuroprotection clinical trials (NCT02168842) and shown to be modestly antidyskinetic in preclinical studies. Two wks post-6OHDA, rats began daily LD (6mg/kg) for 10 days (d). In a 2nd study, to provide unequivocal target validation evidence, rats received intrastriatal injections of rAAV-CaV1.3-shRNA (CaV1.3) to provide continuous, high level silencing of CaV1.3 channels, or a rAAV-Scr-shRNA (Scr) control. One wk post-vector delivery, rats were unilaterally lesioned with 6OHDA and 2 wks post-lesion began daily treatment with escalating doses of LD (6, 9, 12, & 18mg/kg; 10 d/dose). We found that 2x daily israd provided partial protection at d5 (Peak Dose LID: Veh+LD=6.8$\pm$1.6; Israd+LD=4.3$\pm$1.9; mean$\pm$SEM; 37% reduction) against low dose LD (6mg/kg), but the effect was lost by d10. Studies aimed at enhancing israd protection (i.e.:

israd±quinpirole) have been examined and will be reported. Our rAAV-mediated CaV1.3 silencing studies are ongoing, but data thus far confirm that vectored silencing can provide near complete prevention of LID induction following 10d low dose LD (6mg/kg; Peak Dose LID Day 10: Scr=12.6±3.0; CaV1.3=2.7±0.7; $p<0.001$) and 10d moderate dose LD (9mg/kg; Peak Dose LID Severity Day 6: Scr=15.2±2.9; CaV1.3=2.4±0.9; 85% reduction; $p<0.001$).

**Disclosures:** **K.A. Steece-Collier:** None. **L.R. Begg:** None. **T.J. Collier:** None. **J.A. Stancati:** None. **I.M. Sandavol:** None. **C.E. Sortwell:** None. **C.J. Kemp:** None. **B.F. Daley:** None. **N.J. Collier:** None. **N. Kuhn:** None. **F.P. Manfredsson:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.10/T11

**Topic:** C.03. Parkinson’s Disease

**Title:** Validation of p53 inducible gene 3 (PIG3) as a novel therapeutic target for Parkinson's disease using dopaminergic cell lines and patient-specific induced pluripotent stem cell (iPSC) derived neurons

**Authors: *J. C. CHAUFTY**[1], R. ROESSLER[1], K. HA[1], J. RANJAN[1], S. PHAT[1], S. KIM[2], S. AKELLA[1], L. SHANAHAN[1], C. BARLOW[2], K. THAPA[1], M. KIEBISH[1], S. GESTA[1], B. SCHUELE[2], V. VISHNUDAS[1], N. NARAIN[1], R. SARANGARAJAN[1], P. NARAIN[1], J. LANGSTON[2]
[1]Neurol. Dept, Berg Biosystems, Framingham, MA; [2]Parkinson's Inst., Sunnyvale, CA

**Abstract:** Mutations in the *LRRK2* gene represent a major genetic risk factor for both sporadic and familial Parkinson’s disease (PD). However, the mechanistic link between LRRK2 variants and PD-related neurodegeneration remains unclear. We have previously disclosed the application of Berg’s proprietary Interrogative Biology® methodology to compare primary skin fibroblasts from PD patients harboring the $LRRK2^{G2019S}$ mutation, idiopathic PD patients, and their matched mutation-negative controls. This study led to identification of the quinone oxidoreductase, PIG3, as a novel candidate for mechanistically linking $LRRK2^{G2019S}$ to PD pathology. Fibroblasts from LRRK2-PD patients demonstrated elevated steady-state PIG3 protein expression that correlated with upregulation of MKK3/6 activity, p38 MAPK phosphorylation, and accumulation of p53. We have previously shown that human dopaminergic SH-SY5Y cells treated with rotenone and 6-hydroxydopamine (6-OHDA) exhibited an increase in PIG3 mRNA and protein expression that correlated with apoptosis. siRNA directed against *PIG3* significantly reduced rotenone- and 6-OHDA- induced cell death in SH-SY5Y cells. We have also shown that inhibition of p38 MAPK, by SB203580, reduced apoptosis in these models and was associated with attenuated induction of PIG3 expression. Here we demonstrate that inhibition of LRRK2 kinase activity, by

LRRK2-IN-1, similarly reduces neurotoxin-induced PIG3 expression and apoptosis induction. Furthermore, we demonstrate that PIG3 siRNA reduces reactive oxygen species (ROS) production in SH-SY5Y cells. To further examine the role of PIG3 in human PD models *in vitro*, we have successfully reprogrammed fibroblasts from our discovery cohort to generate patient-specific iPS cells. Indeed, the resulting IPSCs express the appropriate pluripotency markers (ie. SOX2, OCT4 and NANOG). These models enable us to assess the potential role of genetic predisposition on PIG3 modulation and apoptosis in response to PD neurotoxins. This study also addresses the effects of attenuated PIG3 expression on neurotoxin susceptibility using patient-specific, iPSC-derived neurons from LRRK2 and alpha-synuclein mutant donors. Taken together, our findings demonstrate that PIG3 contributes to apoptosis in neuronal models of PD and may advance our understanding of the mechanism behind $LRRK2^{G2019S}$ and hypersensitivity to environmental neurotoxins.

**Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.**

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.11/T12

**Topic:** C.03. Parkinson’s Disease

**Support:** Pilot project from MUSC Barmore Fund

NIH/NIGMS 5P20GM103542 (HAB)

Cell and Molecular Imaging Shared Resource at MUSC (P30 CA138313)

Shared Instrumentation Grant S10 OD018113

**Title:** Inhibition of brain-derived neurotrophic factor signaling prevents effects of vagus nerve stimulation in a preclinical model of Parkinson's disease

**Authors: *A. FARRAND**[1], R. GREGORY[2], M. GOOZ[3], D. TOWNSEND[3], V. HINSON[4], K. HELKE[5], H. BOGER[1]
[1]Neurosciences, [2]Comparative Med., [3]Drug Discovery and Biomed. Sci., [4]Neurol., [5]Comparative Med. and Pathology, Med. Univ. of South Carolina, Charleston, SC

**Abstract:** Vagus nerve stimulation (VNS) is currently FDA-approved for the treatment of drug-resistant epilepsy and depression. Previous studies have shown that VNS has beneficial effects on noradrenergic (NE) neurons in the locus coeruleus (LC) and its innervation of target regions. This makes VNS an innovative treatment strategy for Parkinson’s disease (PD) since LC-NE

degeneration typically occurs prior to the dopaminergic (DA) loss in the substantia nigra (SN). Studies from our lab show that VNS can attenuate the loss of tyrosine hydroxylase-positive neurons in the LC and SN observed in a rat lesion model of PD, and can improve locomotor activity. Additionally, previous studies show that VNS increases activation of signaling proteins downstream of BDNF-TrkB activation, suggesting BDNF-TrkB signaling as a potential mechanism of action. Therefore, our hypothesis is that by blocking BDNF signaling via daily administration of a TrkB antagonist (ANA-12), we can reduce the beneficial effects of VNS in a progressive preclinical model of PD. To create the model, we administered DSP-4 (50 mg/kg, i.p.) to rats to lesion the LC-NE system, followed one week later by bilateral intrastriatal 6-OHDA (5 µg/µL) to lesion the SN-DA system. At this time, a subset of rats also had vagus cuffs implanted around the isolated vagus nerve that connect to a headcap to later receive VNS. After eleven days to allow lesion development, rats received two thirty-minute sessions of VNS per day using precise bursts at a set amplitude and rate for ten days, and locomotor activity was measured during the afternoon session each day. Rats received either ANA-12 (0.5 mg/kg, i.p.) or vehicle daily prior to the afternoon VNS session. Immediately following the final session, rats were euthanized, and the SN and LC were dissected out for immunohistochemical assessment of neuronal populations. The right dorsal striatum and frontal cortex were dissected for detection of BDNF, protein thiols, and redox enzyme levels. Lesion + VNS rats had increased levels of BDNF in target regions of LC (frontal cortex) and SN (dorsal striatum), as well as reduced thiol levels and alterations in redox enzymes indicative of decreased oxidative stress, compared to lesion control rats. Additionally, daily administration of ANA-12 prevented improvements of VNS on locomotor activity for lesion rats. Studies are ongoing to determine whether blocking BDNF-TrkB signaling can also prevent the improvements observed after VNS on neuronal populations and oxidative stress. Collectively, these data indicate that VNS works through a BDNF signaling mechanism to attenuate PD-like deficits observed in this preclinical model.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.12/U1

**Topic:** C.03. Parkinson’s Disease

**Title:** Anti-high mobility group 1 antibody suppresses neuroinflammation in the plasma and brain tissue of Parkinson's disease model rat

**Authors: *K. ITTETSU**[1], T. SASAKI[1], M. OKAZAKI[1], K. KUWAHARA[1], J. MORIMOTO[1], K. KIN[1], M. UMAKOSHI[1], Y. TOMITA[1], T. YASUHARA[1], M. KAMEDA[1], I. DATE[1], M.

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**Abstract:** [Objective]
The main pathology of Parkinson's disease (PD) is a loss of dopamine neurons in the substantia nigra pars compacta (SNc), but underlying mechanism has not been fully understood. Recently, neuroinflammation in the pathogenesis of PD has been documented. High mobility group box-1 (HMGB1) exists as a structural nuclear protein in the normal state, but displays an inflammatory cytokine-like activity in the extracellular space under pathological condition. In this study, we investigated the involvement of HMGB1 in the pathology and the neuroprotective effects of anti-HMGB1 monoclonal antibody (mAb) on rat model of PD by focusing on their role in the plasma and brain tissue.
[Material and Method]
Adult female Sprague-Dawley rats were injected with 6-hydroxydopmaine (6-OHDA, 20 μg/4 μl) into the right striatum, then anti-HMGB1 mAb (1 mg/kg), or control mAb was intravenously administered immediately, at 6 and 24 h after 6-OHDA injection. Enzyme-linked immunosorbent assay (ELISA) of HMGB1 in plasma and Western immunoblotting in the striatum and SNc were performed at days 1 ,4 and 7 to measure the expression of HMGB1. Fluorescent immunostaining for HMGB1, Iba1 as a microglia marker, MAP2 as a neuron marker and GFAP as an astrocyte marker were performed at 24 hours, day7 and 14 for histological evaluation. Fluorescent immunostaining for albumin was conducted to assess blood-brain barrier leakage at day1. Polymerase chain reaction (PCR) was performed to assess expression of inflammatory cytokines in the striatum and SNc at day1 and 4.
[Result and Discussion]
The treatment with anti-HMGB1 mAb significantly suppresses the increase of HMGB1 in the plasma and brain tissue compared to the control mAb-treated group. Iba1-positive cells were decreased at day7 and 14 after 6-OHDA injection in anti-HMGB1 groups, whereas HMGB1 translocation was observed in neurons at day1 and astrocytes at day7 in the control mAb-treated group. Anti-HMGB1 mAb inhibited the activation of microglia, blood-brain barrier leakage and the expression of inflammation cytokines such as IL-1β and IL-6. These results suggested that HMGB1 released from neurons and astrocytes was at least partly involved in the mechanism and pathway of degeneration of dopaminergic neurons induced by 6-OHDA exposure.
[Conclusion]
Intravenous administration of anti-HMGB1 mAb suppresses neuroinflammation and attenuates disruption of blood-brain barrier in a rat model of PD.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.13/U2

**Topic:** C.03. Parkinson’s Disease

**Support:** National Research Council of Thailand

**Title:** Oxyresveratrol attenuates motor deficits in rotenone-induced parkinsonism rats via an antioxidative effect

**Authors: *R. RODSIRI**[1], H. BENYA-APHIKUL[1], N. TEERAPATTARAKAN[1], B. SRITULARAK[2], K. LIKHITWITAYAWUID[2]
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**Abstract:** Previous studies have reported the antioxidant and anti-inflammatory effects of oxyresveratrol *in vitro*. The neuroprotective effect of oxyresveratrol has also been demonstrated in animal model of cerebral ischemia. This study aimed to investigate effects of oxyresveratrol in rotenone model of parkinson's disease in rats. Male Wistar rats were assigned randomly into three groups; control, parkinsonism and oxyresveratrol groups. Oxyresveratrol (300 mg/kg p.o.) was given to rats in oxyresveratrol group for 20 days while rats in control and parkinsonism groups received 1% CMC. To induce parkinsonism behaviors, rotenone (3 mg/kg i.p.) was given to rats in parkinsonism and oxyresveratrol groups on day 15, 16, 18 and 20 while control rats received 2% DMSO. Rotarod test was performed on day 1, 16 and 20. At the end of experiment, brains were collected. The malondialdehyde (MDA) levels and trolox equivalent antioxidant capacity (TEAC) were determined in brain tissue. It was shown that rotenone treatment significantly decreased latency to fall of rats in parkinsonism group compared to controls on day 16 and 20 ($p<0.0001$ and $p<0.05$, respectively). On day 16, oxyresveratrol-treated rats had higher latency to fall than that of rats in parkinsonism group ($p<0.01$). However, rotarod performance of rats in oxyresveratrol and parkinsonism groups significantly decreased from controls on day 20 ($p<0.05$). Brain MDA levels of rats in parkinsonism group were higher than that of controls ($p<0.05$) while oxyresveratrol significantly decreased MDA levels compared to parkinsonism rats ($p<0.05$). Oxyresveratrol tended to increase brain TEAC but this result was not significantly different from parkinsonism rats. Overall, the results indicated that oxyresveratrol prevented rotenone-induced parkinsonism behaviors and revealed an antioxidative effect. The protective effects of oxyresveratrol on dopaminergic neurons in the nigrostriatal pathway will be further investigated.

**Disclosures: R. Rodsiri:** None. **H. Benya-aphikul:** None. **N. Teerapattarakan:** None. **B. Sritularak:** None. **K. Likhitwitayawuid:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.14/U3

**Topic:** C.03. Parkinson's Disease

**Title:** Nuetralizing TLR2 alleviates synucleinopathies

**Authors: *C. KIM**[1], B. SPENCER[2], E. ROCKENSTEIN[2], H. YAMAKADO[2], M. MANTE[2], A. ADAME[2], J. A. FIELDS[2], D. MASLIAH[2], H.-J. LEE[3], P. DISPLATS[2], R. RISSMAN[2], S.-J. LEE[4], E. MASLIAH[1]
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**Abstract:** Synucleinopathies are neurological disorders, characterized by neuronal and glial deposition of alpha-synuclein aggregates. Cell-to-cell transmission of these aggregates are thought to be the underlying mechanism of aggregate spreading in patients' brain and perhaps of clinical progression. Interfering with the aggregate transmission can thus be a potential strategy for halting the disease progression. Here, we verify the pathological role of TLR2 in alpha-synucleinopathies. Overexpression of TLR2 aggravates alpha-synuclein neuropathology in alpha-synuclein transgenic mice. Thereby, administration of TLR2 neutralizing antibody alleviates synucleinopathy lesions and neuroinflammation in synucleinopathies animal model. In addition, we demonstrate that TLR2 plays an important role in cell-to-cell transmission of alpha-synuclein aggregates, regulating both secretion and uptake of the aggregates. Therefore, we propose the anti-TLR2 treatment as a therapeutic strategy for Parkinson's disease and related synucleinopathies.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.15/U4

**Topic:** C.03. Parkinson’s Disease

**Title:** N-palmitoylethanolamide prevents parkinsonian phenotypes in aged mice

**Authors: *R. CRUPI**[1], D. IMPELLIZZERI[1], M. CORDARO[1], R. SIRACUSA[1], G. CASILI[1], S. CUZZOCREA[1,2]
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**Abstract:** Parkinson's disease (PD) is a neurodegenerative disease characterized by degeneration of dopaminergic neurons. The canonical symptoms of this disease are: resting tremor, rigidity and hypokinesia. Aging is considered the major risk factor for generating idiopathic PD. Recently, several studies have focused the neuroprotective effects of Palmitoylethanolamide (PEA) alone or in combination with antioxidants, in an experimental model of PD after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induction. The aim of this study was to evaluate the pre-treatment effect of micronized PEA formulation (PEAm), on the neuroinflammation process and on the neuronal death on in vivo model of PD on aged mice. The old animals were pre-treated orally for 60 days with PEAm at dose of 10mg/kg. After pre-treatment, they received four injections of the dopaminergic neurotoxin MPTP and were sacrificed 7 days after induction. On the 8th days, brains were processed for histological and immunohistochemical analysis. Pre-treatment with PEAm significantly ameliorated behavioral deficits, reduced the expression of specific markers of PD such as tyrosine hydroxylase (TH), dopamine transporter (DAT), as well as decreased the upregulation of α–synuclein and β3-tubulin in the substantia nigra after MPTP induction. Moreover PEAm reduced proinflammatory cytokines expression and showed a pro-neurogenic effect in hippocampus. Thus, this strategy could prevent neurodegenerative diseases associated to the old age.

**Disclosures: R. Crupi:** None. **D. Impellizzeri:** None. **M. Cordaro:** None. **R. Siracusa:** None. **G. Casili:** None. **S. Cuzzocrea:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.16/U5

**Topic:** C.03. Parkinson’s Disease

**Support:** IBRO-SfN Travel Grants 2017

Institute of Research and Development, Walailak University, Thailand

**Title:** The neuroprotective effect of Astaxanthin from white shrimp shell on C57BL/6 mice model of MPTP-induced Parkinsonism-like symptoms via antioxidant activity and neuro-inflammatory marker

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**Abstract:** Parkinson's disease (PD) is a progressively debilitating neurodegenerative disorder that characterized the massive loss of dopominergic (DA) neurons, particularly in substantia nigra pars compacta (SNpc), and can be experimentally established by the most useful neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). Subsequently there was accompanied by bradykinesia, resting tremor, and postural instability. The drug treatments have many limitations in terms of side effects and incapability to protect the neurodegeneration. The oxidative stress and neuro-inflammatory maker, Ionized binding adaptor molecule-1 (Iba-1) have been strongly implicated in pathogenesis of PD. Recently, novel treatments associated to natural anti-inflammation and marine products/molecules with neuroprotectant effect are being exploited for therapy, Astaxanthin extract from white shrimp shell to investigate the neuroprotective effect of Astaxanthin and possible mechanisms related to antioxidant and neuroinflammatory marker in models of PD. Thirty-five adult C56BL/6 mice orally received pretreatment with Astaxanthin extracts for 3 weeks and then were divided into seven groups: control group, MPTP treatment (i.p., 15 mg/kg BW in saline, four times for 1 consecutive day at 2-h intervals), MPTP combined with oral administrations of vitamin C (100 mg/kg BW), Tidomet plus (L-dopa+carbidopa 5 mg/kg BW) and Astaxanthin extracts (30, 60, and 100 mg/kg BW) respectively. After 1, 3, and 7 days of MPTP treatment, all mice were subjected to rotarod test and resting tremor score in order to determine motor, coordination, and severity of PD. Moreover, oxidative stress parameters of malondialdehyde (MDA) level, glutathione peroxidase (GPx) activity, and % inhibition of $O_2^{-\cdot}$ and immunohistochemistry of Iba-1 were also examined. Treatment with Astaxanthin extract 60 mg/kg for 7 days significantly improved retention time and resting tremor score compared to untreated-mice and tidomet plus-treated group ($p<0.05$). In addition, Astaxanthin 60 mg/kg exhibited decrease of MDA level while increase of both GPx activity and % inhibition of $O_2^{-\cdot}$ and suppress of Iba-1 expression compared to MPTP-treated group in striatum areas ($p<0.05$). The result of our study proposed that Astaxanthin at dose of 60 mg/kg BW ameliorated behavioral tests by mediating its neuroprotection against MPTP induced-PD via its antioxidant activities and anti-inflammatory effect.

**Disclosures:** **P. Boonruamkaew:** A. Employment/Salary (full or part-time):; School of Pharmacy, Walailak University. **M. Sroyraya:** A. Employment/Salary (full or part-time):; Mahidol University. **W. Klaypradit:** A. Employment/Salary (full or part-time):; Kasetsart University. **P. Chonpathompikunlert:** A. Employment/Salary (full or part-time):; Chandrakasem Rajabhat University.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.17/U6

**Topic:** C.03. Parkinson’s Disease

**Support:** CONACYT No. 233815 and 168356

INNN No. 79/09

**Title:** Copper sulfate pretreatment prevents mitochondrial electron transport chain damage and apoptosis against MPP+-induced neurotoxicity

**Authors: *R. OSORNIO**[1], M. OROZCO-IBARRA[2], S. MONTES[3], E. BRAMBILA[4], A. DIAZ-RUIZ[5], C. RIOS[6], J. GUEVARA[7]
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**Abstract:** Parkinson’s disease (PD) is a neurodegenerative disorder characterized by reduced mitochondrial complex I activity, free radicals' overproduction and reduced antioxidant defenses in the substantia nigra pars compacta (SNpc). Intrastriatal injection of 1-methyl-4-phenylpyridinium (MPP+) is considered a model to reproduce some biochemical alterations observed in PD patients. Copper (Cu) plays an important role in the metabolism and antioxidative responses through its participation as a cofactor in the cytochrome c oxidase (COX), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), and metallothioneins (MTs). We tested the effect of copper sulfate (CuSO4) pretreatment on the mitochondrial electron transport chain (METC) in the striatum after MPP+ toxicity in rats. The results showed that the MPP+ intrastriatal injection reduced mitochondrial complex I, II, IV and V activities, while 10 µmol of CuSO4 pretreatment counteracted this damage. Activities of complexes I, II and IV, were coincident with ATP recovery. Moreover, Cu/Zn-SOD activity was reduced as a consequence of MPP+; however, copper pre-treatment kept the striatal Cu/Zn-SOD activity unchanged in MPP+-damaged animals. We observed that MPP+ also reduced the metallothionein content and that CuSO4 pretreatment maintained baseline values. CuSO4 pretreatment also reduced the striatal caspase-3 and caspase-9 activities that were increased three days after MPP+-induced damage. The present study provided evidence that copper pretreatment reduced MPP+-induced apoptotic damage, probably through direct action on copper-dependent proteins or indirectly on proteins in the apoptotic pathway.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.18/U7

**Topic:** C.03. Parkinson's Disease

**Support:** NRF Grant 2017R1A2B4008456

NRF Grant 2011-0030049

**Title:** GW5074, Raf 1 kinase inhibitor induced neuroprotection and motor improvement in LRRK2 G2019S PD model

**Authors:** ***Y. PARK**, J. KIM, T. KIM, H. SEO
Hanyang Univ., Ansan, Korea, Republic of

**Abstract:** Dopaminergic neurons in substantia nigra are selectively vulnerable under high risk environments with genetic or external cause of Parkinson's disease (PD). LRRK2G2019S mutation has been reported to increase kinase activity inducing neurodegeneration associated with PD. We hypothesized that LRRK2 kinase inhibition by GW5074 [5-Iodo-3-[(3,5-dibromo-4-hydroxyphenyl) methylene]-2-indolinone], synthetic drug that inhibits Raf 1 activity induces neuroprotection in *in vitro* and *in vivo* PD models with LRRK2G2019S mutation. In this study, we found that MPP+ induced cell death of dopaminergic neuron was blocked by GW5074 which activated the neuroprotective signaling pathway. Treatment with GW5074 protects dopaminergic neurons against the neurotoxic effects of MPP+ and glutathione depletion-induced oxidative stress. GW5074 prevented neurodegeneration and improved motor functions in LRRK2G2019S model of PD. Given its neuroprotective effects, in response to neurotoxic stimuli, and in LRRK2G2019S model of PD, GW5074 could be used to study therapeutic mechanism against neurodegenerative pathologies of PD by LRRK2G2019S mutation.
*Y.P. and J.K. equally contributed to this work.

**Disclosures:** **Y. Park:** None. **J. Kim:** None. **T. Kim:** None. **H. Seo:** None.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.19/U8

**Topic:** C.03. Parkinson’s Disease

**Support:** JSPS KAKENHI Grant Number JP23890130

**Title:** Synphilin-1 has neuroprotective effect on Parkinson’s disease-model cells by inhibiting apoptosis

**Authors: *Y. NAGANO**, T. SHISHIDO, M. ARAKI, T. TAKAHASHI, H. MARUYAMA
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**Abstract:** [Background] Synphilin-1 represents a cytoplasmic protein that interacts with α-synuclein in neuron and labels the central core of Lewy bodies which is the pathological hallmark of Parkinson’s disease (PD). The interaction of synphilin-1 with α-synuclein indicates that synphilin-1 may also play a central role in PD pathogenesis. However, the biological functions of synphilin-1 are not fully understood. [Objective] In this study, we investigated whether synphilin-1 would have neuroprotective effects on both *in vitro* and *in vivo* PD-models using $MPP^+$ or MPTP which preferentially cause dopaminergic cell death. [Materials and Methods] *In vitro* study, we established SH-SY5Y cells stably overexpressing synphilin-1. The cells treated with $MPP^+$ were evaluated to check the cell viability, apoptosis and ROS production using immunoblotting and immunostaining methods.
*In vivo* study, we generated transgenic mice overexpressing synphilin-1. To investigate the function of synphilin-1 against MPTP toxicity, synphilin-1 transgenic and non-transgenic mice were treated chronically with MPTP/probenecid. We analyzed the number of dopaminergic neurons in substantia nigra pathologically and immunoreactive bands of tyrosine hydroxylase in midbrain using immunoblotting. [Results] Trypan blue exclusion assay showed that synphilin-1 significantly inhibited neuronal cell death induced by $MPP^+$. Confocal study showed that synphilin-1 partially restored nuclear condensation and fragmentation caused by $MPP^+$. Furthermore, immunoblotting studies indicated that the protein expression levels of cleaved-PARP and cleaved-caspase3 were decreased in synphilin-1 expressing cells compared to control cells. *In vivo* studies showed that synphilin-1 tended to inhibit dopaminergic neuronal death induced by MPTP, however the differences weren't significant. [Conclusion] These data suggested that synphilin-1 could have neuroprotective function in the molecular mechanism of PD.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.20/U9

**Topic:** C.03. Parkinson's Disease

**Title:** Evidence for improved motor function by ceftriaxone despite striatal tyrosine hydroxylase loss following nigrostriatal lesion

**Authors: *E. A. KASANGA**[1], S. M. MEADOWS[4], T. MCINNIS[2], M. CANTU[2], M. F. SALVATORE[3], C. R. BISHOP[5]
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**Abstract:** The beta-lactam antibiotic, ceftriaxone, attenuates tyrosine hydroxylase(TH) loss in striatum in rodent Parkinson's disease (PD) models when given early after nigrostriatal lesion. This protection may be related to the well-known properties of ceftriaxone to increase expression of the glutamate transporter, GLT-1, which increases glutamate uptake. However, it is unknown if ceftriaxone could partially restore striatal TH loss, concomitant with decreased motor impairment, when administered at a time post-lesion when motor function is impaired. The ability of ceftriaxone to decrease motor impairment was evaluated after evidence of motor impairment was observed in 3 tests; forepaw adjustment steps, open-field locomotor activity, and amphetamine-induced rotation. Ceftriaxone (200 mg/kg, i.p.) was given intermittently on days 7-13, 21-27, and 35-38 post-lesion. The FAS test and amphetamine-rotation tests established evidence of motor impairment 7 days after lesion induction. There was a significant increase in FAS taken, with an overall 45% increase in mean FAS of the 3 days evaluated 19,25, and 35 days post-lesion. Amphetamine-induced rotation was reduced by 63% between day 7 and day 14 after lesion. Locomotor activity increased at the end of the study (38 days post-lesion) against the day 7 baseline following ceftriaxone. Expression of striatal TH protein revealed no significant difference in loss between rats receiving ceftriaxone or saline injections in the intermittent injection regimen. Taken together, these preliminary data suggest the possibility that ceftriaxone-mediated increases in locomotor activity following nigrostriatal lesion may be independent of any preservation or possible restoration of striatal TH. Given previous evidence of protection against striatal TH loss when ceftriaxone is given early after lesion, the results also indicate that progression of striatal TH loss is affected by excitotoxic mechanisms.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.21/U10

**Topic:** C.03. Parkinson's Disease

**Support:** Cellular Dynamics International Inc. Research Contract

**Title:** Cryopreserved dopamine neurons derived from human induced pluripotent stem cells survive and partially reverse motor asymmetry in the 6-hydroxydopamine-lesioned athymic nude rat

**Authors: *B. M. HILLER**[1], D. J. MARMION[1], C. W. MCMAHON[2], D. R. WAKEMAN[3], J. H. KORDOWER[1]
[1]Rush Univ. Med. Ctr., Chicago, IL; [2]Cell. Dynamics Intl. Inc., a FujiFilm Co., Madison, WI; [3]RxGen, Inc., Hamden, CT

**Abstract:** Induced pluripotent stem cells (iPSCs) are a renewable source of dopamine neurons and as such are a good candidate for cell replacement therapy in Parkinson's disease (PD). Previously we showed that commercially available cryopreserved iPSC-derived midbrain dopamine (iPSC-mDA) neurons (iCell DopaNeurons) survive and reverse 6-hydroxydopamine (6-OHDA)-induced motor asymmetry in the cyclosporine-immunosuppressed Sprague Dawley rat (Wakeman et al., 2017). In the present study we compared the efficacy of additional iPSC-mDA differentiation protocol variations, including an earlier cryopreservation date (iPSC-mDA-early), omission of FGF-8 (iPSC-mDA-noFGF-8), and alternative purification strategies (iPSC-mDA-purified) in the athymic nude rat. Rats received 6-OHDA injections in the right medial forebrain bundle. Nude rats with unilateral lesions confirmed by ipsilateral amphetamine-induced rotations were then transplanted with $4.5x10^5$ ($1.5x10^5$ cells/µl; 3µl) viable iPSC-mDA neurons in the right striatum. Motor asymmetry was assessed by amphetamine- and apomorphine-induced rotations. Animals were sacrificed at 9 months post-transplantation and brains were processed for immunohistochemistry. Here we show that striatal injections of cryopreserved mDA neurons survived and integrated with the host tissue over a period of 9 months. Robust grafts and fiber integration were seen in all animals that received iPSC-mDA injections. Transplanted cells maintained their dopaminergic phenotype, as indicated by the presence of tyrosine hydroxylase-immunoreactive fibers and cell bodies localized to the striatum. Importantly, we saw little evidence of proliferation indicative of teratoma formation or neural outgrowth in grafted animals. Amphetamine-induced rotations were completely reversed in animals receiving iCell DopaNeurons, iPSC-mDA-purified, or iPSC-mDA-early and partially reversed in animals injected with iPSC-mDA-noFGF-8 compared to vehicle control. Partial reversal in apomorphine-induced rotations was seen for iCell DopaNeurons and iPSC-mDA-

purified. These data support the application of cryopreserved iPSC-mDA neurons as a cell replacement therapy in PD. Further long-term studies with cells made using a fully cGMP-compliant process are currently underway.

**Disclosures: B.M. Hiller:** None. **D.J. Marmion:** None. **C.W. McMahon:** A. Employment/Salary (full or part-time):; Cellular Dynamics International Inc., a FUJIFILM company. **D.R. Wakeman:** F. Consulting Fees (e.g., advisory boards); Cellular Dynamics International Inc., a FujiFilm company. **J.H. Kordower:** 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.; Cellular Dynamics International Inc., a FUJIFILM company. F. Consulting Fees (e.g., advisory boards); Cellular Dynamics International Inc., a FUJIFILM company.

**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.22/U11

**Topic:** C.03. Parkinson’s Disease

**Title:** TRPC3 mediates ROS-dependent regulation and dysregulation of spontaneous firing activity in the midbrain dopamine neurons

**Authors: *S. KIM**, Y. LEE, M. PARK, H. KIM
SKKU Sch. of Med., Suwon, Korea, Republic of

**Abstract:** Midbrain dopamine neurons are pacemakers generating spontaneous firing activity, which triggers dopamine release. Thus, they are continuously exposed to oxidative stress conditions due to dopamine metabolism. Parkinson’s disease (PD)-related risk factors exacerbate mitochondrial dysfunction leading to more reactive oxygen species (ROS) formation. Although oxidative stress is implicated in dopaminergic neurotoxicity in PD, the molecular mechanisms underlying the loss of dopamine neurons are still elusive. We recently found that TRPC3 drives pacemaking and regulates basal firing rate in the midbrain dopamine neurons. Since TRPC3 is a ROS-sensitive channel, we hypothesized that the spontaneous firing activity of the midbrain dopamine neurons may be regulated by intracellular ROS levels via TRPC3. ROS generators including PD-related neurotoxins increased, while ROS blockers decreased the basal firing rate of the acutely isolated midbrain dopamine neurons. Importantly, the effects by ROS generators were totally abolished by pretreatment of a selective inhibitor of TRPC3, implying that TRPC3 may be involved in dopaminergic neurotoxicity. In a PD model using human dopaminergic neuronal cell line LUHMES, knock-down of TRPC3 greatly increased neuronal survival in response to PD-neurotoxin, 1-methyl-4-phenylpyridinium. Immunocytochemistry experiments

confirmed that TRPC3 is present in both isolated midbrain dopamine neurons and differentiated LUHMES cells. Taken together, these results suggest that disruption of spontaneous firing activity through TRPC3 activation by ROS is a possible mechanism for dopaminergic neurotoxicity and TRPC3 may be a therapeutic target for PD.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 573.23/U12

**Topic:** C.03. Parkinson's Disease

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**Title:** Role of gut microbiota-derived metabolites in neurodegenerative disorders involving protein misfolding and C9orf72 expansion

**Authors: *L. HO**[1,2,3], K. RUAN[4], K. ONO[5], T. LLOYD[4], G. M. PASINETTI[1,2,3]
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**Abstract:** There is growing evidence that in many neurodegenerative disorders, cell-to-cell transmission of a pathological, misfolded protein, such as misfolding of α-synuclein (α-syn) in Parkinson's disease (PD), may be a vehicle for the spreading of pathology throughout the brain. This misfolded protein, or seed, further induces misfolding of native proteins within the cell. Pathological misfolded proteins may exist in diverse conformations with distinct cellular and biochemical properties. We investigate whether microbiota-derived metabolites may help to attenuate the misfolding of α-syn and thereby promote resilience against PD phenotypes. We identified 6 biologically available, gut microbiota-derived compounds (GMP10, GMP11,

GMP26, GMP28, GMP39, and GMP44) for investigation. Using independent *in vitro* protein aggregation assays (e.g., photo-induced cross-linking of unmodified proteins assay, thioflavin-T, fluorescence assay, and electron microscopy) we demonstrated that three of the compounds (GMP26, GMP44, GMP28) potently inhibit aggregations of monomeric α-syn (or monomeric β-amyloid peptides) into neurotoxic protein aggregates, *in vitro*. Based on evidence linking the c9orf72 gene with expansions of GGGGCC hexanucleotide repeats and PD, as well as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), we continue to test the neuroprotective ability of our compounds *in vivo* using a Drosophila model with overexpression of GGGGCC hexanucleotide repeats. Overexpression of 30 GGGGCC repeats in the Drosophila eye causes age-dependent photoreceptor neurodegeneration (Zhang K. et al, Nature 2015; 525:56). We treated Drosophila by mixing individual test compounds into the food and found all six compounds significantly suppressed eye degeneration at 10 μM, with compounds GMP26 and GMP11 almost completely suppressing the eye phenotype. The comparative efficacy of the six compounds are GMP26 = GMP11 > GMP39 > GMP10 > GMP44 > GMP28. Outcomes from our studies link gut microbiota with mechanisms underlying PD and suggest the feasibility of developing GMP26 as a means to simultaneously target both α-syn misfolding and C9orf72 expansion to increase the likelihood of therapeutic efficacy in PD, ALS, FTD patients with C9orf72 expansion.

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**Poster**

**573. Parkinson's Disease: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 573.24/V1

**Topic:** C.03. Parkinson’s Disease

**Support:** NIH Grant R21MH101525

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W.M. Keck Foundation

**Title:** Bioluminescent optogenetics activation of transplanted neural precursor cells improves motor deficits in a Parkinson disease mouse model

**Authors: B. L. PALMATEER**, N. DORKA, L.-M. WAGNER, E. D. PETERSEN, M. PRAKASH, *U. HOCHGESCHWENDER
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**Abstract:** The need to develop efficient therapies for neurodegenerative diseases is urgent, especially given the increasing percentages of the population living longer, with increasing chances of being afflicted with conditions like Parkinson's Disease (PD). A promising curative approach towards PD and other neurodegenerative diseases is the transplantation of stem cells to halt and potentially reverse neuronal degeneration. However, stem cell therapy suffers from problems with graft efficiency that can lead to adverse side effects and reduced improvement for patients. By using remote stimulation to optogenetically activate cells we attempted to increase graft efficiency. We generated a neuronal precursor cell line expressing luminopsin 3 (LMO3), a luciferase-channelrhodopsin fusion protein, which responds to the luciferase substrate coelenterazine (CTZ) with emission of blue light that in turn activates the opsin. Neuronal precursor cells were injected bilaterally into the striatum of homozygous aphakia mice, which carry a spontaneous mutation leading to lack of dopaminergic neurons and symptoms of PD. Following transplantation, the cells were stimulated over a period of 10 days by intraventricular injections of CTZ. Mice receiving CTZ demonstrated significantly improved motor skills in a rotarod test compared to mice receiving vehicle. Thus, bioluminescent optogenetic stimulation of transplanted neuronal precursor cells shows promising effects in improving graft efficiency in the aphakia PD mouse model and encourages further studies to elucidate the mechanisms and long-term outcomes of the beneficial effects.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.01/V2

**Topic:** C.04. Movement Disorders

**Support:** NIH Grant R01 NS088192

**Title:** ATAD3A oligomerization causes Huntington's disease-associated neurodegeneration by coupling mitochondrial fragmentation and bioenergetics defects

**Authors: *Y. ZHAO**[1], X. SUN[1], C. HOPPEL[3,2], R. RAMACHANDRAN[1], X. QI[1,3]
[1]Dept. of physiology and biophysics, [2]Dept. of Pharmacol., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [3]center for mitochondrial disease, Cleveland, OH

**Abstract:** Mitochondrial fragmentation and bioenergetic failure manifest in Huntington's disease (HD), a fatal neurodegenerative disease. Hyperactivation of Dynamin-related protein 1 (Drp1), a primary fission protein, causes mitochondrial fragmentation and dysfunction in various HD models. Crucial questions such as how Drp1 hyper-activation mediates mitochondrial

dysregulation, especially those damages that occur inside the mitochondria, and how these processes are linked to neurodegeneration, however, remain to be answered. The factors that couple mitochondrial fusion/fission with bioenergetics and their impacts on neurodegeneration also remain poorly understood. In this study, we use unbiased proteomics analysis for Drp1-interacting proteins in HD patient neuronal cells derived from patient induced pluripotent stem cells (HD-iPS cells), and identify mitochondrial protein ATAD3A (ATPase family AAA-domain containing protein 3A) as an interactor of fission protein Drp1 in HD. ATAD3A is a nuclear-encoded mitochondrial protein and belongs to a family of AAA-ATPase proteins. ATAD3A has a unique structure; its C-terminus containing the conserved ATPase domain which is located in the mitochondrial matrix, whereas its N-terminus has been reported to either expose to the cytosol or associate with mitochondrial outer membrane. Here we document an enhanced interaction between Drp1 and ATAD3A and ATAD3A oligomerization in various HD models, which are required for Drp1-mediated mitochondrial fragmentation. Moreover, disturbance of ATAD3A steady state impairs mtDNA maintenance by disrupting TFAM/mtDNA binding. Blocking Drp1/ATAD3A interaction with a peptide, DA1, abolishes Drp1 hyperactivation and ATAD3A oligomerization, suppresses mitochondrial fragmentation and mtDNA lesion, and reduces bioenergetics deficits and cell death in HD mouse- and patient-derived cells. Treatment with DA1 reduces behavioral and neuropathological phenotypes of HD in HD transgenic mice. Our findings provide novel insights
into the pathogenesis of HD, present evidence for ATAD3A function on maintaining mitochondrial integrity and neuronal survival, demonstrate a key role of ATAD3A in neurodegeneration by bridging Drp1-induced mitochondrial fragmentation with mtDNA defect, and highlight a promising therapeutic strategy for HD and other neurodegenerative disorders featured with mitochondrial dysfunction.

**Disclosures: Y. Zhao:** None. **X. Sun:** None. **C. Hoppel:** None. **R. Ramachandran:** None. **X. Qi:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.02/V3

**Topic:** C.04. Movement Disorders

**Support:** NIH Grant N5096352

Ruth K. Broad Biomedical Research Foundation Award for Graduate Students

**Title:** Striatal projection neurons require Huntingtin for synaptic connectivity

**Authors: *C. BURRUS**, S. U. MCKINSTRY, N. KIM, H. YIN, C. EROGLU
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**Abstract:** Huntington's Disease (HD) is a fatal, inherited disease caused by an autosomal dominant polyglutamine expansion mutation in the N-terminus of Huntingtin (Htt) protein. Patients with HD suffer from progressive motor, cognitive, and psychiatric impairments, along with significant degeneration of the striatal projection neurons (SPNs) of the striatum. The dominant nature of the Htt mutation has led to the widely-accepted hypothesis that HD is caused by a toxic gain-of-function of mutant Htt protein. However, recent findings suggest that loss of function of Htt due to dominant-negative effects of the mutant protein also play important roles in HD. Previously, we have shown that wildtype Htt plays a critical role in regulating the dendrite formation and excitatory synaptic connectivity of cortical pyramidal neurons. However, the role of Htt in synaptic development and connectivity within the striatum is not yet known, leaving critical aspects of HD pathology unexplored. In the current study, we conditionally deleted Htt from specific subpopulations of striatal projection neurons (SPNs) using the Cre-Lox system and have determined that SPNs require Htt for synaptic development and longevity, as well as coordinated motor function. Our findings show that Htt is necessary for proper striatal synaptic connectivity and motor function, indicating that loss of Htt function likely plays a critical role in HD pathogenesis.

**Disclosures: C. Burrus:** None. **S.U. McKinstry:** None. **N. Kim:** None. **H. Yin:** None. **C. Eroglu:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.03/V4

**Topic:** C.04. Movement Disorders

**Support:** NIH/NIGMS grant 1SC3GM096945

NIH/NINDS grant R01082354 (MMR)

Wright State University startup funds (AAV)

**Title:** Neuromuscular transmission and muscle hyperexcitability in Huntington's disease

**Authors: *S. ROMER**[1], A. KHEDRAKI[1], E. J. REED[1], Q. WANG[2], M. M. RICH[2], R. J. TALMADGE[3], A. A. VOSS[1]
[1]Dept. of Biol. Sci., [2]Dept Neurosci, Cell Biol & Physiol, Wright State Univ., Dayton, OH; [3]Dept. of Biol. Sci., California State Polytechnic University, Ponoma, Ponoma, CA

**Abstract:** Huntington's disease (HD) is a fatal and progressive condition with severe debilitating motor defects. While classically recognized as a neurodegenerative disorder, there is increasing evidence of cell autonomous toxicity in skeletal muscle. Recently, our laboratory demonstrated that, despite being fully innervated, the membrane properties of skeletal muscle are hyperexcitable in HD. In this study, we examined the relationship between neuromuscular transmission and skeletal muscle hyperexcitability using an ex-vivo preparation of the levator auris longus (LAL) muscle from late-stage R6/2 HD model mice and age-matched wild-type controls. Spontaneous miniature endplate currents (mEPCs) and nerve-evoked endplate currents (eEPCs) were recorded under voltage clamp, which, unlike current clamp records, were independent of the changes in muscle membrane properties. There was a reduction in the number of vesicles released per action potential (quantal content) in R6/2 muscle, which binomial statics suggest is caused by a reduction in the number of vesicle release sites (*n*) rather than a change in the probability of release (*p*). To assess endplate morphology, we performed a combination of immunohistochemistry with confocal microscopy. There was no evidence of denervation, but neuromuscular junctions were not only smaller, they were less dense, which would result in fewer vesicle release sites. The depressed neuromuscular transmission in R6/2 muscle may help compensate for the muscle hyperexcitability and contribute to motor impersistence.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.04/V5

**Topic:** C.04. Movement Disorders

**Support:** ARO Grant #65594-LS

**Title:** The moonlighting role of $NAD^+$ salvage pathway proteins in huntingtin proteotoxicity protection

**Authors: *A. RUETENIK**[1], A. OCAMPO[2], A. BARRIENTOS[3]
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**Abstract:** Huntington's disease is caused by a single genetic abnormality in which the number of trinucleotide repeats encoding polyglutamine in exon 1 of the huntingtin gene expands to or past a threshold number of repeats to cause disease. This abnormally high number of glutamine repeats cause dysfunction and oligomerization of the resulting huntingtin protein. Neurodegeneration is the main hallmark of the disease, effecting mainly the striatum and cortex,

and patients usually die within 15-20 years of the first noticeable symptoms. As no current treatments exist to prolong lifespan for this terrible disease, we are interested in better understanding the disease mechanism complexities, as well as exploring possible routes for therapeutic targets. Previously, our lab discovered that several proteins that form the $NAD^+$ salvage pathway in yeast protected against deleterious effects caused by the overexpression of human huntingtin exon 1 with a 103 repeat polyglutamine (103Q) track in a yeast model of Huntington's disease. We further found that 103Q oligomers were being degraded in these cells, while 103Q oligomers in cells without overexpression of the $NAD^+$ salvage pathway proteins were stable. Importantly, we also showed that an intact $NAD^+$ salvage pathway was unnecessary for the protection seen by the salvage pathway proteins. Together, this suggests that $NAD^+$ salvage pathway proteins may have an alternative function in the cell, additional to their catalytic role in the $NAD^+$ salvage pathway Here, we have continued the investigation into the mechanisms and ability of $NAD^+$ salvage pathway proteins to confer protection, with the hypothesis that they may have a moonlighting chaperone function. In addition to continuing our studies in our yeast model of Huntington's disease, we have expanded our studies to include *in-vitro* assays and human cells derived from Huntington's disease patients to aid in our research. Preliminary studies *in-vitro* support our hypothesis that at least some of the $NAD^+$ salvage pathways proteins may have intrinsic chaperone function. Additionally, preliminary studies of catalytically inactive $NAD^+$ salvage pathway proteins in our yeast model of Huntington's disease in a variety supports previous findings that the catalytic function within the $NAD^+$ salvage pathway is non-essential for their protective function. These preliminary results suggest that proteins of the $NAD^+$ salvage pathway may have previously unknown functions within the cell, and suggests that these proteins may be possible targets for therapeutic intervention in Huntington's disease and other diseases of protein misfolding.

**Disclosures:** **A. Ruetenik:** None. **A. Ocampo:** None. **A. Barrientos:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.05/V6

**Topic:** C.04. Movement Disorders

**Support:** R01NS080331

**Title:** Mutant huntingtin is secreted via a late endosomal/lysosomal unconventional secretory pathway

**Authors: *K. TRAJKOVIC**, H. JEONG, D. KRAINC
Neurol., Northwestern University, Feinberg Sch. of Medici, Chicago, IL

**Abstract:** Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of a CAG triplet in the gene encoding for huntingtin. The resulting mutant protein huntingtin (mHtt) with extended polyglutamine (polyQ) sequence at the N-terminus leads to neuronal degeneration both in cell-autonomous and non-cell-autonomous manners. Recent studies identified mHtt in the extracellular environment and suggested that its spreading contributes to toxicity, but the mechanism of mHtt release from the cell of origin remains unknown. In this study, we performed a comprehensive, unbiased analysis of secretory pathways and identified an unconventional lysosomal pathway as an important mechanism for mHtt secretion in mouse neuroblastoma cell line. Mutant Htt secretion was dependent on synaptotagmin 7, a regulator of lysosomal secretion, and inhibited by chemical ablation of late endosomes/lysosomes, suggesting a lysosomal secretory pattern. Moreover, mHtt was preferentially targeted to the late endosomes/lysosomes compared to wild-type Htt. Importantly, we found that late endosomal/lysosomal targeting and secretion of mHtt could be efficiently inhibited by the phosphatidylinositol 3-kinase (PI3-kinase) and neutral sphingomyelinase (NS) chemical inhibitors, Ly294002 and GW4869, respectively. Together, our data suggest a lysosomal mechanism of mHtt secretion and offer potential strategies for pharmacological inhibition of neuronal secretion of mHtt.

**Disclosures: K. Trajkovic:** None. **H. Jeong:** None. **D. Krainc:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.06/V7

**Topic:** C.04. Movement Disorders

**Support:** CIHR 20R90174

**Title:** The autophagy receptor p62/SQSTM1 is palmitoylated and significantly decreased in brains of Huntington disease patients

**Authors: *D. D. MARTIN**[1], S. S. SANDERS[1], N. S. CARON[1], Y. T. NYUGEN[1], D. J. KLIONSKY[2], M. R. HAYDEN[1]
[1]Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. of Michigan, Ann Arbor, MI

**Abstract:** Autophagy is essential for the removal of damaged proteins and organelles by delivering them to the lysosome for degradation and recycling. Induction of autophagy requires quick relocalization of many proteins from the cytosol to organellar membranes. A potential mechanism mediating this shift is lipidation. However, lipidation of many autophagy proteins has been overlooked. We recently constructed a compendium of palmitoylated proteins from

proteomic studies and performed enrichment analysis to determine the pathways and diseases that are enriched for palmitoylation. Similar to phosphorylation, palmitoylation involves the dynamic and reversible addition of the fatty acid palmitate to cysteine residues. Schizophrenia, HD and ALS were among the top diseases predicted to be enriched in palmitoylation. A new comparison between all known palmitoylated genes and the autophagy regulatory network shows significant enrichment of palmitoylation in the regulation of autophagy (~50%). Consequently, dynamic and reversible palmitoylation may be crucial for regulating autophagy and allowing the rapid relocalization of its regulators. Subsequently, by focusing on points of convergence within the autophagy pathway, we confirmed that an essential autophagy receptor, sequestosome-1 (SQSTM1, also known as p62), is palmitoylated. p62 is required for the removal of aggregated proteins and damaged mitochondria. Consequently, mutations in *SQSTM1* are associated with neurodegeneration, including frontotemporal dementia and amyotrophic lateral sclerosis (ALS). Dysfunctional p62 cargo loading is also associated with the neurodegenerative disease Huntington disease (HD). We show that palmitoylated p62 is degraded by the lysosome, signifying that palmitoylated p62 is the "active" form of p62. Subsequently, we confirmed that palmitoylation of p62 is significantly decreased in the brains of an HD mouse model and in the cortex of human HD patients. Strikingly, we demonstrate that p62 palmitoylation can be altered in mouse brains by 2 different fasting regimes, thereby demonstrating that palmitoylation can be dynamically regulated in the brain through fasting. **Appeal:** This is study suggests that palmitoylation may have a greater role in regulating autophagy. This is also the first demonstration that palmitoylation can be regulated by diet, suggesting that diseases with dysregulated palmitoylation, like Schizophrenia or HD, may be normalised through diet or fasting. We clearly show that p62 is palmitoylated and significantly decreased in human HD brains suggesting that the deficiency in cargo loading in HD may be related to decreased palmitoylation of p62.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.07/V8

**Topic:** C.04. Movement Disorders

**Support:** NIH NINDS R01 NS079450

**Title:** Mitochondrial iron dysregulation in mouse and human Huntington's disease brain

**Authors: S. AGRAWAL**, J. A. FOX, *J. H. FOX
Vet. Sci., Univ. of Wyoming, Laramie, WY

**Abstract:** Huntington's disease (HD) is an autosomal dominant disorder caused by a CAG repeat expansion in the huntingtin gene. Mitochondria have an important role in HD pathogenesis and are also a major site of iron utilization for heme and iron-sulfur cluster protein synthesis. Brain iron dysregulation is involved in the pathogenesis of HD. We investigated a role for mitochondrial iron dysregulation in disease pathogenesis. Using brain regional sub-cellular fractionation and ICP-MS measurements for total iron, in R6/2 and YAC128 HD models, we demonstrate that brain iron accumulation is present in mitochondrial, but not cytoplasmic fractions. To investigate potential mechanisms of mitochondrial iron accumulation in HD models we completed Western blot analyses of the mitochondrial iron homeostatic proteins mitoferrin 2, ABCB8 and frataxin in R6/2 mice and in human HD brain. Levels of the iron-uptake protein mitoferrin 2 were increased in R6/2 striatum and cortex and in human HD BA9 and BA17 neocortical regions. Levels of the mitochondrial iron export ABCB8 protein were unaltered in R6/2 mice. Levels of frataxin, a protein involved in iron-sulfur cluster synthesis, were decreased in R6/2 striatum and cortex and human HD BA4 and BA9 neocortical regions. We further demonstrate that oxidative stress markers in brain mitochondrial membrane fractions are increased. Specifically, there are significant elevations of mitochondrial lipid peroxidation products and oxidized glutathione in R6/2 mouse striatum and cortex. Findings support a role of dysregulated mitochondrial iron in the pathogenesis of HD as studied in genetic mouse models.

**Disclosures: S. Agrawal:** None. **J.A. Fox:** None. **J.H. Fox:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.08/DP07/V9 (Dynamic Poster)

**Topic:** C.04. Movement Disorders

**Support:** DIM Biotherapy

**Title:** Gene therapy based on SMaRT for Huntington's disease: Fluorescent screening to assess trans-splicing RNA molecule activity

**Authors: *S. C. MAIRE**[1,2], M.-C. GAILLARD[1,2], A. BERGER[3], N. DUFOUR[1,2], C. JOSÉPHINE[1,2], P. HANTRAYE[1,2], E. BROUILLET[1,2], A.-P. BEMELMANS[1,2]
[1]DRF, CEA, Fontenay Aux Roses, France; [2]MIRCen, Neurodegenerative Dis. Lab., Fontenay aux Roses, France; [3]Inst. de la Vision, Paris, France

**Abstract:** Huntington's disease (HD) is an autosomal dominant genetic disorder caused by a CAG repeat expansion mutation located in the first exon of the Huntingtin gene (*HTT*). We use spliceosome mediated RNA *trans*-splicing (SMaRT) to develop an innovative strategy of gene therapy that will significantly reduce or eliminate the expression of the mutant protein while

restoring a physiological level of normal *HTT*. This process is achieved using an artificial RNA, termed pre-*trans*-splicing molecule (PTM), which has the capacity to exchange the mutant exon with a mutation-free exon. In the case of a dominant mutation, therapeutic benefits can only result from high rates of correction. Therefore, our goal is to achieve the most efficient *trans*-splicing (TS) reaction possible by implementing different strategies of PTMs screening in order to select *HTT*-targeting PTMs efficient enough to exhibit therapeutic benefit in affected neuronal population.
Our screening strategy is based on an artificial fluorescent reporter system which results in the production of two fluorescent proteins: (i) a chimeric fluorescent protein (XFP) (ii) a mTurquoise2 protein. XFP sequence is basically composed of YFP sequence in 5' and mTurquoise2 sequence in 3'. Since these two proteins are derived from GFP, the combination of these sequences results in chimeric fluorescent compound at protein level. The innovative strategy with this construction is to insert between these two sequences an *HTT* target intron, exhibiting splicing activity. In case of *cis*-splicing, XFP is produced with varying fluorescence emission levels, dependent on XFP expression driven by the strength of the *HTT* target intron introduced (MaxEnt analysis, Yeo et al, 2003). We constructed PTMs libraries for each *HTT* intron targeted with 5' mTurquoise2 sequence as exchanging exon. When TS occurs with those PTMs, it is supposed to produce the native mTurquoise2 protein with distinct excitation and emission spectra, allowing detection and quantification. PTM libraries were tested to assess their TS activity in HEK293T cells using epi-fluorescence microscopy and flow cytometry. We first screened hundreds of PTMs and assessed their individual TS activity but this strategy has not been successful. Then we combined PTMs with different targets supposing to act in synergy in inhibiting *cis*-splicing process. This new strategy gave us promising results with fluorescence emission significantly ($p=0.007$, T-test, $n=3$) higher than negative control, suggesting a TS activity. We will next adapt PTM constructs to the *HTT* context and validate their potential therapeutic benefits in relevant cellular models such as HD patient-derived iPS cells.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.09/V10

**Topic:** C.04. Movement Disorders

**Support:** NIH R01 NS076631

NIH T32NS007413

**Title:** Mutant Huntingtin disrupts CELF1-MBNL1 regulation in patient brain and model systems

**Authors: *S. RAMACHANDRAN**[1], S. L. COFFIN[1], G. G. CAJKA[1], M. S. KEISER[1], C. A. ROSS[2], B. L. DAVIDSON[1]
[1]Pathology, The Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Div. of Neurobio., Johns Hopkins Med. Sch., Baltimore, MD

**Abstract:** Huntington's disease (HD) is caused by a polyglutamine-repeat expansion in exon 1 of *huntingtin (HTT).* The mutation confers gain of function phenotypes on the HTT protein, as well as on transcripts arising from the *HTT* locus. Additionally, recent analyses show differential gene expression and alternative splicing in HD brain, with RNA-binding proteins as candidate mediators of that misregulation. Here, we show in neurons *in vivo* and in culture, that the presence of mutant huntingtin (mHTT) alters the transcript and protein levels of the RNA binding proteins Muscleblind-like Splicing regulator 1 (MBNL1) and CUGBP/Elav-Like Family Member 1 (CELF1), with CELF1 more stable and abundant in HD tissues, and MBNL1 levels reduced. Moreover, the MBNL1 that is present has partitioned to a less soluble species that co-localizes with mHTT transcripts. CELF1s stability in HD cells results from a combination of a MBNL1-dependent switch in poly(A) site preference on the transcript, and a PKR- and PKC-dependent hyperphosphorylation of the protein. Intriguingly, the extent of CELF1-MBNL1 regulatory loop disruption is correlative with the CAG-repeat length. Knockdown of m*HTT* rescues the misregulation of the CELF1-MBNL regulatory loop, providing further support for a gain-of-function role for m*HTT* transcripts.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

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**Topic:** C.04. Movement Disorders

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The Solomon H. Snyder Department of Neuroscience Graduate Program

National Institute of Neurological Disorders and Stroke

Brain Science Institute

CIRM Training Grant

**Title:** Mutant huntingtin disrupts the nuclear pore complex

**Authors: *J. C. GRIMA**[1], J. G. DAIGLE[1], N. ARBEZ[1], K. C. CUNNINGHAM[1], K. ZHANG[1], J. OCHABA[2], C. GEATER[2], E. MOROZKO[2], J. STOCKSDALE[2], J. C. GLATZER[1], J. T. PHAM[1], I. AHMED[1], Q. PENG[1], H. WADHWA[1], O. PLETNIKOVA[1], J. C. TRONCOSO[1], W. DUAN[1], S. H. SNYDER[1], L. P. RANUM[3], L. M. THOMPSON[2], T. E. LLOYD[1], C. A. ROSS[1], J. D. ROTHSTEIN[1]
[1]The Solomon H. Snyder Dept. of Neurosci., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Psychiatry and Human Behavior, Univ. of California Irvine, Irvine, CA; [3]Mol. Genet. and Microbiology, Univ. of Florida, Gainesville, FL

**Abstract:** Huntington's disease (HD) is caused by an expanded CAG repeat in the Huntingtin (HTT) gene. The mechanism(s) by which mutant HTT (mHTT) causes disease is unclear. Nucleocytoplasmic transport, the trafficking of macromolecules between the nucleus and cytoplasm, is tightly regulated by nuclear pore complexes (NPCs) made up of nucleoporins (NUPs). Previous studies offered clues that mHTT may disrupt nucleocytoplasmic transport and a mutation of an NUP can cause HD-like pathology. Therefore, we evaluated the NPC and nucleocytoplasmic transport in multiple models of HD, including mouse and fly models, neurons transfected with mHTT, HD iPSC-derived neurons, and human HD brain regions. These studies revealed severe mislocalization and aggregation of NUPs and defective nucleocytoplasmic transport. HD repeat-associated non-ATG (RAN) translation proteins also disrupted nucleocytoplasmic transport. Additionally, overexpression of NUPs and treatment with drugs that prevent aberrant NUP biology also mitigated this transport defect and neurotoxicity, providing future novel therapy targets.

**Disclosures: J.C. Grima:** None. **J.G. Daigle:** None. **N. Arbez:** None. **K.C. Cunningham:** None. **K. Zhang:** None. **J. Ochaba:** None. **C. Geater:** None. **E. Morozko:** None. **J. Stocksdale:** None. **J.C. Glatzer:** None. **J.T. Pham:** None. **I. Ahmed:** None. **Q. Peng:** None. **H. Wadhwa:** None. **O. Pletnikova:** None. **J.C. Troncoso:** None. **W. Duan:** None. **S.H. Snyder:** None. **L.P. Ranum:** None. **L.M. Thompson:** None. **T.E. Lloyd:** None. **C.A. Ross:** None. **J.D. Rothstein:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.11/V12

**Topic:** C.04. Movement Disorders

**Support:** CHDI Foundation

**Title:** Changes in PT and IT corticostriatal projections in the Q175 mouse model of Huntington's disease

**Authors: *T. PANCANI**, J. KONDAPALLI, J. SURMEIER
Dept. of Physiol., Northwestern Univ. Feinberg Sch. of Medicin, Chicago, IL

**Abstract:** Huntington's disease (HD) is a neurodegenerative disease characterized by severe motor alterations. The neuropathological occurrences underlying behavioral changes are extensive and include significant loss of striatal and cortical parenchyma. Direct communication between cortex and striatum is ensured by glutamatergic corticostriatal (CTX-STR) fibers which play a pivotal role in shaping motor behavior. Not surprisingly, a growing body of evidence is revealing significant alterations of these fibers in several mouse models of HD. CTX-STR axons are mainly originating from two distinct classes of cortical neurons: intratelencephalic (IT) and pyramidal tract (PT) neurons. IT neurons project within the telencephalon ipsi- and contralaterally to cortex and striatum forming the main component of the callosal commissure. Conversely, PTs project only ipsilaterally to extratelencephalic regions such as thalamus or brainstem forming also *en passant* synapses in striatum. The severe and progressive reduction of corpus callosum fibers seen in HD clearly suggest that CTX-STR IT projections are altered in HD. Moreover, the loss and/or alteration of SMI32-immunopositive long-projecting pyramidal neurons, seen in M1 cortex, suggests that PT projections are also affected in HD. However, alterations of corticostriatal fibers originating from specific cortical neuronal subpopulations has not been studied in the context of HD. Using the Q175 mice and IT and PT - Cre expressing mouse lines, together with optogenetics and electrophysiology techniques, we found that IT CTX-STR glutamatergic transmission onto both direct and indirect pathway SPNs is surprisingly increased in 7-8 - month-old Q175 mice while the opposite trend is seen at PT - SPNs synapses. At IT synapses, we also found that the amplitude of $Sr^{2+}$-mEPSCs is unaltered in SPNs from HD mice compared to control, suggesting increased glutamate release sites rather than increased neurotransmitter's release probability. To address this issue, we used sCRACM and mGRASP to determine specific topological alterations of CTX-STR IT (and also PT) synapses onto SPNs. Furthermore, it is well established that mAChRs can potently modulate CTX-STR glutamatergic transmission and a recent report show that this effect is altered in HD. Interestingly, we also found that muscarinic receptors (mAChR) activation has a differential modulatory influence on CTX-STR IT versus PT synapses and this effect is altered in Q175 symptomatic mice.

**Disclosures:** **T. Pancani:** None. **J. Kondapalli:** None. **J. Surmeier:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

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**Program#/Poster#:** 574.12/V13

**Topic:** C.04. Movement Disorders

**Support:** Chinese Ministry of Science and Technology Grant 2016YFC0905100

NSFC grant 91649105

**Title:** Targeting Gpr52 lowers mutant huntingtin levels and rescues huntington's disease-associated phenotypes

**Authors:** *B. LU[1], ***B. LU**[1], X. XIE[2], H. SONG[1]
[1]Fudan Univ., Shanghai, China; [2]CAS Key Lab. of Receptor Research, Natl. Ctr. for Drug Screening, Shanghai, China

**Abstract:** Lowering the levels of disease-causing proteins is an attractive treatment strategy for neurodegenerative disorders, among which Huntington's disease (HD) is an appealing model to test this strategy because of its monogenetic nature, allowing early diagnosis/treatment and establishment of reliable genetic disease models. HD is mainly caused by cytotoxicity of the mutant huntingtin protein (mHtt) with an expanded polyglutamine repeat (polyQ). Lowering mHtt is a promising HD treatment strategy, but this is hard to be achieved by small molecule drugs due to a lack of effective drug targets. Here we demonstrate that knocking-out an orphan G protein-coupled receptor (GPCR), Gpr52, significantly reduces mHtt levels in the striatum and rescues HD-associated behavioral phenotypes in a knock-in HD mouse model. Importantly, a novel Gpr52 antagonist E7 also reduces mHtt levels and rescues HD phenotypes in cellular and mouse models. Our study provides a proof-of-concept in treating HD by targeting GPR52 with small molecule drugs aiming at lowering the mHtt level.

**Disclosures: B. Lu:** None. **X. Xie:** None. **H. Song:** None.

**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.13/V14

**Topic:** C.04. Movement Disorders

**Support:** Huntington's Disease Society of America

Canadian Institutes of health Research

**Title:** Cerebrospinal fluid mutant huntingtin concentration: What does it mean?

**Authors:** ***A. L. SOUTHWELL**[1], N. S. CARON[2], C. YANICK[1], S. E. SMITH[3], Y. XIE[2], J.-J. SONG[4], I. SEONG[5], B. LEAVITT[2], M. HAYDEN[2]
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**Abstract:** A variety of huntingtin (HTT) lowering strategies have shown preclinical promise for the treatment of Huntington disease (HD), and the first HTT lowering clinical trial is now underway. Based on our previous work demonstrating that CNS-wide suppression of HTT in mice results in correlative reduction of cerebrospinal fluid (CSF) mtHTT, CSF mtHTT is being used as a marker of target engagement. However, there is very little known about CSF mtHTT; where it comes from, how it enters CSF. Thus, interpretation of treatment-induced changes, or lack thereof is not currently possible. We are using our ultrasensitive immunoprecipitation and flow cytometry (IP-FCM) mtHTT detection assay to address these questions and enable substantive use of CSF mtHTT as an HD biomarker. Unlike mice, HTT lowering in man is not expected to be CNS-wide. The agent under trial is expected to be predominantly active in the cortex, but partial cortical reduction may be insufficient to result in measureable CSF reduction. This could erroneously indicate failure to suppress HTT in the cortex. The same applies to agents delivered by viral vector that may only be active in the basal ganglia or in specific cell types. Thus, we are interrogating the source of CSF mtHTT protein using BACHD HD model mice (floxed mtHTT exon 1) crossed to brain region and cell type-specific cre mice. These crosses generate mice with selective KO of mtHTT and enable evaluation of the contribution of the region or cell type at study to the pool of CSF mtHTT protein. Additionally, we do not know how mtHTT enters CSF. If it is mostly released passively from dying cells, any neuroprotective therapy would be expected to reduce CSF mtHTT, but if it is actively cleared from the brain, only HTT lowering therapeutics targeting regions or cells that substantially contribute to CSF mtHTT would be expected to reduce it. Furthermore, therapeutics that increase brain HTT clearance could even increase CSF mtHTT. We have previously shown that mtHTT is not detected in CSF of all premanifest HD mutation carriers and that acute brain injury causes a transient increase in CSF mtHTT. This suggests that HTT is passively released to CSF from dying cells. However, we have detected high concentrations of mtHTT in the CSF of a mouse that lacks neurodegeneration, suggesting that there may be both passive release and active clearance mechanisms at play. We are ectopically delivering intracellular and extracellular mtHTT to investigate the kinetics and mechanism of mtHTT clearance to CSF. Delineating the source and mechanism of entry of CSF mtHTT protein will greatly enhance the utility of this measure as an HD biomarker.

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**Poster**

**574. Cell Biology of Huntington's Disease II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 574.14/V15

**Topic:** C.04. Movement Disorders

**Support:** CHDI

Dake Family Fund

NS RO1 NS100529

**Title:** Evidence for altered regulation of the Rac-Actin pathway in mouse and human models of Huntington's disease

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**Abstract:** Neuronal loss in HD striatum is preceded by as yet unexplained changes in dendritic morphology including the marked loss of spines, onto which most synaptic contacts occur. The small GTPase Rac1 regulates Actin-dependent morphology changes including changes in dendritic spines, growth cone shape and motility. Striatum of HD knock-in mice (Q140/Q140) had significantly higher levels of Rac1 activity at 6 weeks followed by a significant decrease at 4.5 months compared to age matched wild-type controls. Furthermore, human neural stem cells (NSCs) derived from induced pluripotent stem cells (IPSCs) with 42Q, 50Q, and 72Q in Huntingtin had increased basal Rac1 activation compared to controls and correction of the 72Q repeat to 21Q by homologous recombination reduced Rac1 activation levels in NSCs. Human neuronal cultures from HD IPSCs differentiated towards medium spiny neurons with 50Q showed increased Rac1 activation compared to wild-type cultures derived from two independent control lines. Morphology driven processes such as motility and growth cone development which are regulated by Rac1 were also changed in HD cells compared to controls. At a molecular level, immunoprecipitation of endogenous wild-type and mutant Huntingtin co-precipitated the p85α regulatory subunit of PI 3-kinase, Rac1 and Alpha-actinins. Alpha-actinins can crosslink and bundle Actin filaments, regulate spine shape, and can be regulated by Rac1 during cell adhesion. In control human fibroblasts where the microfilament cytoskeleton is easily observed, we show localization of Huntingtin to Actin stress fibers, Vinculin-positive adhesion contacts, and co-localization with Alpha-actinin-1 at membrane ruffles and lamellapodia. Depletion of Huntingtin

by small interfering RNA (siRNA) altered cell morphology and increased basal Rac activation. Finally, reducing Huntingtin protein using siRNA in fibroblasts altered the stimulation dependent localization of Alpha-actinin-1 at the plasma membrane. All together, these data provide evidence that Rac activation is changed in mouse and human HD models resulting in diverse phenotypic changes and that normal Huntingtin is important for Rac1 regulation and localization of Alpha-actinins necessary for cell morphology.

**Disclosures: K.B. Kegel:** None. **A. Tousley:** None. **J. Alexander:** None. **E. Sapp:** None. **E. Weisman:** None. **P. Vodicka:** None. **M. Iuliano:** None. **L. Gatune:** None. **H. Cho:** None. **H. Richardson:** None. **J. Ritch:** None. **X. Li:** None. **N. Aronin:** None. **N. Zhang:** None. **L.M. Ellerby:** None. **D. Irimia:** None. **M. Difiglia:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.01/V16

**Topic:** C.04. Movement Disorders

**Support:** CIHR Grant FDN-143-210

**Title:** A novel automated home-cage system to assess learning and performance of a skilled motor task in a mouse model of Huntington’s disease

**Authors: *C. L. WOODARD**, F. BOLAÑOS, J. D. BOYD, G. SILASI, T. H. MURPHY, L. A. RAYMOND
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**Abstract:** Behavioural testing is a critical step in assessing the validity of rodent models of neurodegenerative disease, as well as evaluating the efficacy of pharmacological interventions. In models of Huntington’s disease (HD), a gradual progression of impairments is observed across ages, increasing the need for sensitive, high-throughput and longitudinal assessments. Recently, a number of automated systems have been developed to perform behavioural profiling of animals within their own home-cage, allowing for 24-hour monitoring and minimizing experimenter interaction. However as of yet, few of these have had functionality for the assessment of skilled motor learning, a potentially relevant behaviour for movement disorders such as HD. To address this, we have adapted a novel paradigm that incorporates a lever positioning task into the mouse home-cage. Animals first learn a simple version of the task, before moving to a second phase where they must learn to hold the lever for progressively longer in a rewarded position range. Testing with this paradigm has revealed the presence of distinct phenotypes in the YAC128 mouse model of HD at 2 months, 4 months and 6 months of age. YAC128 mice at 2 months, but not older, displayed a decreased ability to adapt to changes in

task demands when shifting to a more difficult task. In contrast, 4 and 6 month-old YAC128 mice exhibited circadian abnormalities and alterations on several kinematic measures, suggesting an impairment in motor control. This paradigm holds promise for facilitating high throughput behavioural assessment of HD mouse models for preclinical therapeutic screening.

**Disclosures: C.L. Woodard:** None. **F. Bolaños:** None. **J.D. Boyd:** None. **G. Silasi:** None. **T.H. Murphy:** None. **L.A. Raymond:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.04. Movement Disorders

**Support:** Stewart & Dake Family Gift

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WeHaveAFace.org

**Title:** Longitudinal analysis of mutant allele specific silencing in the YAC128 mouse using transcription activator-like effectors

**Authors: *P. DENG**[1], J. N. A. M. HALMAI[2], A. KOMARLA[3], G. T. THARMARAJAH[4], I. M. SANDOVAL[5], F. P. MANFREDSSON[6], D. J. SEGAL[1], J. A. NOLTA[7], K. FINK[7]
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**Abstract:** Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the presence of a misfolded mutant *Huntingtin* (muHTT) protein. Strategies aimed towards reducing expression of muHTT through gene silencing may prove to be an effective strategies towards attenuating disease progression. We have previously shown allele-specific silencing of the muHTT transcript in patient-derived fibroblasts via transcriptional

activator-like effectors (TALE) by targeting a single nucleotide polymorphisms (SNP) that is highly associated with the mutant allele. Furthermore, we have demonstrated robust distribution following unilateral striatal injection of our lipid nanoparticle-encapsulated TALE (LNP-TALE) across the ipsilateral striatum, sub-ventricular zone, corpus collosum, and cerebral cortex as well as presence of LNP-TALE in the contralateral striatum and cortex. Molecular analysis of those regions demonstrated a significant reduction of the muHTT and an observable reduction of the muHTT protein 48 hrs following injection.
In this study we examine LNPs and adeno-associated virus (AAV) as putative delivery vehicles for our therapeutic TALE transgene in our novel NOD-SCID gamma YAC128 (YAC128xNSG) transgenic HD mouse model. Both delivery platforms have been explored in human clinical settings and offer multiple avenues in successfully delivering therapeutic transgenes *in-vivo*. In addition, the YAC128xNSG provides a model for future toxicity studies of potential humanized immune responses towards our novel therapeutics. We have executed a longitudinal study evaluating an optimized LNP-TALE formulation and AAV9-TALE for distribution of the TALE, duration of transgene expression over the course of 3 months, duration of mutant allele silencing, and attenuation of HD-related neuropathology and behavioral phenotypes. Identification of a potent, widespread delivery vehicle and assessment of the long-term duration of expression and effect of our therapeutic transgene will be vital in the evaluation of our TALE as a viable therapeutic for HD.

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**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.03/V18

**Topic:** C.04. Movement Disorders

**Title:** $^{18}$F-fallypride pet imaging of d2/d3 receptors in huntington’s disease mouse model q175dn

**Authors: *T. HUHTALA**[1], P. POUTIAINEN[2,3], J. RYTKÖNEN[1], A. AIRAKSINEN[4], T. KOIVULA[4], T. PARKKARI[1], I. KASANEN[1], O. M. KONTKANEN[1], C. DOMINIQUEZ[5], L. C. PARK[5]
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**Abstract:** Dopamine, a neurotransmitter, plays an important role in the mediation of movement, cognition, and emotion. Dopamine receptors are involved in the pathophysiology of neuropsychiatric diseases, such as Huntington's disease, Parkinson's disease, Alzheimer's disease, and schizophrenia. $^{18}$F-Fallypride is a selective, high-affinity antagonist of D2/3R's, which has been used to study receptor occupancy and density in neuropsychiatric disorders and aging in humans. In this study, comparison of $^{18}$F-fallypride binding between heterozygous (HET, n=12) and wild-type (WT, n=12) Q175DN Huntington's mice was performed. Mice were studied at the age of 9 and 12 months. Prior the experiment, bile acid concentration (indication of liver shunt) was confirmed from plasma samples. Also, minimum of 2 weeks prior the PET scan, the males were housed with single females to minimize stress of the animals. Based on published material in rodents, primates and humans, stress and dominant behavior can affect $^{18}$F-fallypride PET imaging outcome. The least stressful housing for male mice is together with females, i.e. one male with one female (Prof. Koolhaas laboratory, University of Groningen). $^{18}$F-Fallypride was synthetized with high specific activity (SA, 298 - 360 GBq/µmol EOS) and excellent radiochemical purity (>99.8%). For the PET scan, animals were anesthetized using isoflurane, cannulated and put in the scanner. PET scan was started and $^{18}$F-fallypride (10 - 15 MBq) was administrated as a bolus. List mode PET scan over 90 min was performed, after which a CT scan was performed. For the PET analysis, 3D OSEM reconstruction was performed. To calculate $BP_{ND}$ for each individual, simplified reference tissue modeling was applied (PMOD 3.7). VOI delineation was preformed using in-house generated MRI images from HET and WT mice.Small variation in binding potential ($BP_{ND}$) was seen within genotypes and no mass effect was observed when SA of $^{18}$F-fallypride was >100 GBq/µmol at the time of injection. HET showed 30.2% decrease in $BP_{ND}$ ($p<0.0001$) when compared to WT at 9 mo and 51.6 % decrease ($p<0.0001$) at 12 mo. Further, 23.6 % decrease ($p<0.05$) was analyzed during aging in HET mice. As a summery, significant decrease in D2R/D3R availability in striatum of HET mice was observed compared to WT in 9 and 12 mo. PET imaging provides a powerful and translational research tool in Huntington's disease allowing comprehensive evaluation of disease progression and treatment interventions for in vivo studies.

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**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.04/V19

**Topic:** C.04. Movement Disorders

**Title:** Characterization of behavioral changes, MRI brain volumetry and MR spectroscopy in ZQ175 knock-in delta neo minus mouse model of Huntington's disease

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**Abstract:** zQ175 knock-in (KI) mice have been considered a valuable mouse model for Huntington's disease (HD) research. Especially mice heterozygous for the KI of an expanded CAG tract in exon 1 of the mouse huntingtin (Htt) gene mimic well the human mutation causing HD. Recently, a modified zQ175 KI mouse line was introduced. In these mice, a neomycin resistance cassette in the Htt gene locus was removed resulting in increase in the amount of mutated Htt in the mice. In this study, we aimed to characterize the behavioral phenotype, brain volumetric changes and concentrations of striatal metabolites in the newly developed zQ175 KI delta neo (DN) mice. We also wanted to compare the results to those previously obtained in zQ175 KI with existing neomycin cassette. Total of 40 female wild-type and heterozygote (HET) zQ175 KI DN mice were used. The motor behavioral tests including fine motor kinematic analysis, open field, rotarod, tapered beam balance and grip strength tests were performed at 2, 6, 9, 12 and 15 months of age. At the same age points MRI measurements of whole brain, striatum and cortical volumes, and the 1H-MR spectroscopy measurements of the striatal metabolites, including, amongst others, glutamine, myo-inositol, taurine and choline, were performed. The HET zQ175 KI DN mice had decreased body weight starting from 30 weeks of age in females and 25 weeks of age in males. Beam balance performance started to decline at 6 months and rotarod performance from 9 months onwards. Fine motor kinematic revealed genotype differences already at 2 months of age, showing clear and progressive deficits starting from 6 months of age. MRI volumetric and striatal metabolic changes were similarly progressive starting from 6 months of age. Taken together, in HET zQ175 KI DN mice the age of onset of HD-like symptoms appeared to be earlier and occurring with a more rapid progression than previously observed in KI mice of HD. These changes were most clear in the fine motor capabilities and gait.

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**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.05/V20

**Topic:** C.04. Movement Disorders

**Title:** Deficient object location/paired associates learning in Q175KI mouse model of Huntington's disease

**Authors: *M. KOPANITSA**, T. O. PIIPONNIEMI, T. PARKKARI, A. J. NURMI
Charles River Discovery, Kuopio, Finland

**Abstract:** Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by uncontrolled movements, psychiatric disturbances and cognitive impairment. Numerous mouse models of HD have been developed, but cognitive performance of such mutants is often based on approaches that are not easily translatable to clinical setting.
We examined performance of 10-12-month-old Q175 knock-in (Q175 KI) mice, expressing human mHTT allele with expanded CAG repeat (~179 repeats) within the native mouse huntingtin gene, and their age-matched wild-type (WT) counterparts in a highly translational task of object location/paired associates learning (OL/PAL). This touchscreen-based task is similar to Paired Associates Learning (PAL) test of CANTAB panel of human touchscreen tasks. Impaired performance of HD patients in the human version of PAL task has been previously reported.
During basic operant pretraining for OL/PAL task, Q175 KI and WT mice exhibited similar performance during "Initial touch", "Must touch", and "Must initiate" tasks, whereas during the last "Punish Incorrect" pretraining task, mutants achieved the criterion ~3-fold slower. This impairment stemmed from low selectivity of touches (target vs. blank) and lower number of trials attempted by Q175 KI mice. In addition, during "Punish Incorrect" pretraining task, mutant mice were slower to poke both into the image (target touch) and into the blank area of the screen (blank touch). These alterations were unlikely due to locomotor disturbances because Q175 KI and WT mice made a similar number of beam breaks and chamber traversals during the first exposure to touchscreen chamber.
During 50 36-trial OL/PAL sessions, WT mice demonstrated clear learning of the task, improving their performance from initial chance level (50%) to over 80% correct response rate during the last five sessions. In contrast, Q175 KI mice showed very little learning of the task as their performance, even at later stages, was only slightly higher than chance level (57.1 ± 1.6%). As was during the "Punish Incorrect" pretraining stage, Q175 KI also required much more days to complete the required number of trials.
We conclude that impaired learning of OL/PAL touchscreen task by Q175 KI mice parallels deficient performance of individuals diagnosed with Huntington's disease in human PAL task. Lower responding rate of Q175 mice may be an indication of an apathic phenotype. Because apathy is an important symptom in HD, assessment of Q175 KI mice in more specific tests of motivational behavior and reward-related decision making, such as progressive ratio or effort-related choice, is warranted.

**Disclosures: M. Kopanitsa:** None. **T.O. Piiponniemi:** None. **T. Parkkari:** None. **A.J. Nurmi:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

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**Topic:** C.04. Movement Disorders

**Support:** NIH Grant NS036232

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**Title:** Use of CRISPR/Cas9 to alter huntingtin expression in Huntington's disease mice

**Authors: *H. YANG**, S. YANG, R. CHANG, S.-H. LI, X.-J. LI
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**Abstract:** Huntington’s disease (HD) is a genetically inherited autosomal dominant neurodegenerative disease characterized by motor impairments, cognitive disorders and psychiatric symptoms. Expanded polyglutamine repeats (> 35) in the N-terminal region of huntingtin (mHtt) lead to the neuropathology and symptoms in HD. It is well known that N-terminal region of mHtt is responsible for the gain-of-function and that loss of Htt can cause embryonic lethality in mice. However, how N-terminal region of mHtt contributes to the neuronal survival and pathology in HD remains to be elucidated. Also, current HD mouse models that express N-terminal mutant Htt were generated by expressing exogenous Htt via the transgenic approach. Adeno-associated virus (AAV) mediated delivery of CRISPR/Cas9 *in vivo* has been used to modify endogenous genes in adult neurons in the mouse brain, which would allow us to examine the effects of altered expression of endogenous mHtt. We used AAV-CRISPR/Cas9 to remove the exon1 of mutant Htt in HD 140Q KI mouse brains. We found that permanent suppression of the endogenous expression of mHtt via CRISPR/Cas9 in the striatum of HD140Q knock-in mice can effectively deplete Htt aggregates and early neuropathology. The reduction of Htt expression in striatal neuronal cells in the adult HD140Q KI mice does not affect their viability, but alleviates their motor deficits. These findings suggest that CRISPR/Cas9 can be used to efficiently remove N-terminal region of mHtt to mitigate the neuropathology and symptoms. To further address the role of N-terminal mHtt in the mouse brain, we have also performed injection of CRISPR/Cas9 into HD KI mice to generate new HD mice that express truncated Htt with an expanded polyQ repeat at the endogenous level. We will characterize these HD mice to examine their phenotypes that are caused by endogenous N-terminal mHtt.

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**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

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**Topic:** C.04. Movement Disorders

**Support:** Conacyt Grant 291911 to FPS

**Title:** Study of the neuroprotector mechanism induced by PPARbeta/delta activation in HD models

**Authors: *A. MORALES**[1,2], A. ZAMORANO- CARRILLO[2], S. MONTES[1], H. PEDRAZA-ESPITIA[1], L. RAMOS-LANGUREN[3], C. RIOS[1], F. PÉREZ-SEVERIANO[1]
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**Abstract:** PPAR beta/delta is a Peroxisome Proliferator-Activated Receptor (PPARs) member of the nuclear receptor superfamily that can be activated by endogenous (fatty acids) or exogenous (thiazolidinediones and fibrates) ligands. From the three isoforms of PPARs (alpha, beta/delta and gamma), PPAR beta/delta is highly expressed in the CNS and their activation has been related to a neuroprotector effect. Our aim was to explore the PPARs-induced mechanism and to evaluate the therapeutic effect of PPAR beta/delta ligand preventing the damage into HD models, induced by quinolinic acid (QUIN, NMDAR agonist) and R6/1 transgenic, both in mice. (I)Male C57 black mice (25-30 g) were divided into 3 groups (n=12): 1. Vehicle-treated, administered with the striatal saline solution (SS). 2. Vehicle-treated, administered intrastriatal with QUIN to induce injury 3. L165041 (a PPAR-beta/delta selective ligand) (10 mg/kg), administered with striatal QUIN. Three days after injury, apomorphine was administered and circling behavior was evaluated. Oxidative stress was evaluated in striatum homogenate, 2 hours after QUIN injection, (reactive oxygen species and lipid peroxidation). (II) Mice R6/1 and Wild-type of 11 and 24 weeks were administrated icv a specific agonist L 165041 (120µg) and they were divided into 6 groups(n=8-10). 1. Wild Type-untreated, 2. HTT-untreated, 3.Wild Type-vehicle treated, 3. HTT-vehicle treated, 4. Wild Type-L 165041 treated, 5. HTT- L 165041 treated. Spontaneous activity and rotarod test were performed. Oxidative parameters were also measured. Our data in the QUIN-model shows that a pre-treatment with L165041 was able to reduce circling behavior and oxidative stress. Mice pre-treated recovered QUIN-induced lowered GABA levels, promoting a neuroprotector effect in QUIN-induced injured mice and exerting a higher antioxidant effect also in the transgenic model. These effects were reflected improving the response in the motor behavior to 24 weeks. In conclusion, our results suggest that the

stimulation of PPARbeta/delta by L165041 in experimental models could be a therapeutic resource to limit the progression of HD. Supported by Grant Conacyt 241911 to FPS.

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**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

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**Program#/Poster#:** 575.08/V23

**Topic:** C.04. Movement Disorders

**Support:** the CHDIF (AR)

The Methodist Hospitals Endowed Professorship in Neuroscience (AR).

**Title:** Striatal projection neuron pathology in heterozygous Q175 knock-in Huntington's disease mice

**Authors: *Y. DENG**, H. WANG, M. JONI, A. REINER
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

**Abstract:** Progressive basal ganglia neurodegeneration is a well-known feature of Huntington's disease (HD). The Q175 knock-in mouse is a slowly progressing HD model, but its basal ganglia pathology is not well characterized. Our previous studies have reported that in 18 month-old Q175 mice, although total striatal neuron numbers and striatal volumes were not significantly changed, significantly fewer striatal projection neurons were positive for DARPP32. As enkephalin (ENK) message in indirect pathway striatal projection neurons (iSPNs) was also significantly decreased, but substance P (SP) message in direct pathway striatal projection neurons (dSPNs) was unchanged, the DARPP32 perikaryal reduction appears to be preferential for ENK iSPNs. Consistent with this, we found reduction in DARPP32 in the striatal terminals in globus pallidus externus (GPe) but not in striatal terminals in globus pallidus internus (GPi) or substantia nigra pars reticulata (SNr). Despite the reduced ENK message and unaltered SP message in striatum, ENK was increased in striato-GPe terminals and SP was increased in striatal terminals in GPi and SN in 18 month-old Q175 mice. In the present study, we examined striatal projection neuron pathology in 6 and 12 month-old heterozygous Q175 mice. We found again that fewer striatal projection neurons immunolabeled for DARPP32 at 12 months, ENK message in iSPNs was significantly reduced, but SP message in dSPNs was not. Increased ENK+ striato-GPe terminals and SP+ striato- GPi and striato-SN terminals were also observed in both 6 and 12 month Q175 mice, reflecting either early occurring striatal neuron dysfunction or a

developmental abnormality. Of note, the DARPP32 reductions in striatal projection neurons and striato-GPe fibers, and the increased ENK+ and SP+ terminals in striatal target areas were all correlated with motor abnormalities such as reduced time on rotarod, and/or shorter distance traveled, slowed speed, and increased turning (a sign of chorea) in open field. Our results suggest that striatal projection neuron abnormalities are present early in Q175 knock-in mice

**Disclosures:** **Y. Deng:** None. **H. Wang:** None. **M. Joni:** None. **A. Reiner:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.09/V24

**Topic:** C.04. Movement Disorders

**Support:** Huntington's society of Canada

**Title:** Tumour necrosis factor-αlpha alters striatal synapses in YAC128 mouse model of Huntington's disease

**Authors: *P. KOMAL**[1], G. M. LEWITUS[2], H. PRIBIAG[3], H. ALTIMIMI[4], D. STELLWAGEN[5]
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**Abstract:** Huntington's disease (HD) is a neurodegenerative disease caused by an autosomal dominant mutation resulting from a variable expansion of a CAG repeat encoding polyglutamine. It is an inherited disorder principally characterized by neuroinflammmation and neurodegeneration in the striatum. In this study, we used yeast artificial chromosome (YAC) mouse model of HD which expresses human huntington's gene containing 128 CAG repeats (YAC128). We have previously reported that TNF-α contributes to homeostatic plasticity and differentially regulates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking. Here, we investigated whether TNF-α signaling altered striatal synaptic function in medium spiny neurons (MSNs) in HD mouse model. We performed whole-cell voltage-clamp recording on MSNs in acute dorsal striatal slices from adult (8-10 week old) mice. The strength of direct pathway cortico-striatal glutamatergic input was measured by targeting fluorescent dopamine D1 receptor (D1+) expressing neurons and recording the ratio of AMPAR to N-methyl-D-aspartate receptors (NMDAR) mediated excitatory post-synaptic currents (EPSCs) both in wild type and HD mice. We found YAC128 mice showed significantly higher AMPA/NMDA ratio, higher AMPA rectification and a substantial increase in the AMPA input-output curve, as compared with WT littermates. In contrast, no change in AMPA/NMDA ratio

was found in YAC128 on a TNF-α -/- background and was indistinguishable from TNF-α -/- alone. Pharmacological blockade of TNF-α signaling similarly restored normal AMPA/NMDA ratios on D1-MSNs. However, there were no significant changes in AMPA/NMDA ratios in dopamine D2 receptor expressing MSNs in any condition. Furthermore, while exogenous incubation of striatal slices with a moderate concentration (100ng/ml; 1-2hr) results in AMPAR endocytosis, we find that a high concentration of TNF-α (1mg/ml, 1-2 hr) significantly increased AMPA/NMDA ratio. This suggests that YAC128 mice have high endogenous levels of TNF-α. Collectively, our findings suggest that TNF-α regulates striatal synapses and alters corticostriatal glutamatergic inputs to MSNs during Huntington's disease.

**Disclosures: P. Komal:** None. **G.M. Lewitus:** None. **H. Pribiag:** None. **H. Altimimi:** None. **D. Stellwagen:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.10/V25

**Topic:** C.04. Movement Disorders

**Support:** ORIP/NIH Grant OD010930

National Center for Research Resources Grant P51RR000165

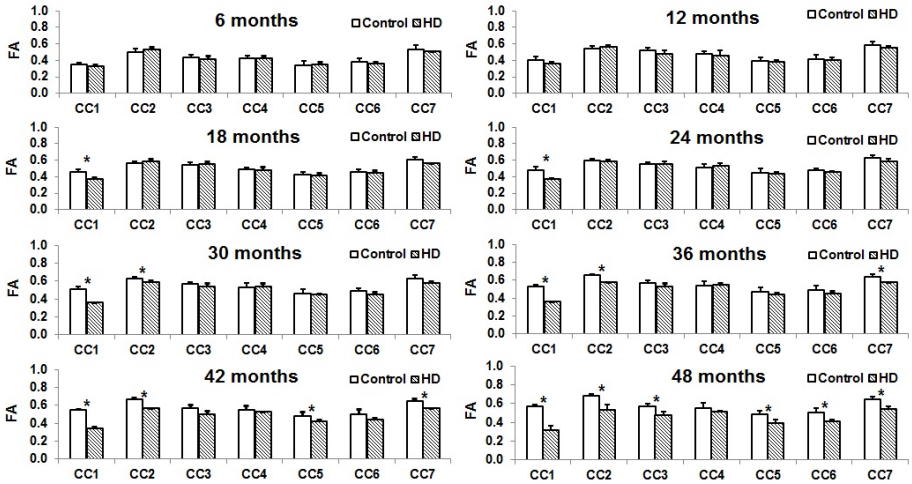
Office of Research Infrastructure Programs / OD Grant P51OD011132

**Title:** Microstructural alterations of corpus callosum in developing brains of macaques with Huntington's disease

**Authors: *Y. MENG**[1], J. BACHEVALIER[4,2], A. W. CHAN[5,3], X. ZHANG[1,3]
[1]Yerkes Imaging Ctr., [2]Div. of Developmental and Cognitive Neurosci., [3]Div. of Neuropharm. and Neurologic Dis., Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [4]Dept. of Psychology, [5]Dept. of Human Genet., Emory Univ. Sch. of Med., Atlanta, GA

**Abstract:** It is poorly understood when corpus callosum (CC) changes emerge during development with Huntington's disease (HD). HD rhesus monkeys (male, n=3) were generated using lentiviral-mediated transgenesis (Yang et al., Nature, 2008) and four age-matched wild-type non-transgenic rhesus macaques (2 males and 2 females) were used as controls. Animals were anesthetized with 1.0% isoflurane mixed with 100% $O_2$. Diffusion tensor imaging (DTI) was conducted on a Siemens 3T Trio with parallel echo planar imaging (EPI) sequence with isotropic voxel size 1.3 mm and a single b-value of 1000 s/mm$^2$ with 30 gradient directions. $T_1$-weighted images acquired were used for structural identification and template construction. FA maps were nonlinearly registered to a population-specific template and then skeletonised with

TBSS toolbox (FMRIB, Oxford). FA in the skeleton was averaged for each of the seven CC segments (from CC1 to CC7: rostrum, genu, rostral body, anterior mid-body, posterior mid-body, isthmus, and splenium) (Witelson et al., Brain, 1989). A multivariate analysis of variance (MANOVA) was performed to examine group difference in FA of each CC segments with significant level 0.05. No significant group differences in FA were noted in any CC segments before 18 months ($p>0.2$) (Fig. 1). Group differences were shown in rostrum from 18 months ($p<0.02$) and in genu from 30 months ($p<0.05$). From 36 months, splenium began showing group differences. At 48 months, most CC segments of the HD monkeys ($p<0.05$) except anterior mid-body ($p=0.22$) had FA values significantly different from controls. Altered rostrum and genu connect inter-hemispheric prefrontal cortical areas, paralleling the impairment in motor planning skills reported previously in the same HD animals at 36 months (Chan et al., PLOS One, 2015). CC alterations were observed as early as at 18 months and became more severe with ages. They parallel the findings in patients with progressive HD stages (Rosas et al., Neuroimage, 2010). The results suggest that DTI may provide valuable early neural markers for the pre-diagnosis of HD.




**Disclosures:** **Y. Meng:** None. **J. Bachevalier:** None. **A.W. Chan:** None. **X. Zhang:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.11/V26

**Topic:** C.04. Movement Disorders

**Support:** CIHR Grant FDN-143210 (to L.A.R.)

Canadian Graduate Scholarship Master's Level (to E.K.)

**Title:** Measuring glutamate transmission in Huntington disease using iGluSnFr, an optogenetic probe

**Authors: *E. KOCH**, C. WOODARD, M. SEPERS, L. A. RAYMOND
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**Abstract:** Huntington disease (HD) is a progressive neurodegenerative disorder caused by an autosomal dominant genetic mutation, and characterized by motor dysfunction, psychiatric disturbances and cognitive deficits. Degeneration of striatal medium spiny neurons is a key feature of the disease, along with cortical atrophy. Glutamate is an important excitatory neurotransmitter and dysfunction in its signalling is associated with HD. The YAC128 transgenic mouse model of HD was previously demonstrated to exhibit increased glutamate release at 1 month of age and decreased release at 12 months, and glutamate receptor signalling is altered in HD. However, little is known about alterations in mechanisms or modulation of cortical glutamate release onto striatal neurons in HD. An optogenetic probe, the iGluSnFr, is a genetically-encoded reporter that allows for sensitive, real-time measurement of glutamate dynamics. We exposed brain slices to conditions that are known to decrease cortical-striatal glutamate release, including low calcium and pharmacological activation of autoreceptors. Our results showed that the iGluSnFr signal decreases under these conditions, verifying this probe as an accurate measure of glutamate release. We are now testing the effects of pharmacological manipulation of presynaptic receptors, particularly glutamatergic NMDA receptors and cannabinoid receptor type 1, on glutamate release at corticostriatal synapses in YAC128 HD and wild-type mouse brain slices. The iGluSnFr provides a new approach to measuring glutamate release that can help us understand HD pathology and contribute to the development of treatments.

**Disclosures: E. Koch:** None. **C. Woodard:** None. **M. Sepers:** None. **L.A. Raymond:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.12/W1

**Topic:** C.04. Movement Disorders

**Support:** CHDI Grant A-7601

**Title:** Pathological changes in descending cortical projections in the zQ175 mouse model of Huntington's disease

**Authors: *N. FOSTER**[1], M. BECERRA[1], I. BOWMAN[2], M. ZHU[1], M. S. BIENKOWSKI[3], H. HINTIRYAN[1], H. DONG[4]

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**Abstract:** Huntington's disease is a neurodegenerative disorder of genetic etiology in which the basal ganglia suffers profound degeneration. Of late, the corticostriatal pathway has emerged as a key site of early disease-induced change. Previously we examined changes in the density of axonal terminals arising from several corticostriatal pathways, including primary and secondary motor cortex. This was accomplished by injections of anterograde axonal tracers into the cortex, followed by quantification of the axonal terminal fields in the caudoputamen, in 2, 6, and 12 month old zQ175 heterozygous male mice. We found selective alterations, with some pathways showing reductions in terminal density, others increases, and others no change. Yet in addition to the striatum, the cortex provides axonal input to other parts of the basal ganglia. We have therefore quantified axonal terminal densities arising from these injections and terminating in the subthalamic nucleus, i.e., the so-called hyperdirect pathway, a key component of the cortico-basal ganglia-thalamic circuit. An overview of the concerted changes in connectivity within this circuit will provide a roadmap illustrating the pathological and compensatory effects that occur in the progression of Huntington's disease.

**Disclosures: N. Foster:** None. **M. Becerra:** None. **I. Bowman:** None. **M. Zhu:** None. **M.S. Bienkowski:** None. **H. Hintiryan:** None. **H. Dong:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.13/W2

**Topic:** C.04. Movement Disorders

**Title:** Mptp mouse model of parkinson's disease - imaging modalities for metabolic, anatomical and 1h-spectroscopic changes

**Authors: *K. LEHTIMÄKI**[1], T. HUHTALA[1], J. RYTKÖNEN[1], P. POUTIAINEN[2,3], R. O. PUSSINEN[1], J. KURKIPURO[1], J. T. PUOLIVÄLI[1], A. J. NURMI[1]
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**Abstract:** Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and non-motor symptoms, including, rigidity, difficulty of movement, and impaired with walking and gait. The main pathological feature of PD is the cell death of dopaminergic neurons located in the substantia nigra pars compacta (SNc) and substantial loss of dopamine (DA) and its

metabolites, and reduced dopamine active transport (DAT) activity. Imaging technologies have been used to monitor the progression of PD as well is supporting criteria for Parkinson's diagnosis. DA plays an important role in the mediation of movement, cognition and emotion. Loss of DA-containing neurons in striatum, results in a loss of DA transporters (DAT) in the presynaptic nerve terminals and hence the reduction of DAT density is inversely correlated with the severity of motor dysfunction. In this work we have used various imaging tools in mouse model of systemic administration of MPTP by using T2-MRI, 1H-MRS and PET imaging (DAT and glucose consumption). MPTP was administered intraperitoneally (i.p.) or subcutaneously (s.c.) at different doses (20 mg/kg) two times a day with 3h-interval on two consecutive days. On week 2 and 5 MPTP treated mice showed small but non-significant trend towards anatomical atrophy in various brain structures. In addition, on same time points MPTP mice showed significant changes in cellular metabolites in striatum when compared to vehicle treated mice. PET imaging of $^{18}$F-FE-P2I was performed on week 5, clear and significant decrease in DAT activity was seen in MPTP mice. Furthermore, and supporting the 1H-MRS findings we observed that glucose consumption by FDG-PET was significantly decreased at week 6 after the MPTP challenge when compared to healthy mice. Taken together, MPTP challenge creates a permanent and long term phenotype which can be evaluated by various imaging techniques, in addition to conventional biochemical and histological and immunohistochemical means.

**Disclosures: K. Lehtimäki:** None. **T. Huhtala:** None. **J. Rytkönen:** None. **P. Poutiainen:** None. **R.O. Pussinen:** None. **J. Kurkipuro:** None. **J.T. Puoliväli:** None. **A.J. Nurmi:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.14/W3

**Topic:** C.04. Movement Disorders

**Title:** Identification of chemical compounds that interfere with Supt4h/Supt5h complex formation and suppress mutant HTT gene expression

**Authors: *Y.-Y. WU**[1,2]
[1]Natl. Yang-Ming Univ. / Molecule Med., Taipei, Taiwan; [2]Inst. of Biochem. and Mol. Biol., Taipei, Taiwan

**Abstract:** Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from expression of mutant huntingtin (*HTT*) allele that carries an expanded CAG tri-nucleotide repeat in the exon 1 of coding sequence. HD afflicted individuals suffer from progressive motor, cognitive, and psychiatric impairments, and usually die 10 to 15 years after the onset of disease. Lowering the level of toxic mutant HTT alleviates the behavior symptoms and pathological features of HD. However, since normal HTT is required for neuronal

development and functions, it is desirable to develop a therapeutic intervention that preferentially suppresses mutant HTT for countermeasures against HD.
Supt4h and Supt5h form a protein complex that aids RNA polymerase II moving persistently along the chromatin template during transcription elongation. Our earlier studies demonstrated that interference of Supt4h/Supt5h complex formation by lowering Supt4h results in a substantial decrease of transcript production from mutant HTT allele while having a marginal effect on the wild-type allele. Moreover, the motor function deficits and lifespan shortening of HD mice are ameliorated by genetic knockout of Supt4h, suggesting targeting Supt4h or Supt4h/Supt5h complex formation is a feasible approach to suppress mutant HTT and likely against HD.
Here, we employ bimolecular fluorescence complementation assay to design a novel screening system that enables us to identify reagents preventing the formation of Supt4h/Supt5h protein complex. We have identified and validated multiple hits with the capability of lowering mutant HTT expression in mouse striatal neural cells or lymphoblastoid cells derived from HD patients. These chemical compounds also show a rescue effect on rough eye and declined eclosion rate that are caused by expression of mutant HTT in Drosophila HD models, supportive of their potential use in HD.
Fibromyalgia 2016
June 15-16, 2016

**Disclosures: Y. Wu:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.15/W4

**Topic:** C.04. Movement Disorders

**Title:** Age-related decline in complex cognitive function in the HttQ111/+ mouse model of Huntington's disease

**Authors: R. MARX**, K. CRICHTON, R. GATLIN, L. HOFFMANN, J. O'SELL, Y. RYBALKA, J. CARROLL, *J. M. FINLAY
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**Abstract:** Complex cognitive deficits significantly affect the daily lives of people living with Huntington’s disease (HD) even before the characteristic motor symptoms of the disease emerge. In HD mutation carriers, the prevalence and severity of cognitive symptoms increases with age; however, few studies have examined the time course of these deficits in mouse models of the disorder. Our research examines cognitive function in the $Htt^{Q111/+}$ knock-in mouse model (n=17) and wildtype mice (n=14) at 9 months and 18 months of age. Cognitive function was assessed using a 5-choice serial reaction time task (5-CSRTT) in which mice were trained to perform a

nosepoke response to a brief, random illumination of one of five apertures. Correct responses were rewarded with delivery of a food pellet. Our findings indicate that there is a greater age-related decline in cognitive function in $Htt^{Q111/+}$ than wildtype mice. For example, performance deficits in $Htt^{Q111/+}$ mice in the 5-CSRTT under baseline conditions [1.6 s stimulus duration (SD)] were most apparent at 18 months. At 9 months, $Htt^{Q111/+}$ accuracy was ~5% lower and omissions were ~8% higher than that of age-matched wildtype mice ($Htt^{Q111/+}$: 85±1 & 27±3; wildtype: 89±1 & 25±4; all data are presented as mean percent accuracy or omissions ± SEM). When the mice were retested at 18 months, $Htt^{Q111/+}$ accuracy was ~10% lower and omissions were ~25% higher than that of wildtype ($Htt^{Q111/+}$: 77±3 & 39±4; wildtype: 85±2 & 31±4). Correct response latency was ~25% longer in $Htt^{Q111/+}$ mice than wildtype mice at both 9 and 18 months of age (9 months: 1.5±0.1 & 1.2±0.1 sec; 18 months: 1.4±0.1 & 1.1±0.1 sec). We examined whether increasing cognitive demand exacerbated performance deficits in $Htt^{Q111/+}$ mice by testing under conditions of reduced SD, variable intertrial intervals, and reduced stimulus intensity. Again, performance deficits in $Htt^{Q111/+}$ mice were most apparent at 18 months. When mice were tested at 9 months of age under reduced SD (0.4, 0.8, 1.2, & 1.6 sec), $Htt^{Q111/+}$ accuracy was ~2% lower and omissions were ~12% higher than that of wildtype mice ($Htt^{Q111/+}$: 80±2 & 36±3; wildtype: 81±3 & 32±4). When the mice were retested at 18 months, $Htt^{Q111/+}$accuracy was ~15% lower and omissions were ~28% higher than that of wildtype ($Htt^{Q111/+}$: 68±3 & 46±4; wildtype: 80±4 & 36±5 omissions). Our results suggest that, like human HD mutation carriers, $Htt^{Q111/+}$ mice experience progressive impairments in attention and impulsivity. Results of the present study differ from those of a recent publication in which Yhnell and colleagues reported that cognitive deficits in the $Htt^{Q111/+}$ mouse model of HD are not progressive [PLoS ONE. 2016, 11(10):e0164072].

**Disclosures: R. Marx:** None. **K. Crichton:** None. **R. Gatlin:** None. **L. Hoffmann:** None. **J. O'Sell:** None. **Y. Rybalka:** None. **J. Carroll:** None. **J.M. Finlay:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.16/W5

**Topic:** C.04. Movement Disorders

**Title:** Polyq huntingtin levels in ecf, csf, and brain tissue of q175 mice

**Authors: M. S. HEINS**[1], K. HUININK[3], A. RASSOULPOUR[2], *M. MONBUREAU[2], K. LO[4], S. DIJKSTRA[4], G. MCALLISTER[5], D. MACDONALD[6], I. MUNOZ SANJUAN[6], R. CACHOPE[7], L. MRZLJAK[6]
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Cambridge, United Kingdom; [6]CHDI Management/CHDI Fndn., Los Angeles, CA; [7]Translational Biol., CHDI Mgmt. / CHDI Fndn., Los Angeles, CA

**Abstract:** Huntington's disease (HD) is a neurodegenerative disorder caused by a mutation in the gene encoding the Huntingtin protein (HTT) resulting in a poly-glutamate (polyQ) expansion at the N-terminus of the protein. Due to this increased number of glutamate repeats (> 36) the HTT protein becomes unstable and neurotoxic. We optimized the conditions for *in vivo* microdialysis and sample collection to enable quantitation of polyQ HTT in striatal extracellular fluid (ECF) of Q175 mice. All samples were analysed by Charles River (the Netherlands). We found that polyQ HTT levels in the Q175 mice were increasing with age and that levels in homozygotes were higher than in age-matched heterozygotes, indicating increased levels of polyQ HTT aggregation in these mice. Additionally, we compared of the levels in striatal ECF, CSF and brain tissue, which showed that the polyQ HTT levels in ECF have a correlation with the disease progress, while levels in CSF and brain tissue show an inverse correlation.

**Disclosures: M.S. Heins:** None. **K. Huinink:** None. **A. Rassoulpour:** None. **M. Monbureau:** None. **K. Lo:** None. **S. Dijkstra:** None. **G. McAllister:** None. **D. Macdonald:** None. **I. Munoz SanJuan:** None. **R. Cachope:** None. **L. Mrzljak:** None.

**Poster**

**575. Animal Models of Huntington's Disease**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 575.17/W6

**Topic:** C.04. Movement Disorders

**Support:** CHDI Foundation

**Title:** Development of primary neuronal cortico-striatal co-cultures derived from knock-in mice as a model for Huntington's disease therapy

**Authors: K. BOEKHOORN**[1], R. VAN DE BOSPOORT[1], A. STRIJBOSCH[1], S. LACHIZE[1], N. VAN DEN BERG[1], W. GRERNRUM[1], L. GEERTS[1], S. DIJKSTRA[1], M. DA SILVA[1], P. HALONEN[1], *D. F. FISCHER[2], G. MCALLISTER[2], S.-W. JANG[3], I. MUNOZ-SANJUAN[3] [1]Discovery, Charles River, Leiden, Netherlands; [2]Discovery, Charles River, Saffron Walden, United Kingdom; [3]CHDI Fndn., Los Angeles, CA

**Abstract:** Huntington's disease (HD) is a severe neurodegenerative disorder characterized by motor, cognitive and psychiatric effects. HD is an autosomal dominant, inherited disease that is caused by an unstable trinucleotide repeat expansion (CAG repeats) in the *huntingtin (HTT)* gene. To better understand HD pathogenesis in a relevant cellular context, we developed a primary neuronal cortico-striatal co-culture platform derived from one of well-characterized

knock-in models, zQ175, further engineered to remove a neomycin cassette for genomic integrity. In particular, a robust HTT quantification assay was established in the co-cultures to study the effect of candidate therapeutics on endogenous soluble HTT levels. First, Homogeneous Time Resolved Fluorescence (HTRF) genotyping was performed, allowing for rapid and reliable identification of the knock-in mice in mixed genotype litters. Cortical and striatal neurons were harvested and subsequently subjected to electroporation separately with different fluorescent-tagged reporters and plating on top of a rat astrocyte feeder layer. Different treatment set-ups related to compound addition and readout time points were tested to obtain the most optimal assay set-up. Soluble mutant HTT levels were quantified using a Meso Scale Discovery (MSD) platform. Cell survival and toxicity were measured using high content assays for different reporters and viability. Treatments using previously characterized reference compounds showed that the best window of lowering soluble HTT over toxicity occurred at the later time points. Our results demonstrate that primary neuronal cortico-striatal co-cultures derived from the knock-in mice can be utilized as a robust model system, in particular, for the study of different candidate therapeutics for HTT lowering. In order to further develop physiologically relevant primary neuronal co-cultures, current efforts are focused on assessing the survival of the co-cultured cells using a mouse astrocyte feeder layer. Moreover, additional MSD assays for the quantification of total and mutant Htt levels, *e.g.* in an aggregated form, are also under development.

**Disclosures: K. Boekhoorn:** None. **R. van de Bospoort:** None. **A. Strijbosch:** None. **S. Lachize:** None. **N. van den Berg:** None. **W. Grernrum:** None. **L. Geerts:** None. **S. Dijkstra:** None. **M. da Silva:** None. **P. Halonen:** None. **D.F. Fischer:** None. **G. McAllister:** None. **S. Jang:** None. **I. Munoz-Sanjuan:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.01/W7

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** BMS Program

NIH NS072454

**Title:** Calcium regulation of dorsal root ganglion neurons after peripheral nerve injury

**Authors: *M. WALTERS**, D. R. LADLE, M. J. SONNER
Neuroscience, Cell Biol. and Physiol., Wright State Univ., Dayton, OH

**Abstract:** Peripheral nerve injuries (PNIs) occur when a nerve is damaged often as a result of motor vehicle accidents, falls, sport events, and other types of movement-related traumas. The failure to effectively treat PNIs means patients are often left with disability, neuropathic pain, social and economic hardships, and a decreased quality of life. Full functional recovery is dependent on the recovery of spinal reflexes whose feedback fine-tunes our movements. After PNI, reflexes remain abnormal, limiting motor coordination and functional improvement. The goal of this study is to better characterize the mechanisms responsible for these reflex-abnormalities with hopes that this knowledge will translate to better outcomes for patients who have suffered PNIs. Research has shown that neurons of the peripheral nervous system, whose cell bodies reside in dorsal root ganglia (DRGs), become hyperexcitable after injury due to changes in calcium and potassium currents. However, the majority of these studies have been *in vitro* , limiting their applicability to living animals. By employing less invasive methods than previous studies via calcium imaging, we are able to examine the functional properties of DRG neurons in an intact reflex system, using an *ex vivo* preparation of an adult animal. This study utilizes transgenic mice in which proprioceptive neurons express a fluorescent marker of neuronal activity (GCaMP6s). Calcium plays an essential role in neurons as it is responsible for regulating metabolic activity through activation of receptors, channels, and second messenger pathways. Therefore, determining the calcium signature of neurons in response to physiologically relevant stimuli provides an indirect means of assessing the function and activity of a cell . The functional properties of DRG neurons will be explored via calcium imaging at three time points after sciatic crush injury: during inflammation at 48 hours, during regeneration at two weeks, and after regeneration is complete at three months. Comparing the calcium signatures of animals that underwent surgery to sham-surgery controls at these key time points will reveal how calcium metabolism changes over the course of regeneration to achieve a final, hyperexcitable state in DRG neurons. These experiments will test the hypothesis that abnormal calcium regulation contributes to spinal reflex dysfunction after PNI and regeneration. This central hypothesis puts forth a novel mechanism to explain why spinal reflexes fail to recover after nerve injury. Identifying the mechanism underlying reflex failures will be beneficial in providing direction for research targeted at treatment.

**Disclosures:** **M. Walters:** None. **D.R. Ladle:** None. **M.J. Sonner:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.02/W8

**Topic:** C.10.Tauopathies, Tau-dementias, and Prion diseases

**Title:** Pupil diameter as biomarker of tauopathy-related degeneration of the locus coeruleus

**Authors: *H. LEFUMAT**[1], D. J. IRWIN[2], J. LEVINE[1], S. JOSHI[1], J. L. GOLD[1]
[1]Dept. of Neurosci., [2]Ctr. for Neurodegenerative Dis. Res., Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Frontotemporal lobar degeneration (FTLD) is a leading cause of dementia, similar in prevalence to Alzheimer's disease in patients younger than 65. FTLD has heterogeneous clinical features of dementia that encompass progressive behavioral, language, and motor dysfunctions. FTLD also typically involves specific protein abnormalities in the brain, or proteinopathies. The main two proteinopathies that characterize FTLD entail the accumulation of either TAU or TDP-43 proteins. These proteinopathies have different progressions and patterns of degeneration in the brain and thus are ideally associated with different courses of treatment. In many cases, however, such tailored treatments cannot be used because these proteinopathies can often only be distinguished from each other post mortem, at autopsy. One striking finding from these post-mortem studies is that FTLD-Tau involves severe neurodegeneration in the brainstem nucleus locus coeruleus (LC), whereas FTLD-TDP involves mild LC deficits that occur only in advanced-stage cases. The goal of this study is to investigate if and how indirect measures of LC activation, including changes in pupil diameter and certain event-related potentials (ERPs) can be used to assess LC function in FTLD patients and potentially as a biomarker to distinguish FTLD-TAU from FTLD-TDP.
We are using three complementary approaches. First, we are extending ongoing studies in awake monkeys of relationships between spiking activity of individual LC neurons and pupil diameter to include ERP measurements (including the P3 response and spectral components), which show LC-related modulations under both passive conditions and in response to surprising sounds. Second, we are testing how pupil diameter and ERPs are modulated under a broad range of conditions in healthy young human subjects, to establish a baseline for measurements in FTLD patients. In particular, we are using an auditory oddball task in which preliminary data indicate that manipulations of motor responses, distractors, and the discriminability of the oddball and standard stimuli all have different effects on these indirect measures of LC function. Third, we will use the task conditions and physiological measurements that we develop in the monkey and healthy human studies to develop effective, non-invasive approaches to assess LC function in healthy and FTLD-afflicted older adults. This study thus may culminate in a novel, non-invasive method to providing ante mortem diagnosis of FTLD-Tau to improve clinical trials.

**Disclosures: H. Lefumat:** None. **D.J. Irwin:** None. **J. Levine:** None. **S. Joshi:** None. **J.L. Gold:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.03/W9

**Topic:** C.10.Tauopathies, Tau-dementias, and Prion diseases

**Title:** Transmission of scrapie into sheep after passage through white-tailed deer results in a phenotype change

**Authors: *R. KOKEMULLER**[1], S. J. MOORE[2], M. H. W. GREENLEE[1], J. J. GREENLEE[2]
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**Abstract:** Transmissible spongiform encephalopathies (TSE's) are neurodegenerative diseases that result in misfolding of the prion protein from the normal cellular form ($PrP^{C}$) to the diseased form ($PrP^{Sc}$). TSE's affect sheep (scrapie), deer (chronic wasting disease; CWD), cattle (bovine spongiform encephalopathy), and humans (Creutzfeldt-Jakob disease). TSE agents can have strain variations that influence disease phenotype (eg. incubation time, location of $PrP^{Sc}$ deposition, etc.) and may affect susceptibility in animals with different prion protein gene (*PRNP*) sequences. Susceptibility of sheep to disease is associated with 3 specific polymorphisms in *PRNP*; 136 valine (V), 154 arginine (R), and 171 glutamine (Q) (VRQ). Sheep resistant to scrapie are known to have the genotype 136 alanine (A), 154 histidine (H), and 171 arginine (R) (AHR). The most influential codon being 171 where QQ171 is susceptible and RR171 is resistant. Inter-species transmission of TSE's causes genetic and phenotypic variability, which may affect sampling and eradication of prion-infected animals. Since deer and sheep may use the same grazing land, it is important to understand the potential transmission of TSEs between these species. The US scrapie isolate (No.13-7) has a 100% attack rate in white-tailed deer (WTD) after oronasal challenge. The purpose of this study was to determine if sheep are susceptible to oronasal challenge with the scrapie agent from WTD. Suffolk lambs of *PRNP* genotype $VV_{136}RR_{154}QQ_{171}$, $AA_{136}RR_{154}QQ_{171}$, and $AV_{136}RR_{154}QQ_{171}$ were challenged by the oronasal route with a 10% brain homogenate from scrapie-affected WTD. Upon development of clinical signs, sheep were euthanized and necropsied. Tissues were tested for $PrP^{Sc}$ by enzyme immunoassay (EIA), western blot (WB), and immunohistochemistry (IHC). The first sheep to develop clinical signs at approximately 29 MPI had the VRQ/VRQ genotype. Sheep of the ARQ/ARQ genotype also developed clinical signs, but at a mean of 52 MPI. This is in contrast to the original No.13-7 inoculum that has a faster incubation period in sheep with the ARQ/ARQ genotype (20 MPI). This work is intriguing because the original No. 13-7 scrapie isolate had a faster incubation time in ARQ/ARQ sheep, but after being passaged through deer, the incubation time was much shorter in VRQ/VRQ sheep indicating a phenotype change. This is significant because it raises the concern that scrapie infected deer could transmit disease to sheep and potentially promote the propagation of a new strain of scrapie.

**Disclosures: R. Kokemuller:** None. **S.J. Moore:** None. **M.H.W. Greenlee:** None. **J.J. Greenlee:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.04/W10

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** John and Lucille van Geest Foundation

BBSRC Babraham Institute Signalling ISPG

MRC Project Grant MR/N004582/1

**Title:** Wallerian degeneration: Is it regulated by NAD, NMN or both?

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**Abstract:** The degeneration of transected axons (Wallerian degeneration) can be slowed tenfold by overexpression of a variety of NAD-synthesizing enzymes, such as isoforms of NMNAT or the related mutant fusion protein, $WLD^S$. Wallerian degeneration is also delayed by deletion of TLR adapter protein SARM1, a protein recently reported to promote NAD degradation. It is important to understand fully the mechanism of Wallerian degeneration because related mechanisms contribute to axon loss in a number of disease models, including models of peripheral neuropathies, Parkinson's disease, multiple sclerosis and glaucoma. We will also present new data implicating a role in hereditary spastic paraplegia.
While depletion of NAD is an attractive hypothesis for the mechanism of Wallerian degeneration, especially as NAD can be increased by dietary methods, it cannot explain a number of key observations. FK866, an inhibitor of NAMPT, blocks the NAD salvage pathway and strongly depletes NAD, including within axons. However, instead of killing axons as the NAD hypothesis would predict, it does precisely the opposite: it phenocopies the protective effect of $WLD^S$. Moreover, ectopic expression of the bacterial enzyme NMN deamidase, a protein absent in mammals, protects injured axons both in transgenic mice and in primary neuronal cultures, but it has no effect on NAD levels either under basal conditions or in degenerating nerves. These observations fit better with a proposed toxic role for the NAD synthesis intermediate NMN, a model that can also explain the protective effect of $WLD^S$. This poster will discuss the strengths and limitations of each hypothesis, including new data, and explore models to explain the changes in both NMN and NAD.

**Disclosures:** **M.P. Coleman:** None. **A. Loreto:** None. **M. Di Stefano:** None. **C. Angeletti:** None. **J. Gilley:** None. **C. Hung:** None. **N. Raffaelli:** None. **G. Orsomando:** None. **L. Conforti:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.05/W11

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Pontificia Universidad Javeriana Grant 0006962

**Title:** Structural plasticity in rat cortical neuron induced by Pentacalia nitida

**Authors:** ***S. L. ALBARRACIN**, D. M. RAMIREZ, R. VERA, J. J. SUTACHAN
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**Abstract:** Processes of formation, growth and branching of the dendritic tree are fundamental in learning and memory. In addition, it has been found that a reduction in the complexity of the dendritic tree is a common feature share by neurodegenerative diseases, suggesting that the maintenance of the dendritic arborization is critical for the normal function of the nervous system. Therefore, the search for molecules that can modulate structural plasticity in these diseases has recently gain interest, especially for those non-traditional molecules derived from medicinal plants. *Pentacalia nitida* is a plant of the asteraceae family that is commonly used for the regulation of signaling pathways that regulate inflammation. Although, the prospective use of this plant, there are not studies evaluating the potential of *Pentacalia nitida* extracts in regulating structural plasticity in neurons. In the present work, extracts from leaves of *Pentacalia nitida* were obtained by hydro-alcoholic solution and by a solid-liquid phase using solvents with different polarities and evaluated for their capacity to regulate the dendritic branching of rat cortical neurons *in vitro*. Toxicity assays showed that the methanol extract did not affect the viability of cortical neurons. In addition, methanol extracts at concentrations of 25 µM and 2.5 µM increased the complexity of the dendritic tree and the levels of MAP2. These effects were inhibited by the use of the LY294002 y PD98059 inhibitors, suggesting that extracts achieves its stimulatory effect by regulating the $PI_3$ and MAP kinase pathways. The obtained results suggest that *Pentacalia nítida* have molecules that can regulate the neuronal dendritic tree and may have therapeutic potential.

**Disclosures:** **S.L. Albarracin:** None. **D.M. Ramirez:** None. **R. Vera:** None. **J.J. Sutachan:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.06/W12

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

**Support:** CAPES - Brazilian Government

FAPEMIG - State of Minas Gerais - Brazil

**Title:** Thalamic proteome changes and behavioral impairments in thiamine deficient rats

**Authors: *A. M. RIBEIRO**, P. T. NUNES, D. P. GOMEZ-MENDOZA, C. P. REZENDE, H. C. P. FIGUEIREDO
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**Abstract:** Thiamine deficiency (TD) has been used as an experimental model in rodents to study the molecular mechanisms of neurodegeneration process. The aims of the present study were to investigate: (i) the spatial cognitive performance of pyrithiamine-induced thiamine deficiency (PTD) in adult rats using the Morris Water Maze task (MWM) and (ii) the thalamic proteome alterations occurred by a TD episode, using a combination of liquid chromatograph with mass spectrometry (LC-MS/MS) analysis. After the onset of the last neurological signs, the TD was interrupted and after two weeks of recovery, spatial cognitive tasks in MWM were performed. One day after the behavioral test, the animals were killed and the thalami were dissected. The PTD rats exhibited deficits during the learning process, which was reverted by repeated training. The neuroproteomic analysis, using label-free quantification, revealed deregulation of 183 proteins, among the 1440 detected by LC-MS/MS. From the 183 proteins, 153 and 30 were down and up-regulated, respectively. Using bioinformatics tools, the deregulated proteins were categorized according to the Gene Ontology **(**GO) functional annotation and metabolic pathways. TD affects proteins involved in different biological processes, such as oxidative stress, neuroplasticity and neurotransmission release, including several proteins involved in the synaptic vesicle cycle function, such as complexin 2, dynamin 3, AP2 adaptor complex, V-ATPase. In addition, one of the proteins up-regulated by TD is the leucine-rich repeat kinase 2 (gene: Lrrk2 - accession: F1LNJ1-LRRK2), that is also involved with synaptic processes. Mutations in this protein have been found in hereditary and sporadic form of Parkinson’s Disease pathogenesis. One important conclusion from these data is that not only thiamine-dependent enzymes are affected by TD, indicating that thiamine and its phosphate forms might have relevant roles in central nervous system distinct from those well-known co-enzymatic functions. The observed deregulation of antioxidant thalamic proteins and anti-inflammatory cytokines represents a disruption of inflammatory response induced by TD. In addition, proteins that are components of different metabolic pathways, such as fatty acid and glycolysis were deregulated in thalamus of

the PTD rats. Some of these targets are indirectly involved in the glutamate and GABA syntheses. Finally, the present data highlight some relevant molecular targets for future studies focusing on neurodegenerative process and/or on the neurobiological mechanisms related to the spatial cognitive deficits induced by TD.

**Disclosures: A.M. Ribeiro:** None. **P.T. Nunes:** None. **D.P. Gomez-Mendoza:** None. **C.P. Rezende:** None. **H.C.P. Figueiredo:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.07/W13

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Natural Sciences and Engineering Research Council of Canada

Canada Foundation for Innovation

Heart and Stroke Foundation of Canada

Saskatchewan Health Research Foundation

**Title:** The anti-diabetic drug metformin prevents neurodegeneration and hippocampal-dependent learning deficits after chronic systemic administration of adenosine A1 receptor agonist

**Authors: *A. AMAH**[1], F. S. CAYABYAB[2]
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**Abstract:** Our lab recently reported that the A1R agonist $N^6$cyclopentyladenosine (CPA) or focal cortical ischemia caused hippocampal neurodegeneration, which was prevented by A1R antagonist dipropylcyclopentylxanthine (DPCPX). We hypothesized that aging-related elevation of adenosine leads to changes in adenosine receptors and increased neurodegeneration in Parkinson's disease (PD). The anti-diabetic drug metformin has also been reported to slow the progression of PD. Therefore, we determined whether metformin exhibits its neuroprotective effect by altering the adenosine signalling pathways. Male Sprague-Dawley rats received intraperitoneal injections of CPA (3mg/kg, daily for 7 days) or vehicle control, and some rats also received metformin 30min prior to CPA. Rats treated with metformin for 7 days showed a dose-dependent reduction in the CPA-induced hippocampal neurodegeneration (5 and 10 mg/kg metformin prevented, but 2 mg/kg metformin did not) as shown by propidium iodide uptake and increased FluoroJadeB staining. Consistent with hippocampal neuronal loss, CPA caused significant hippocampal-dependent learning and memory deficits (Y-maze test), increased depressive behaviour (by forced swim test) as well as enhanced anxiety behaviour (by open field

test). Metformin at 10mg/kg improved all behavioural deficits. Electrophysiological fEPSP recordings from acute 400μm hippocampal slices revealed that 1-30μM metformin increased baseline synaptic transmission, which was accompanied by increased paired pulse depression. Metformin (5μM) reduced the synaptic depression produced by CPA (100nM). Taken together, metformin protects hippocampal neuronal damage and learning deficits by preventing the adenosine A1 receptor-mediated neurotoxicity enhancing signalling pathways.

**Disclosures: A. Amah:** None. **F.S. Cayabyab:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.08/W14

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Columbia TTFI program in CIPN and sensory neuroscience

Fondation Roger De Spoelberch

**Title:** Role of CAMKK2-AMPK stress response pathway in chemotherapy induced peripheral neuropathy

**Authors: *A. SAHASRABUDHE**[1], F. BARTOLINI[2], F. POLLEUX[1]
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**Abstract:** Chemotherapy Induced Peripheral Neuropathy (CIPN) is a serious side effect of many drugs using for chemotherapy including paclitaxel-based (Taxol) cancer treatments and is frequently the main reason for reduction or discontinuation of the therapytreatment. General clinical signs of CIPN involve numbness, paresthesia, dysesthaesia and tingling often aggravated by neuropathic pain. The supposed pathogenesis of CIPN is thought to be the onset of axonopathy through dying back axonal damage, however the exact molecular events underying these cellular damages are largely unknown. We recently identified the CAMKK2-AMPK kinase pathway as a critical stress response pathway in early stages of neurodegenerative conditions such as Alzheimer's disease (Mairet-Coello et al. *Neuron* 2013). We decided to explore if this pathway is involved in CIPN.
Using cultured adult Dorsal Root Ganglia (DRG) neurons, we show found that there is a marked upregulation of AMPK phosphorylation upon taxol treatment. This upregulation over-activation is transient, lasting for about 12 hours followed by a steep decrease in the pAMPK levels and subsequent increased mTOR activation up to 72 hours. Morphological analysis of DRG axons in culture at 72 hours post- taxol treatment leads to formation of retraction bulbs at the tip of the axons, morphologiically mimicking the dying back mechanism of the axon described for CIPN.

Genetic analysis using AMPK$^{-/-}$ DRG neurons showed that the axon morphology defects described above can be significantly reversed prevented using AMPK-null mouse DRG neurons to an extent suggesting a role for AMPK in the process of axon fragmentation degeneration up induced on by taxol treatment. To delineate identify the upstream regulators of AMPK, we used LKB1-null$^{-/-}$ neurons and or an pharmacological inhibitionor for CAMKK2 (STO609). While CAMKK2 inhibition partially reversed the axon phenotypes described above to an extent, removal genetic ablation of LKB1 did not have any effect on the formation of retraction bulbs. Our preliminary results are suggestivesupport of a role for $Ca^{2+}$-CAMKK2-AMPK pathway in the process of taxol CIPN and in particular taxol-mediated induced axonal damagedegeneration. Following up on these lines weWe are currently testing the activation of calpainsif Calpain activation is a downstream target of taxol-induced CAMKK2-AMPK overactivation and also determining the source of $Ca^{2+}$ that might leads to the activation of CAMKK2.

**Disclosures:** **A. Sahasrabudhe:** A. Employment/Salary (full or part-time):; Columbia University. **F. Bartolini:** A. Employment/Salary (full or part-time):; Columbia University. **F. Polleux:** A. Employment/Salary (full or part-time):; Columbia University.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.09/W15

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Colciencias Contrato No.616-2014

**Title:** Neuroprotection generated by ischemic preconditioning involves protein O-GlcNAcylation in a rat model of cerebral ischemia

**Authors: *J. RENGIFO**[1], C. F. CARDOZO[1], E. VIVEROS[1], A. VERA[3], J. GONZALEZ[2], L. V. BECERRA-HERNANDEZ[4]
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**Abstract:** Ischemic tolerance is an endogenous cytoprotection mechanism that allows a cell, previously exposed to a sub-lethal ischemic event, survive a lethal one. In the heart it has been shown that a series of brief ischemia/reperfusion cycles limit the extent of the cardiac infarct generated in a subsequent prolonged ischemic event. The experimental protocols of ischemia/reperfusion, designed to activate ischemic tolerance, are termed ischemic preconditioning (IPC) and although in the last decades the understanding of the cellular mechanisms involved in this cytoprotection in the heart has increased, there is still a lot to learn

about ischemic tolerance in other organs, like the brain. Evidence about the participation of the post-translational protein modification of O-GlcNAcylation in cytoprotective mechanisms has been increasing in different tissues. O-GlcNAcylation is a dynamic and ubiquitous protein modification much like phosphorylation and, for many proteins, it has been demonstrated that phosphorylation and O-GlcNAcylation are reciprocal modifications that regulate protein function. In multiple forms of cellular stress, including ischemic stress, an acute increase of O-GlcNAc levels on proteins is an endogenous response to the stress and strategies that augment O-GlcNAcylation have been shown to be pro-survival. Here we evaluate a protocol of IPC as a protective strategy against cerebral ischemic injury in the rat Middle Cerebral Artery Occlusion (MCAO) model. We have found that the IPC protocol reduces the size of the infarct and increases the level of protein O-GlcNAcylation suggesting a possible participation of this protein modification in the neuroprotection generated. We have also investigated how IPC affects the expression of a protein kinase known as Akt or PKB which is a crucial component of cellular survival pathways that can block apoptosis. Our results indicate that the IPC protocol generates a significant increase in Akt phosphorylation at residues necessary for the kinase activity, providing additional insight into the probable cellular pathways involved in the cytoprotection elicited by ischemic preconditioning in the brain.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.10/W16

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

**Support:** National Science Foundation of China 81573416

**Title:** Multiple target of hAmylin on rat primary hippocampal neurons

**Authors: *W. ZHANG**
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**Abstract:** Alzheimer’s disease (AD) and type II diabetes mellitus (DM2) are the most common aging-related diseases, characterized by β-amyloid and amylin accumulation, respectively. Multiple studies have indicated a strong correlation between these two diseases. Amylin oligomerization in the brain appears to be a novel risk factor for developing AD. Although amylin aggregation has been shown to induce cytotoxicity in the neurons via altering $Ca^{2+}$ homeostasis, the underlying mechanisms have not been fully explored. Here, we investigated the effects of amylin on the rat hippocampal neurons using calcium imaging and whole-cell patch

clamp recordings.. We showed that $Ca^{2+}$response induced by low concentration of hAmylin was abolished by Amylin receptor antagonist AC187. However $Ca^{2+}$response induced by higher concentration of human amylin (hAmylin) was independent with amylin receptor. This effect relied on extracellular $Ca^{2+}$. Additionally, blockade of L-type $Ca^{2+}$ channels partially reduced hAmylin-induced $Ca^{2+}$ response. In whole-cell recordings, hAmylin depolarized membrane potential. Moreover, application of transient receptor potential (TRP) channel antagonist ruthenium red (RR) attenuated hAmylin-induced $Ca^{2+}$ increase. Single-cell RT-PCR showed that transient receptor potential vanilloid 4 (TRPV4) mRNA expressed in most of hAmylin-responsive neurons. Meanwhile, selective knockdown TRPV4 channel inhibited hAmylin-evoked $Ca^{2+}$ response. These results indicated that different concentration of human amylin (hAmylin) act via different pathways. Amylin receptor mediates the excitatory effects of low concentration of hAmylin. While for the high concentration of hAmylin, hAmylin aggregation precipitated on the neuron membrane activated TRPV4 channels and then triggered membrane voltage-gated calcium channel opening followed by membrane depolarization. Hence, out data suggest that TRPV4 is a key molecular mediator for the cytotoxic effect of hAmylin on hippocampal neurons.

**Disclosures: W. Zhang:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.11/W17

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH Grant U54NS083932

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**Title:** Assessment of lead and tellurium induced neurotoxicity *In vitro*

**Authors: *J. A. EATMAN**[1], T. SAAFIR[2], K. R. SHEPHERD[3]
[1]Morehouse Sch. of Med., Atlanta, GA; [2]Morehouse Sch. of Med., Atlanta, Georgia; [3]Pharmacology/Toxicology, Morehouse Sch. of Med., Atlanta, GA

**Abstract:** *Neuroscience: Neurotoxicology*
Combinational toxicity is the aggregate effect of multiple environmental contaminants, including metals, insecticides, and pesticides, on living systems. Many recent studies have demonstrated that metals cause degeneration of the central nervous system, however, the mechanisms underlying toxicity resulting from the effect of multiple metal types, or metals in conjunction

with other classes of environmental contaminants, has yet to be identified. The purpose of this study is to understand the threat of combinational toxicity, in addition to identifying the molecular basis of neurotoxicity as a result of cross exposure to two environmental contaminants, Lead (Pb) and Tellurium (Te). We hypothesized that Te would exacerbate Pb induced neurotoxicity. To test this hypothesis, we 1) quantified the production of reactive oxidative species (ROS) in differentiated human neuroblastoma (SH-SY5Y) cells after exposure to Pb and Te, 2) assessed the effects of Pb and Te on mitochondrial function/cell viability, and 3) determined the presence of cell death cascades through immunohistochemistry assays using cleaved caspase-3 antibody. The results show that Pb significantly increased ROS levels after 24 hours; Te also increased ROS levels after this time period. Pb at 1uM and 10 uM significantly decreased mitochondrial function/cell viability after 48hrs. The reduction in mitochondrial function/cell viability was associated with increased labeling of caspase-3 in SH-SY5Y cells, further demonstrating the cytotoxic effects of Pb. In addition, 10 uM Te caused labeling of caspase-3 in SH-SY5Y cells, suggesting that this dose caused injury/cytotoxicity. Combined effects on mitochondrial functioning/cell viability and caspase-3 labeling was also assessed. Taken together, the findings from the present study suggest that the presence of trace elements such as Te may exacerbate Pb induced neurotoxicity.

**Disclosures:** **J.A. Eatman:** None. **T. Saafir:** None. **K.R. Shepherd:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.12/W18

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** T32 ES007051

**Title:** Methamphetamine produces deficits in egocentric and allocentric learning and memory and in brain monoamines after a single dose in rats

**Authors: *A. GUTIERREZ**[1], M. T. WILLIAMS[2], C. V. VORHEES[3]
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**Abstract:** Chronic methamphetamine (MA) abuse results in long-term problems in cognitive function, including in working, declarative, and procedural memory. Neurochemical alterations include depletion of dopamine and serotonin in the striatum (STR) and hippocampus (HIPP). Rodent models produce similar neurochemical alterations, and we demonstrated that a 4 x 10 mg/kg MA regimen at 2 h intervals produced deficits in egocentric learning and memory in the

Cincinnati Water Maze (CWM) and in allocentric spatial learning and memory in a large Morris water maze (MWM). The purpose of the present study was to examine the effects of a single 40 mg/kg MA dose on egocentric and allocentric learning and memory rather than multiple distributed doses. We were also interested in differences in navigational strategy after MA, therefore, we assessed navigational preference using an 8-arm Star water maze (SWM). Monoamines were assessed by high performance liquid chromatography with electrochemical detection in STR, nucleus accumbens (NAcc), and HIPP, regions important for the aforementioned navigational strategies in naïve and behaviorally tested rats. Beginning 2 weeks after MA, Sprague-Dawley rats were tested in the CWM for 18 days, 2 trials/day, in the SWM for 20 days, 2 trials/day with a probe trial every 5 days, and finally in 3 phases of the MWM (acquisition, reversal, and shift) with 6 platform days and one probe trial on day 7. In the CWM, MA-treated rats were impaired for latency and errors. In the SWM, a preference for egocentric navigation was seen in saline-treated rats while MA-treated rats had no preference in the day 5 probe. In the MWM, impairments in the MA-treated rats were seen on acquisition and shift but not on reversal, although there was an effect on the reversal probe trial. Dopamine and serotonin levels in the STR were reduced in MA-treated rats 2 weeks post-treatment in groups of behaviorally untested animals and at the end of behavioral testing compared with controls. Monoamines in the NAcc and HIPP are being analyzed. The data show that deficits in both types of navigation are induced by MA, and MA reduces the inherent egocentric preference when rats can choose a strategy to use. MA-induced monoamine depletions in the STR persisted and showed no recovery even after behavioral testing. These findings provide a simpler, 1 dose model for further examination of interventions that may permit determination of the neurochemical effects that are critical for the induction of MA-associated cognitive deficits.

**Disclosures: A. Gutierrez:** None. **M.T. Williams:** None. **C.V. Vorhees:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.13/W19

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** 2016/24863-6 FAPESP

**Title:** Investigation of the autophagic process in the hippocampal neurons response to anhydroecgonine methyl ester exposure, a cocaine pyrolysis product

**Authors: *S. D. PRATES**
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**Abstract: Background**: Cocaine is worldwide used as a recreational drug and can be found as hydrochloride form (taken by intranasal aspiration) and as cocaine freebase (crack cocaine) that is smoked. Crack is a thermo stable substance that vaporizes at temperatures above 93°C and 50-80% of cocaine is converted into
anhydroecgonine methyl ester (AEME) during the heat, which is inhaled with volatilized cocaine. There are evidences that cocaine can induce necrosis and/or apoptosis at several encephalic areas, however little is known about the mechanism underlying AEME neurotoxicity. Previous studies of our lab described that AEME is a neurotoxic compound with an additive effect when associated with cocaine after 24h of exposure and the mechanism of its neurotoxicity may involve apoptosis. **Aims**: To investigate the autophagic process involvement of cocaine (C), AEME (A) and the association of both substances (CA) in a primary hippocampus culture. **Methods**: Primary hippocampus neurons (E18) were exposed to 2 mM of C, 1 mM of A and 2 mMC+1 mM A (CA) for 3, 6 or 12h. For the flow cytometry analysis (Accure BD) it was used $5x10^5$ cells and after the exposure, the supernatant was discarded and was incubated with acridine orange (1µg/mL) during 15min. Then, the cells were washed once with PBS and collected by tripsinazation. We analyzed green versus red fluorescence and considerate the percentage of double marked cells. For LC3 evaluation by Western blotting, we used 40 µg of total protein in a 17% acrylamide gel and a PVDF membrane. **Results and conclusion**: At 3h (n=12) we observed an increase in the percentage of cells with acid vesicles when exposed to A ($p<0.05$) and CA ($p<0.01$) compared to the control group. C group showed lower acid vesicles when compared to A and CA (both $p<0.001$). No statistical differences were observed in 6h (n=5). After 12h (n=12) of exposure, only A showed more cells with acid vesicle than C group ($p<0.05$). Western blotting analysis showed at 3h that all treatment presented LC3 I and II fraction, but LC3 II was more evident at CA group.
At 6h and 12h, LC3 II was more evident at C group. Our results show that 3h is a critical point for neurons protective response to A and CA, and at posterior periods other mechanisms than autophagy can be triggered. **Acknowledges**: FAPESP, CAPES

**Disclosures: S.D. Prates:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.14/W20

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NSF-CREST HRD-1137725

**Title:** Phthalates affect synaptic growth and stability at the *Drosophila* nmj

**Authors: *K. M. DE LEON**[1], B. MARIE[2]
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**Abstract:** Dibutyl phthalate (DBP) is a commonly used plasticizer in everyday products. Phthalates are not chemically bound to their polymer matrix and can leach out into the environment. Little is known about the effects of DBP on the nervous system. Studies have shown that DBP acts as a teratogen and as an endocrine disruptor. In addition, recent studies have linked DBP to apoptosis and neurotoxicity in mouse and rat models. In the present study, we address the effect of DBP at the neuromuscular junction (NMJ). We characterized a variety of synaptic markers: the vesicle marker synapsin, the microtubule associated protein Futsch (MAP1B homolog), the active zone associated structural protein Bruchpilot (CAST homolog), the postsynaptic marker discs large (Dlg; PSD-95 homolog), and the presynaptic membrane marker HRP. The animals were reared with a milieu containing environmentally relevant low concentrations of DBP (0.02, 0.2, 2 or 20 ppm). Animal reared in the contaminated milieu presented a reduction in synaptic growth. Indeed, synapses from animals reared in milieu at different concentrations of DBP showed a growth that was 80% of the control synapses. In addition, the animals reared in milieu with DBP showed synaptic retractions. These retractions are defined by the presence of postsynaptic markers and the absence of a subset of presynaptic markers. We found that the frequency of retraction to be dose dependent, where up to 30% of the synapses observed, showed synaptic retractions phenotype at 20 ppm. We observed that the animals exposed to DBP have boutons deficient in MAP1B and CAST. We also noted that when there is a synaptic retraction, neighboring boutons show abnormal accumulation of presynaptic proteins such as CAST, MAP1B and synapsin. We expanded our research to determine if different phthalates had similar effects on the synapse. Indeed, when exposed to di-2-ethylhexyl phthalate (DEHP) animals showed a reduction in synaptic growth and 41% retraction frequency. In contrast, exposing animals to benzylbutyl phthalate (BBP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) had no effect on synapse growth nor retraction frequency. These results correlate with the literature describing higher chained phthalates safer than shorter chained. This study shows that specific phthalates, such as DBP and DEHP can affect synaptic growth and stability. We are now focusing on the consequences of DBP exposure on glutamate receptor clustering and synaptic release.

**Disclosures: K.M. De Leon:** None. **B. Marie:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.15/W21

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** ANPCyT-PICT 0921

CONICET PIP 0707

**Title:** Epigenetic control of early neurodegenerative events in Diabetic Retinopathy by the histone deacetylase SIRT6. Involvement of Müller glia

**Authors: *D. M. SILBERMAN**[1], M. A. ZORRILLA-ZUBILETE[2], A. YESTE[3], F. J. QUINTANA[3,4], D. TOIBER[5], R. MOSTOSLAVSKY[6]
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**Abstract:** Diabetic retinopathy (DR) is one of the common complications associated with diabetes mellitus (DM) and the leading cause of blindness worldwide. Recent research has demonstrated that DR is not only a microvascular disease but may be a result of neurodegenerative processes and that glucose-induced neuron and glial cell damage may occur shortly after the onset of diabetes making the disease hard to diagnose at early stages. SIRT6, a NAD-dependent sirtuin deacylase, modulates aging, energy metabolism and neurodegeneration. In previous studies we showed that SIRT6 deficiency causes major retinal neurotransmission defects, changes in expression of glycolytic genes and elevated levels of apoptosis. Given the importance of glucose availability for retinal function and the critical role for SIRT6 in modulating glycolysis, we aim to analyze SIRT6 involvement in early neurodegenerative events during the development of experimental DR. Using non-obese diabetic mice (NOD/ShiLtJ), we determined by Western blot that two weeks after the onset of the disease high glucose concentrations (>250 mg/dl) induced retinal increased of a neovascularization promoting factor (VEGF, $p<0,01$) and loss of a neuroprotective factor (BDNF, $p<0,01$). At this stage, no evident vascular abnormalities were observed. Notably, reduced levels of SIRT6 ($p<0,05$) and increased acetylation levels of its substrates (H3K9 and H3K56, $p<0,01$) were observed suggesting a deregulation of key neural factors at early stages of the disease. Noteworthy, retinas from Central Nervous System (CNS) conditionally deleted SIRT6 mice (C57BL6/J, Nes-Cre-SIRT6$^{-/-}$) showed a resemblance to diabetic retinas exhibiting lower levels of BDNF and increased levels of VEGF. Moreover, cultured Müller glial cells subjected to high glucose concentrations (25mM) exhibited decreased levels of SIRT6 and increased levels of H3K56 acetylation ($p<0,05$). Our findings suggest that neurodegenerative events regulated epigenetically may occur early during diabetes preceding proliferative and vascular changes observed later in a diabetic retina.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.16/W22

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Gestational and chronicexposure to inorganic As (iAs) generates up regulation of Xc- system with changes in the hippocampus glutamatergic neurotransmission

**Authors: *J. NELSON-MORA**, M. GONSEBATT, L. MASSIEU-TRIGO, M. L. ESCOBAR, T. MONTIEL, L. RODRÍGUEZ-DURÁN, V. RODRÍGUEZ
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**Abstract:** More than 200 million people in the world are estimated to be exposed to iAs levels above the security standards suggested by the World Health Organization. Polluted water is the main exposition way. iAs exposure is associated with different types neurological disorders that can also cause memory and learning impairment in children. Our lab has developed a murine model of iAs gestational exposure, in order to seek for the physiological, biochemical and molecular mechanism underlying the cognitive disorders seen in iAs exposure. It has been shown that iAs can induce ROS elevation. Moreover, glutathione (GSH) the main antioxidant molecule in the nervous system participates in iAs metabolism, consequentlty iAs exposure can deplete GSH. The aminoacid cysteine is a limiting resource in GSH synthesis which enters the cell via the Xc- antiporter importing one cystine molecule and exporting one glutamate molecule. We have previously observed and increased expression of Xc- in male hippocampus. This higher Xc- system activity could be associated to elevated extracellular glutamate that can lead to exitotoxicity, and to glutamatergic synapse alterations in hippocampus. CD1 animals were exposed to 20 ppm in drinking water, during gestation and then until three months of age. Extracellular glutamate concentrations were measured by *in-vivo* microdialysis while GSH/GSSG concentrations were determined with the fluorescence probe o-phthalaldehyde (OPA). The glutamatergic transmission status was assessed measuring changes in GluA1 and GluA2 AMPA subunits expression of glutamate receptors by Western Blott in cortex and hippocampus. Also, induction of LTP phenomena in hippocampal dentate gyrus in anesthetized animals, and Morris water maze were used to evaluate the impact of iAs exposure in memory and learning.. Our results show that GSH and GSSG levels were increased suggesting Xc- system activation. Also, extracellular glutamate levels were higher in iAs exposed mice. Both AMPA subunits were down-regulated and an impairment in LTP induction was observed.
Financial Support This work was supported by CONACYT, (02287) and PAPIIT (IN207611) and by the Red de Salud Ambiental Infantil CONACYT 251229 J. Nelson is a CONACYT fellow at the Posgrado en Ciencias Biológicas.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.17/W23

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Recombinant human erythropoietin protects hippocampal neurons against excitotoxic damage through expression change of mediators signaling

**Authors:** ***S. F. CORNELIO-MARTINEZ**[1], C. BEAS ZARATE[2], A. FERIA-VELASCO[3], M. RIVERA CERVANTES[4]
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**Abstract:** Erythropoietin (EPO) is a cytokine required for proliferation, differentiation and maturation of red cells. However, it has been reported EPO acts as a pleiotropic cytokine beyond hematopoietic system, in other tissues including the brain, reproductive tract, in myoblasts and in kidney. In central nervous system EPO, performs neuroprotective functions against neuronal damage in various neurodegenerative disorders. It has anti-inflammatory, anti-oxidant, anti-apoptotic, neurotrophic, neurogenic, angiogenic and anti-excitotoxic effects essential for tissue repair. The signaling pathways involved in these responses have not been fully clarified but are known to involve the erythropoietin receptor (EPOR), moreover beta common receptor (βcR) a subunit. The main purpose of this work was to evaluate the neuroprotective effect of recombinant human EPO (rh-EPO) and determine the change expression of EPO, EPOR and βcR mRNAs in the hippocampus of neonate rats, under excitotoxic conditions, with monosodium glutamate (MSG). First we administered (4 mg/g bw) of MSG at 1, 3, 5 and 7 postnatal day (PD) to induce excitotoxic damage, then administered three different doses of rh-EPO (250UI/kg body weight (bw), 500UI/kg bw and 1,000UI/kg bw) intravenously at 8 PD, and we evaluated the cellular damage at 14 PD using Nissl technique, because it has been found that hippocampus present greater damage at this age in treated animals with MSG. Finally, we determine the change of EPO, EPOR and βcR gene expression at 2, 6 and 24 h after the administration of rh-EPO. Results of this work demonstrated that after administration of EPO, cell damage in hippocampal CA1 region decrease compare with those animals that were administered with MSG only and the most effective dose of EPO was 1,000 UI/kg bw. We also demonstrate that EPO gene expression increase at 2 h after the administration of rh-EPO, meanwhile, EPOR gene increase at 6h and βcR gene increase at 2h then decrease at 6h and increase at 24h again. Our

findings demonstrate that rh-EPO has a neuroprotective role against excitotoxic damage induce by MSG. therefore, EPO may acts through its binding to EPOR and βcR to activate its signaling pathway as a cellular response to excitotoxic injury to prevent damage and cellular lost and it can be used for the treatment of neurodegenerative diseases

**Disclosures: S.F. Cornelio-Martinez:** None. **C. Beas Zarate:** None. **A. Feria-Velasco:** None. **M. Rivera Cervantes:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.18/W24

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** AHA Grant 14FTF-19970029

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**Title:** MicroRNA-200b attenuates increases in cytosolic calcium and improves survival from oxidative stress in neuronal N2a cells

**Authors: J. D. BELL**[1], X. SUN[2], *C. STARY[2], R. G. GIFFARD[2]
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**Abstract:** Oxidative stress mediated by reactive oxygen species (ROS) is an important common pathway in cerebral ischemia, traumatic brain injury and neurodegenerative disease. Excessive production of ROS leads to disruption in intracellular calcium handling and induction of cell death pathways. Modulation of the cellular response to ROS therefore has widespread implication for neuroprotection across modalities. MicroRNAs (miRs) are short non-coding RNA molecules which bind to the 3' untranslated region (UTR) and inhibit the translation of mRNA strands to protein. MiR-200b has been shown to target protein kinase C alpha (PKCα), a known mediator of transient receptor potential cation channel-M2 (TRPM2)-mediated calcium influx in response to oxidative stress. Here we examined the effects of miR-200b mimic on alterations in cytosolic calcium and neuroprotection against oxidative injury in neuronal cells. N2a cells were grown in low serum and low density to induce a neuronal phenotype, characterized by extensive neuritic processes. Cells were transfected with miR-200b mimic or vehicle control and gene changes were verified with RT-qPCR. Oxidative stress was induced by 400 υM $H_2O_2$ plus serum deprivation, and cell death was assayed via propidium iodide after 24 hr. Fura-2 fluorescence was used to examine changes in cytosolic calcium. PKCα protein levels were assessed by immunoblot, and colocalization with TRPM2 was verified with

immunofluorescence. A one-way ANOVA with post-hoc multiple comparisons was used in all analyses to assess significant ($p<0.05$) differences. Oxidative injury induced an increase in cytosolic calcium and PKCα, which was attenuated in cells treated with miR-200b mimic. In N2a cells miR-200b mimic afforded significant protection 24 hr following oxidative stress, however, in separate experiments, miR-200b did not protect primary astrocyte cultures from oxidative injury. Notably, astrocytes do not express TRPM2 channels, validating the specificity of the effect of miR-200b on ROS-induced injury. These results describe a novel method of cellular neuroprotection against ROS-induced calcium influx and subsequent injury, with potential implication for other models of brain injury and disease.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.19/W25

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NINDS R01NS086423

**Title:** Increased cellular glutathione with a thiol-containing compound to attenuate neuroinflammation

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**Abstract:** Neuroinflammation and oxidative stress are key pathogenic factors in the etiology and progression of many neurological disorders. Mechanistically, however, the link between these processes is unclear. Research has shown that modulation of cellular redox status can influence inflammatory processes in different diseases. Hence, we hypothesized that the modulation of intracellular levels of glutathione (GSH) would decrease oxidative stress and reduce neuroinflammation *in vitro*. We previously identified several thiol compounds with sulfhydryl side groups which could increase cellular glutathione (GSH) levels by a novel mechanism: post-translational activation of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH biosynthesis. Of the compounds tested, 2,3-dimercapto-1-propanol (DMP) was most potent in increasing GSH ($p<0.001$ versus (vs) vehicle control) and decreasing pro-inflammatory cytokine production ($p<0.0001$ vs vehicle control; $p<0.001$ vs lipopolysaccharide (LPS)) in different cell types (JBC: Vol.292, No.13, pp5532-5545, 2017). Based on this, we hypothesized that an FDA approved drug used as a systemic protective agent against chemotherapy, 2-mercaptoethane

sulfonate (MESNA) having a thiol group would increase intracellular GSH levels thereby ameliorating oxidative stress and neuroinflammation *in vitro*. Non-cytotoxic doses of MESNA were chosen based on its ability to release lactate dehydrogenase. At 24h, MESNA (10μM) increased intracellular GSH levels in a rat dopaminergic neuronal cell line (N27) (57% increase over control) as well in mixed rat primary cortical cultures (20.3% increase over control). Next, we determined if treatment with MESNA impacted the generation of reactive oxygen species induced by Antimycin A (mitochondrial respiratory chain complex III inhibitor) in mixed rat primary cortical cultures. To visualize and quantify the oxidative stress response in these cells, we utilized a highly sensitive and selective fluorescent probe that could detect endogenous superoxide levels. Co-treatment with MESNA decreased Antimycin A-induced superoxide levels to control values. The data suggest that MESNA increases cellular GSH and decreases superoxide levels. Furthermore, the data suggest that thiol-containing compounds could inhibit neuroinflammation by modulation of cellular GSH levels.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.20/W26

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** RO1-NS076772

**Title:** Hydrogen peroxide-specific sensors for *In vivo* measurements using carbon-fiber microelectrodes

**Authors: *L. R. WILSON**[1], S. PANDA[2], L. A. SOMBERS[3]
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**Abstract:** Hydrogen peroxide ($H_2O_2$), whose role in the complex environment of the brain is not well understood, has been implicated in the slow destruction of dopaminergic neurons in Parkinson's disease (PD). This neurodegenerative disease affects more than one million people in America, creating a critical need to identify the mechanisms through which $H_2O_2$ interacts with dopaminergic neurons. Real-time in vivo detection of this analyte has recently been described using fast-scan cyclic voltammetry at carbon-fiber electrodes. However, selectively identifying hydrogen peroxide from interferents such as adenosine and pH shifts remains a challenge. Additionally, some chemical agents used to pharmacologically verify the presence of hydrogen peroxide, such as mercaptosuccinic acid (MCS), oxidize at a potential close to that of the target analyte, further convoluting the characterization of $H_2O_2$ dynamics in the brain. We have addressed these problems by fabricating a mechanically robust $H_2O_2$-selective electrode.

1,3-phenylenediamine (mPD) was electrodeposited onto the surface of a carbon-fiber electrode to render it sensitive to small molecules such as $H_2O_2$ fluctuations and pH shifts, but not other analytes. Since pH changes generate a well-characterized and distinct voltammogram, they can easily be removed from the signal using principal component regression to reveal an electrochemical signal due solely to the oxidation of $H_2O_2$. This technology was fully characterized, and the work will facilitate the selective detection of $H_2O_2$ in a variety of preparations, opening the door for further elucidation of the neurodegenerative role it plays in PD, as well as other neuropathies involving oxidative stress.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.21/W27

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** BAP 2015-64 HDP

**Title:** The effect of adenosinergic modulation on oxidative stress in convulsive seizure induced by pentylenetetrazol

**Authors: *F. DEDE**[1], S. KARADENİZLİ[2], O. OZSOY[3], D. SAHIN[4], F. ERALDEMIR[3], N. ATES[4]
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**Abstract:** Epilepsy is an important pathological conditions characterized by recurrent seizures and it affects approximately 1% of the population. Adenosinergic system and oxidant-antioxidant mechanisms are thought to be involved in the pathogenesis of many neurological diseases such as epilepsy. Adenosine is an ATP metabolite, has a number of important roles in physiologic processes in the body. Adenosine contributes to cytoprotection depending on its receptor types. Caffeine has anti-oxidant property against oxidative stress originated from various toxic drugs. The aim of this study is to investigate the effect of adenosine and caffeine on oxidant parameters in convulsive seizure induced by pentylenetetrazol (PTZ). Male Wistar-albino rats were used for the study. Convulsive seizures were generated by injecting PTZ intraperitoneally (ip). Rats were divided into three groups randomly; PTZ (60 mg/kg/i.p., n = 8), Adenosine + PTZ Group (500 mg/kg ip adenosine administered 15 minute prior to PTZ injection, n = 8), Caffeine + PTZ group (5 mg/kg/ip caffeine administered 30 minute prior to PTZ injection, n = 8). Seizure activity was scored by observing seizure behavior and onset time. Rats were anesthesized with ether, blood samples were collected from heart. Brain tissues samples obtained and homogenized. Blood

samples were centrifuged to collect serum samples. Total protein amounts of the cortex tissue and serum samples were determined by using Lowry Method. MDA and GSH levels have been measured both in cortex tissue and serum samples. PTZ + caffeine administration reduced MDA and GSH levels in cortex tissue and enhanced the same parameters in serum compared to PTZ group. In addition, PTZ+adenosine administration decreased MDA and GSH levels in cortex, but there was not any change in serum levels of these parameters compared to PTZ group. Adenosine and caffeine caused a significant decline in cortex MDA level in PTZ induced seizure group ($p<0,05$). But changes in GSH levels have not been found significant. According to results; adenosine and caffeine reduced MDA level in cortex at oxidative stress conditions induced by PTZ. Adenozinergic modulation by adenosine and caffeine might protective effect on lipit peroxidation in oxidative stres process.

**Disclosures: F. Dede:** None. **S. Karadenİzlİ:** None. **O. Ozsoy:** None. **D. Sahin:** None. **F. Eraldemir:** None. **N. Ates:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

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**Program#/Poster#:** 576.22/W28

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH/NINDS R01 NS051445-09

**Title:** Increased striatal vulnerability to 3-nitroproponic acid in male mice lacking interleukin-1r1

**Authors: *M. ALLEN**, S. J. HEWETT
Dept. of Biology, Program in Neurosci., Syracuse Univ., Syracuse, NY

**Abstract:** Huntington's disease (HD) mice bred onto an IL-1R1 null background demonstrate less Huntingtin aggregation in brain, and reduced severity of motor symptoms, indicating a potential protective role for IL-1β (Molecular Brain 3:33, 2010). In HD, mutant Huntingtin aggregates adversely affect mitochondrial dynamics and function, leading to oxidative stress. Previous work from our lab demonstrated that IL-1β protects neural cells against oxidant injury *in vitro* (Glia 63: 1568-1580, 2016). This led us to the question whether IL-1β might protect against the cascade of processes leading to cell death in HD *in vivo* as incurred by the systemic treatment of mice with the phyto/fungal mitochondrial toxin, 3-nitropropionic acid (3-NP). Toward this end, we compared the extent of behavioral and histological injury between mice wild type (WT) and null for the IL-1β signaling receptor, IL-1R1. Both male and female mice (16-18 wks.) were treated with 3-NP intraperitoneally twice per day, over a twelve-day period for a total cumulative dose of 920mg/kg. Analysis of behavioral and striatal lesion pathology

were performed by an investigator blind to the animal's genotype. Motor symptomology — including reduced general locomotor activity, hind limb and truncal dystonia, and postural instability —increased progressively with increasing days of dosage with 3-NP, in both sexes regardless of genotype. Despite this, histological analysis of the striatum, measured over seven slices ranging from +1.22 to -0.18 from bregma, revealed that IL-1R1 null male, but not female, mice had a greater incidence of striatal lesions that were also larger in size as compared to their WT littermate controls. Our results indicate that endogenous IL-1β signaling mitigates 3-NP induced structural but not functional striatal injury in male but not female mice. This study was supported by NIH/NINDS R01 NS051445-09.

**Disclosures: M. Allen:** None. **S.J. Hewett:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.23/W29

**Topic:** B.05. Transporters

**Support:** DFG, RA 1804/2-1

**Title:** DLK/Wnd MAP kinase signaling promotes synapse loss in mutants defective for axonal transport

**Authors: *E. ASGHARI ADIB**[1], J. LI[2], Y. V. ZHANG[4], T. M. RASSE[4], C. A. COLLINS[3]
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**Abstract:** The Dual Lucine Zipper Kinase DLK, known as Wallenda (Wnd) in Drosophila, controls a highly conserved signaling pathway that becomes activated in damaged axons, and is required for multiple responses of neurons to axonal damage. In some damage models, including NGF withdrawal, glaucoma, and excitotoxicity, Wnd/DLK signaling mediates cell death. However, for neurons of peripheral nervous system (PNS) that can regenerate axons, Wnd/DLK is required for the initiation of new axonal growth. A unifying feature of these different injury models is the activation of signaling by Wnd/DLK, which raises the question of how Wnd becomes activated to 'sense' axonal damage. We report here our findings in Drosophila motoneurons that Wnd is restrained by the kinesin 3 motor protein unc-104/imac/KIF1A. Unc-104 mutants show elevated levels of Wnd signaling, and phenocopy wnd gain-of-function mutations in many respects: they have overgrown axon terminals and a more robust axonal regeneration response to injury. We also found that previously described defects in synapse assembly in unc-104 mutants, which include impaired assembly of presynaptic active zones (AZ), reduced mini frequency and EJPs, and altered bouton morphology, are due to over-active

Wnd signaling, as they are rescued in unc-104/wnd double mutants and phenocopied in wnd gain-of-function mutants.
Synapse development and maintenance requires the synthesis and assembly of synaptic machinery at specific locations in neurons. A kinesin-3 family member, Unc-104/Imac/KIF1A, is required for the localization of many pre-synaptic components to nascent synapses. We found that the synaptic defects of Drosophila unc-104 mutants could be rescued by inhibiting the Wallenda (Wnd)/DLK MAP kinase signaling pathway, which was previously identified as a regulator of axonal damage signaling. Wnd/DLK signaling becomes activated when Unc-104's function is reduced, and inhibits synapse structure and function independently of Unc-104's transport functions by inhibiting the expression level of active zone (AZ) and synaptic vesicle (SV) components. Wnd signaling also becomes activated when presynaptic proteins are ectopically over-expressed. These findings suggest a model that the Wnd pathway functions within a stress response pathway to fine-tune the expression level of presynaptic proteins, whose localization requires an intact axon and functional axonal transport machinery.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

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**Program#/Poster#:** 576.24/W30

**Topic:** E.06. Posture and Gait

**Support:** NIH 5R01NS081936-03

**Title:** Reperfusion reoxygenation brain injury following rabbit fetal hypoxia-ischemia increases reactive nitrogen species

**Authors: A. SHARMA, 48201**[1], Z. SHI[1], M. MAHASETH[1], K. LUO[1], G. NATARAJAN[1], J. VASQUEZ-VIVAR[2], *S. TAN[1], M. BAJAJ[1]
[1]Pediatrics, Wayne State Univ., Detroit, MI; [2]Med. Col. of Wisconsin, Milwaukee, WI

**Abstract: Introduction**: We have shown previously production of reactive nitrogen species (RNS) in fetal brains following antenatal hypoxia-ischemia (HI). We have identified a specific type of injury due to oxidative stress using MRI during HI that occurs in the reperfusion-reoxygenation period (RepReOx injury). Previous attempts at identifying biomarkers of RNS in parts of the brain using nitrotyrosine Western Blot have not been successful.
**Hypothesis:** Biomarker detection of RNS production is found in RepReOx injury in fetal brains following antenatal HI.
**Methods:** In vivo global HI of fetuses was induced in pregnant New Zealand White rabbits with

uterine ischemia, in a 3T magnet. MRI delineated 4 groups of HI fetuses including No injury group, without drop in brain ADC and RepReOx injury group, with ADC continuing to drop after end of HI. The presence of RepReOx predicts postnatal hypertonia. Fetal brains were extirpated 20 min after the end of HI. Using automated Western blot (WES, Protein Simple), nitration of tyrosine was measured using an antibody to nitrotyrosine (Abcam, ab7048), and suitable controls were run.
**Results:** In RepReOx, peaks on the chromatogram showed significantly increasing areas with increasing antibody with automatic Western Blot. Specificity of immunodetection was determined by positive control of using a pretreated antibody to nitrotyrosine confirming that positive peaks that are detecting tyrosine nitration.
**Conclusions:** There is evidence of increased production of RNS during RepReOx in fetal brains following HI. Quantification and differentiating non-specific binding of antibody with true nitrotyrosine production has become easier using Automatic Western Blot methods.

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**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.25/W31

**Topic:** B.04. Ion Channels

**Title:** Regulation of activity-dependent synaptic plasticity and synaptic expression of glutamate receptors by the neurosteroid pregnenolone sulfate: Implications for learning and memory

**Authors: *V. KUMARESAN**, *V. KUMARESAN, M. H. RATNER, K. SUGUNAN, S. DOWNING, A. CASARELLA, N. LI, A. JOYAL, D. H. FARB
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**Abstract:** Neurosteroids and synthetic analogs may be the basis for the development of adjunctive treatments for multiple CNS disorders ranging from super-refractory status epilepticus to neurodevelopmental disorders such as schizophrenia. Clinical trials suggest that the endogenous neurosteroids pregnenolone (PREG) with its immediate metabolite pregnenolone sulfate (PregS) show promise in the treatment of cognitive disorders (Marx et al., 2014, Psychopharm. 231 (17):3647). For example, therapeutic approaches aimed at restoring steroidogenesis in patients with PCDH-19 female limited epilepsy have recently been reported (Trivisano et al., Epilepsia,1-5, 2017). 110xC19 steroids and PregS might serve as clinically useful biomarkers of disease control in 21-hydroxylase deficiency (Turcu et al., 2017 J. Clin. Endocrinol Metab. 2016-3989). We recently reported that low nanomolar concentration of PregS, induced a delayed onset increase of the neuronal response to NMDA and trafficking of

NMDAR to the cell surface through an intracellular calcium ([Ca2+]i) dependent mechanism (Kostakis et al., 2013, Mol. Pharm. 84:261). Moreover we have shown that low picomolar PregS increases [Ca2+]i and CREB phosphorylation and the frequency of spontaneous excitatory postsynaptic currents (Smith et al., 2014, Mol. Pharm. 86:390)
Here we report that picomolar PregS stimulates synaptic expression of GluA1-containing AMPA receptor puncta on proximal spines of hippocampal neurons in culture in a synaptic GluN2B- and L-Type voltage gated $Ca^{++}$ channel-dependent manner. cAMP-dependent PKA and casein kinase 2 are mechanistically involved in this process. Recent reports show that repeated injections of the neurosteroid PregS, in rats have resulted in enhanced memory, spatial orientation and object discrimination (Plescia et al., 2014, Behav. Brain Res. 258 :193). To ask whether PREG and/or PregS might modulate spatial memory, we examined the effect of escalating doses of PregS and Preg (0.1, 1.0 and 10.0 mg/kg s.c.) on place cell dynamics. PregS but not PREG induced a dose dependent increase in spatial information content during repeated foraging-based exploration of a familiar environment. These findings suggest that spatial specificity of place cell firing is increased by PregS but not PREG.

**Disclosures: V. Kumaresan:** None. **M.H. Ratner:** None. **K. Sugunan:** None. **S. Downing:** None. **A. Casarella:** None. **N. Li:** None. **A. Joyal:** None. **D.H. Farb:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.26/W32

**Topic:** B.01. Neurotransmitters and Signaling Molecules

**Support:** R01 MH075916-07

**Title:** Inhibition of Src-PSD-95 interaction may enhance NMDA receptor function in schizophrenia

**Authors: *A. BANERJEE**[1], M. HELLER[1], A. SENGAR[2], M. SALTER[2], S. SIEGEL[3], K. E. BORGMANN-WINTER[4], C.-G. HAHN[1]
[1]Dept. of Psychiatry, Univ. Pennsylvania, Philadelphia, PA; [2]Neurosci. and Mental health, The Hosp. for Sick Children, Toronto, ON, Canada; [3]Psyciatry and Behavioral Sci., USC, LosAngeles, CA; [4]Dept. of Psychiatry, Univ. Pennsylvania and The Children Hosp. of Philadelphia, Philadelphia, PA

**Abstract:** Previously, we have reported that NMDA receptor hypoactivity in schizophrenia is partly mediated by reduced Src kinase activity (Banerjee et al. 2015) resulting from altered protein interactions of Src with its binding partners, including PSD-95, ErbB4, Dysbindin, rPTPa. Of these, PSD-95 is of particular interest as it inhibits Src kinase (Kalia et al. 2006) and

its association with synaptic NMDA receptors is increased in schizophrenia. We hypothesized that inhibiting protein interactions between Src and PSD-95 may increase Src activity and rescue NMDA signaling deficit in the illness. SAPIP (Src activating PSDS-95 inhibitory peptide) is a peptide that competes with PSD-95 for its binding domain and overcomes inhibition of Src activity by PSD-95 specifically in synaptic membrane. We first examined whether SAPIP increase Src activity in synaptic membranes of human postmortem and rodent cortex. When 20 ugs of synaptic membranes of human and rodent cortices were incubated with 200 ng of SAPIP, Src activity was increased by about 20% ($P<0.05$ for human and mouse). To increase the membrane permeability of the peptide for in vivo administration, Tat- SAPIP construct was designed and recombinant proteins were purified using nickel affinity chromatography. Tat-SAPIP was then tested for its binding with Src -PSD-95 complex in synaptic membrane fractions derived from mice cortex. To test its membrane permeability, cultured rat cortical neurons (DIV 5 or 12) were incubated with SAPIP (0, 1.3 or 6.5 ng/ul) for 45 min. Tat-SAPIP decreased interaction between PSD-95 and Src on membrane fractions while no effect was observed on the cytosol. We also observed a concomitant increase in src pY416 phosphorylation on the membrane correlated with increase in src activity. The results of this study demonstrate that Tat-SAPIP is membrane permeable, and perturb protein interactions between Src and PSD-95, thereby increasing Src activity. We thus propose Tat-SAPIP be considered a potential therapeutic strategy targeting Src mediated NMDA signaling deficit in schizophrenia.

**Disclosures:** **A. Banerjee:** None. **M. Heller:** None. **A. Sengar:** None. **M. Salter:** None. **S. Siegel:** None. **K.E. Borgmann-Winter:** None. **C. Hahn:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.27/W33

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Optimization of culture conditions and evaluation of cell health in human induced pluripotent stem cell-derived neurons using quantitative live-cell analysis

**Authors: A. C. OVERLAND**, J. N. RAUCH, L. OUPICKA, D. M. ROCK, *C. SCHRAMM, D. M. APPLEDORN
Essen BioScience Inc, Ann Arbor, MI

**Abstract:** The ability to culture, monitor and analyze cells that accurately reflect disease phenotypes of human nervous system disorders has been a major limitation in neuroscience research. The introduction of human induced pluripotent stem cell (hiPSC)-derived neurons has provided a promising new approach aimed at modeling human neurological diseases. However, hiPSC-derived neurons are not well characterized, and the capacity to monitor neuronal

morphology and cell health is critical for the evaluation of these novel model systems in long-term culture. Traditional approaches rely on endpoint assays and imaging techniques that require immunochemical staining, yet they provide limited real-time kinetic information. In this study, we present data outlining optimal culture conditions for cell viability and neurite outgrowth in hiPSC-derived neurons from Cellular Dynamic International (CDI, iCell Neurons). We also evaluated neurite outgrowth and cellular viability in iCell Gluta Neurons from CDI using a quantitative, live-cell imaging approach with the IncuCyte S3® over days/weeks in culture. Testing multiple culture and plate coating conditions, we find iCell Neurons respond best with a combination of BrainPhys media (Stem Cell Technologies) and iCell DOPA Neuron Supplement/iCell Nervous System Supplement (CDI) combined with a plate coating of polyethyleneimine and laminin. To demonstrate a real-time imaging approach using hiPSC-derived neurons, we evaluated glutamate- and kainate-induced excitotoxicity using the IncuCyte S3® phase/fluorescent NeuroTrack applications multiplexed with Annexin V reagents in iCell Gluta Neurons. Glutamate and kainate produced a concentration- and time-dependent decrease in neurite length with a concomitant increase in red or green object count (indicating cell death) over 72 hours. Glutamate and kainate produced a similar effect when measured by IncuCyte™ Cytotox Red, IncuCyte™ Cytotox Green and caspase 3/7 reagents. Treatment with the NMDA receptor antagonist MK-801 and the AMPA receptor antagonist NBQX reduced the glutamate- and kainate-induced effects on neurite length and cell death. These data highlight optimal culture conditions for iCell Neurons and exemplify the ability of the IncuCyte S3® approach for real-time, long-term quantitative analysis of iPSC-derived neuronal cell health.

**Disclosures:** **A.C. Overland:** A. Employment/Salary (full or part-time):; Essen BioScience. **J.N. Rauch:** A. Employment/Salary (full or part-time):; Essen BioScience. **L. Oupicka:** A. Employment/Salary (full or part-time):; Essen BioScience. **D.M. Rock:** A. Employment/Salary (full or part-time):; Essen BioScience. **C. Schramm:** A. Employment/Salary (full or part-time):; Essen BioScience. **D.M. Appledorn:** A. Employment/Salary (full or part-time):; Essen BioScience.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.28/W34

**Topic:** C.10.Tauopathies, Tau-dementias, and Prion diseases

**Support:** NIA Grant T32 AG00216-23

**Title:** NiPSCEs propagate human tau in the mouse brain

**Authors: *C. N. WINSTON**[1], E. M. ROCKENSTEIN[1], J. C. AKERS[2], S. YUAN[1], R. A. RISSMAN[1]
[1]Neurosci., UC San Diego, LA Jolla, CA; [2]Neurosurg., UC San Diego, La Jolla, CA

**Abstract: INTRODUCTION:** The interest in exosomes biology has grown dramatically over the past few years, specifically with their role in cell-to-cell communication and as potential biomarkers for staging neurodegenerative diseases. We previously reported the pathogenic potential of neuronally-derived exosomes (NDEs) as carriers of phosphorylated tau (p-tau) species and other AD-related proteins. Here, we utilize an *intro model* to investigate the role that secreted exosomes play in AD pathogenesis. **METHODS:** Exosomes secreted into the cell culture media were isolated from neuronally - differentiated, human induced pluripotent stem cells that express the tau repeat domain with the with P301L and V337M mutations (NiPSCEs - tau(LM)). Exosome preparations were characterized by size (Nanosight) and extracted protein cargo were quantified by Western blot (WB). NiPSCEs - tau(LM) suspensions was injected into normal mice CNS and pathological changes were characterized by IHC at one and two months' post injection. Following NiPSCEs - tau(LM) injection and animal sacrifice, mouse plasma exosomes were extracted, precipitated and enriched for neuronal source by anti-L1CAM antibody absorption and extracted protein cargo were quantified by WB. **RESULTS:** Size distribution for non-tau transduced hiPSCs exosome preparations (NiPSCEs) fluctuated significantly while NiPSCE - tau(LM) were uniform and smaller in size as demonstrated by Nanosight. KJ9A, a pan-human tau antibody recognized endogenous human tau only in NiPSCEs-tau(LM) samples as demonstrated by WB. KJ9A immunoreactivty was visualized in the CA1 region of the hippocampus while PHF-1 was visualized in the hippocampus, entorhinal cortex, and subcortical thalamic regions at one and two months post - injection. MAP2 staining revealed laminar disorganization and neuronal cell loss in the hippocampus and cortex of injected mice at both time points. Lastly, plasmas NDEs isolated from injected mice contained tau species suggesting the trafficking of exosome cargo proteins from the CNS to the periphery. **CONCLUSIONS:** We report secreted exosomes isolated from our *in vivo model* of hiPSCs propagate human tau and p-tau in mouse CNS with high pathogenic potential. Additionally, we report that NiPSCE - tau(LM) cargo traffic AD-related proteins from the CNS to the periphery by being encapsulated in NDEs isolated from mice plasma.

**Disclosures: C.N. Winston:** None. **E.M. Rockenstein:** None. **J.C. Akers:** None. **S. Yuan:** None. **R.A. Rissman:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.29/W35

**Topic:** B.12. Glial Mechanisms

**Support:** JSPS KAKENHI (Grant-in Aid for Young Scientists (B) 16K21080)

**Title:** Ifitm3 regulates polyI:C-inducced neuronal impairment via rab small gtpases

**Authors: *N. ITOH**[1], T. NAGAI[1], D. IBI[2], A. NAKAJIMA[3], T. NABESHIMA[4], K. YAMADA[5]
[1]Nagoya University, Grad. Sch. of Med., Nagoya, Japan; [2]Chem. Pharmacol., Meijo Univ., Nagoya, Japan; [3]Fac. of Agr. and Life Sci., Hirosaki Univ., Hirosaki, Japan; [4]Advanced Diagnos. Syst. Res. Lab., Fujita Hlth. Univ. Grad. Sch. of Hlth. Sci., Toyoake, Japan; [5]Nagoya Univ. Grad Sch. Med., Nagoya, Japan

**Abstract:** Virus infection during perinatal period induces aberrant neuronal development and increases the vulnerability for neuropsychiatric disorders. Clinical studies suggest that serious viral infection in the central nervous system during the postnatal stage is involved in the etiology of psychiatric disorders. However, little is known about the neurodevelopmental mechanism underlying the association between perinatal virus infection and brain dysfunction in later life. Previously, we reported that treatment of polyriboioinic-polyribocytidylic acid (poly I:C), a synthetic double strand RNA which induces natural immune reaction, in neonatal mice lead to schizophrenia-like behavioral abnormality including increased anxiety, memory impairment, and social behavior deficit in adulthood. We also showed interferon-induced transmembrane protein 3 (Ifitm3) in astrocytes is the key molecule in poly I:C-induced behavioral alteration. However, the molecular mechanism underlying roles of Ifitm3 in CNS remains unknown. To understand how Ifitm3 causes developmental abnormalities in CNS, we screened novel Ifitm3-interacting proteins by combining immunoprecipitation and liquid chromatography-tandem mass spectrometry (LC-MS/MS). As a result, we identified Rab GDP dissociation inhibitor (RabGDI) as a novel Ifitm3-interactig protein. Interaction of Ifitm3 with RabGDI was confirmed by pull down assay in HEK293 cells. Ifitm3 was partially co-localized with RabGDI in cultured astrocyte. Expression of Ifitm3 increased size of EEA1-positive endosome and level of active Rab5. These results suggest that Ifitm3 regulates Rab5 activity through interacting with RabGDI, and promotes early endosome fusion.

**Disclosures:** **N. Itoh:** None. **T. Nagai:** None. **D. Ibi:** None. **A. Nakajima:** None. **T. Nabeshima:** None. **K. Yamada:** None.

**Poster**

**576. Mechanisms of Neurodegeneration II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 576.30/W36

**Topic:** B.12. Glial Mechanisms

**Title:** The neuropeptide substance P elicits cortical spreading depolarization (CSD) in adult rats via activation of the NK1-receptor - could this be important in brain pathophysiology?

**Authors: F. RICHTER**[1], J. LEUCHTWEIS[1], A. EITNER[1], *A. LEHMENKUHLER[2], H.-G. SCHAIBLE[1]
[1]Univ. Hosp. Jena, Jena, Germany; [2]Pain Inst., Dusseldorf, Germany

**Abstract:** It is known that substance P (SP) acts as a vasodilator, induces vasogenic edema, and is able to increase neuronal membrane excitability. Topical application of $10^{-5}$ M substance P (SP) to the surface of rat dura mater induced a significant plasma extravasation [1]. Such processes could contribute to brain damage if large enough amounts of SP are released, e.g. after brain injury or stroke. The particular pathophysiological processes through which SP induces secondary damage have not been clarified yet. In this study we tested, whether SP is able to induce CSD in the healthy brain, and whether this effect is specific to the action of this neuropeptide on its receptor. In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSD were recorded in cerebral cortex with two pairs of glass micropipettes (distance 5-6 mm) at depths of 400 and 1200 µm in two areas of the cortex, separated by a wall. In one area, CSD was elicited by a microinjection of 1 M KCl (tip diameter 5 µm, 100 kPa, 300 ms-1 s) into the grey matter at intervals of 30 min. In the remote area 100 µl of SP at concentrations from $10^{-8}$ M to $10^{-5}$ M were applied topically and left there. To test for specificity, the remote area was pretreated in some experiments with 250 nM of the NK-1 receptor antagonist L703.606 and SP was applied afterwards. Plasma extravasation into the brain was visualized by evans blue. In all rats tested, a pulse of KCl elicited a single propagating CSD. The topical application of SP to the brain surface induced a series of self-regenerating CSD (3-7 within the first 30 min of application) that originated in the SP-treated area, and 87 % of the CSD propagated into the untreated cortical area. Microinjection of KCl to elicit CSD usually halted the self-regenerating waves. In the treated area, amplitudes of CSD elicited by KCl increased significantly (400 µm depth: control 21.3±3.0 mV, after two hours 23.2±4.8 mV; 1200 µm depth: control 21.7±1.4 mV, after two hours 27.4±4.0 mV). CSD elicited by KCl propagated faster after SP (control 2.4±0.1 cm/min, after two hours 2.8±0.7 cm/min). Plasma extravasation was seen in the superficial cortical layers. The NK-1 receptor antagonist prevented the occurrence of self-regenerating CSD by SP. However, it did not prevent the increase in CSD amplitudes and the faster propagation of the CSD waves after KCl microinjection. Our results confirm that SP is a candidate to elicit CSD via the NK-1 receptor independently from other depolarizing agents. Therefore SP-induced CSD might contribute to secondary brain damage besides the other pathophysiological effects of SP. [1] Ebersberger et al., Ann Neurol. 2001;49(1):7-13.

**Disclosures:** **F. Richter:** None. **J. Leuchtweis:** None. **A. Eitner:** None. **A. Lehmenkuhler:** None. **H. Schaible:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.01/X1

**Topic:** C.09. Brain Injury and Trauma

**Title:** Plasticity-related gene protein type 3 modulates neurite outgrowth inhibition through the RHOA-ROCK pathway

**Authors: *C. AGBAEGBU**
Georgetown University/Nih, Bethesda, MD

**Abstract:** Axonal outgrowth is severely limited after injury to the Central Nervous System (CNS) and the mechanisms through which both intrinsic and extrinsic factors regulate plasticity after injury is not completely understood. Here we report that PRG-3, an intrinsic factor, attenuates CSPG induced neurite outgrowth inhibition. Additionally, overexpression of PRG-3 abrogated lysophosphatidic acid (LPA) induced neurite retraction. PRG-3 is a member of the Plasticity-related gene (PRG) proteins, a subclass of the lipid phosphate phosphatase (LPP) superfamily. They are integral membrane proteins characterized by six transmembrane domains. There are five members, PRG1 - 5 that are sequence and structurally similarity to the lipid phosphate phosphatases, LPPs, however, they do not exhibit any phosphatase activity that characterizes the LPPs. Overexpression of PRG-3 has been shown to dramatically change the morphological phenotype of neuroblastoma cells by inducing membrane protrusions. Furthermore, cells overexpressing PRG-3 show a decrease in myosin light chain II phosphorylation after LPA treatment, indicating that PRG-3 modulates the RhoA-ROCK pathway. Although CSPG and LPA act on different receptors, these extrinsic factors converge upon the RhoA-ROCK signaling pathway. In summary, our data indicates that PRG-3 protein modulates neuronal response to CSPGs and LPA, both inhibitory molecules to axonal outgrowth, via the RhoA-ROCK pathway, and therefore may mediate neuronal plasticity, providing a target for investigation to improve therapeutic strategies after injury to the CNS.

**Disclosures: C. Agbaegbu:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.02/X2

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH Grant NS085568

NIH Grant NS091585

NIH Grant NS075338

VA National Merit grant RX000666

AHA Predoctoral Fellowship Award 0840110N

**Title:** Long-term evaluation of vascular remodeling after traumatic brain injury in mice

**Authors: *M. R. MCCRARY**[1], J. Y. ZHANG[2], P. SETHAPUTRA[2], K. K. JESSON[2], X. GU[2], L. WEI[2], S. YU[2]
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**Abstract:** Traumatic brain injury (TBI) is a leading cause of mortality and morbidity and contributes to about 30% of all injury deaths in the US. Vascular regeneration entails a series of complex events including angiogenesis, arteriogenesis, and pericyte recruitment. In the brain, these events are intimately tied to neurovascular and gliovascular unit regeneration and functional recovery. While vascular remodeling has been intensively studied after ischemic stroke, the timeline of neovascularization is less characterized in TBI, particularly in the chronic phases of tissue recovery. In the present investigation, we studied long-term vascular regeneration events in transgenic mice expressing the smooth muscle actin α-SMA-GFP. Adult mice were subjected to a controlled cortical impact stereotactically directed to the sensorimotor cortex. The proliferation marker BrdU was administered daily after injury, and peri-contusion blood flow was measured weekly using laser Doppler. Images of stained slides were analyzed using ImageJ under double-blind conditions. Brain tissues were isolated from the peri-contusion region and the contralateral cortex. Western blot analyses of tissue collected from early time points (1, 3, 7, and 14 days) after injury suggest VEGF rather than EPO is a major factor controlling vascular regeneration after TBI. Laser Doppler readings showed that local blood flow in the peri-contusion region decreased within 1 week after injury, then steadily recovered to pre-injury levels after ~5 weeks. TBI animals were sacrificed 2, 5, and 8 weeks after injury for histologic examination of macro- and micro-vessels. Low power images of macrovessels in the per-contusion and core zones were acquired of the whole brain. The complexity and density of both macrovessels and microvessels, as well as pericytes recovery over time were analyzed. Despite normalization of blood flow by 5 weeks, neovascularization events were noted weeks later. Taken together, these data suggest that vascular regeneration occurs over the course of months after traumatic brain injury and may have ramifications on our approach to the treatment of this disease. Future studies will elucidate the complexities of neovascularization and its implication for tissue regeneration after brain injury.

**Disclosures:** **M.R. McCrary:** None. **J.Y. Zhang:** None. **P. Sethaputra:** None. **K.K. Jesson:** None. **X. Gu:** None. **L. Wei:** None. **S. Yu:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.03/X3

**Topic:** C.09. Brain Injury and Trauma

**Support:** VA Merit Review

NIH HD061963

**Title:** Acute and chronic behavioral outcomes following closed head injury in adolescent mice are dependent on sex

**Authors: *L. PLYLER**, M. ZEMEL, M. BRANHAM, R. RAGHUPATHI
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**Abstract:** Nearly 5.3 million Americans are currently living with disabilities resulting from traumatic brain injury (TBI) of which almost 80% are a consequence of mild TBI. Moreover, mild TBI is a significant health-related issue in adolescents and young adults engaged in contact sports. Girls who sustain mild TBI often exhibit different symptoms compared to their male counterparts and also take longer to recover from these symptoms. To better understand the cellular basis for these sex differences, a mouse model of closed head injury was developed in adolescent (35-42-day-old) male and female C57Bl/6 mice. Anesthetized mice received an impact to the intact skull along the midline suture halfway between bregma and lamba; sham-injured mice underwent an identical procedure without impact. On days 1-3 following injury, both male and female mice displayed deficits in spatial learning/acquisition, but not recall, tested in the Morris water maze. In week 4 after injury, an increase in open arm time, suggestive of impulsive behaviors, was observed in male brain-injured mice compared to their sham-injured counterparts in the elevated plus maze. In contrast, no differences in open arm times were observed between brain- and sham-injured female mice. Closed head injury did not induce a depression-like phenotype measured using the forced swim test in either male or female mice. At 5 weeks post-injury, male brain-injured mice demonstrated an increased periorbital sensitivity to Von Frey filaments compared to the sham-injured group, suggestive of a headache phenotype. Interestingly, brain-injured female mice exhibited hyposensitivity to the filaments suggestive of a decrease in sensory function. These data add to the ongoing characterization of the long-term functional deficits following closed head injury, and provide the basis for differential evaluation of post-traumatic effects in men and women.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.04/X4

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH Grant RO1 NS050465

NIH Grant R01 DK104363

**Title:** Short-term fructose ingestion disrupts peripheral and central metabolism and aggravates the outcome of brain trauma

**Authors: *Z. YING**[1], L. ROYES[1], A. JIMÉNEZ MALDONADO[1], S. REGE[1], G. KRISHNA[1], F. GOMEZ-PINILLA[1,2]
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**Abstract:** Traumatic brain injury (TBI) results in high rates of morbidity and mortality in sports, military and domestic environments. The fact that TBI per se disrupts brain cell metabolism suggests that TBI pathology is highly susceptible to metabolic perturbations. Fructose is abundant in the Western diet and a major cause of metabolic syndrome (MetS), diabetes, and obesity. Chronic exposure to fructose has been associated with protracted brain plasticity and function, assumingly secondary to MetS. We wanted to evaluate the impact of short-term fructose ingestion (prior establishment of MetS) on the response of the brain to a traumatic lesion. A moderate fluid percussion injury (FPI, 2.7 atm) decreased glucose tolerance ($p<0.05$) as short as after 1 week postlesion. Intact animals exposed to fructose consumption for 3 weeks (15% w/v, 3wks) before FPI onset, showed no alterations in peripheral metabolism. However, fructose consumption potentiated an increase in plasma insulin ($p<0.01$) and a decrease in glucose tolerance ($p<0.05$) in animals exposed to FPI. These results indicate that fructose exacerbates the disruptive action of TBI on peripheral glucose regulation. The short-term fructose consumption increased escape latency in the Barnes maze test ($p<0.01$) in animals exposed to FPI, but fructose by itself was not sufficient to alter latency in intact animals. Fructose consumption reduced hippocampal dry weight which was accompanied by a reduction in the neuronal NeuN and Myelin Protein (MBP). Fructose also reduced levels of the peroxisome proliferator activated receptor gamma, coactivator 1 alpha (PGC-1α) and Cytochrome c oxidase subunit II (COX2), suggesting compromised mitochondrial function. We have previously shown that 6 weeks of fructose consumption significantly disrupts memory performance in the Barnes maze in intact rats (Agrawal et al., J Neurophysiol, 2012), and aggravates memory dysfunction after TBI (Agrawal et al, JCBFM, 2016). Our new results show that a short period of fructose consumption poses a risk for the outcome of TBI, compromising metabolic homeostasis in the brain and periphery, with subsequent effects on the progress of the pathophysiology of TBI.

These studies are particularly significant on the light of recent studies showing the pervasive effects of fructose and TBI on many genes associated with important neurological and psychiatric disorders (Meng et al., eBiomedicine, 2016, 2017).

**Disclosures: Z. Ying:** None. **L. Royes:** None. **A. Jiménez Maldonado:** None. **S. Rege:** None. **G. Krishna:** None. **F. Gomez-Pinilla:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.05/X5

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH R01NS045702

P01NS062686

IDDRC U54HD090257

**Title:** Differential regulation of Sirt1 and Sirt2 in white matter after neonatal hypoxia

**Authors: *B. JABLONSKA**, L.-J. CHEW, V. GALLO
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**Abstract:** Diffuse white matter injury (DWMI) is a major form of brain injury, which results in chronic neurological and behavioral disabilities in prematurely born infants, including a broad spectrum of cognitive and learning disabilities until young adulthood. To understand the mechanisms underlying white matter dysmaturation caused by injury, we have used a mouse model of neonatal hypoxia (Hx) that reproduces all the landmarks of brain injury observed in premature infants, including DWMI. In this model, we have previously demonstrated that Hx causes hypomyelination one week after injury (P18). This is due to delayed oligodendrocyte (OL) maturation and increased proliferative response of oligodendrocyte progenitor cells (OPCs) through Sirt1-activated Cdk2 signalling. Hx increases the expression of Sirt1 in white matter lysate, and elevates the number of $NG2^+Sirt1^+$ progenitors, whereas the number of mature $CC1^+$ oligodendrocytes expressing Sirt1 is unchanged. Since Sirt2 is implicated in OL maturation, here, we determined whether neonatal Hx altered Sirt2 expression and function in white matter. We found that Hx reduced Sirt2 expression and the number of $Sirt2^+$ cells in white matter. The number of $Sirt2^+Olig2^+$ cells decreased after Hx but $NG2^+Sirt2^+$ cells remained unchanged, indicating that Hx may affect primarily mature OLs. Indeed, we observed a significant reduction of Sirt2 expression in mature $CC1^+$ and $CNP^+$ oligodendrocytes after Hx. A role in differentiation was supported by Sirt2 loss-of function experiments, in which siRNA-mediated knockdown of Sirt2 in normoxic and Hx cultured cells was performed. In normoxic cultures,

knockdown of Sirt2 caused a significant reduction in the percentages of $Olig2^+$, $GalC^+$ and $O4^+$ cells, as compared to scrambled control cultures. In scrambled controls, Hx caused a reduction in the number of mature oligodendrocytes. This effect was more pronounced after Sirt2 siRNA treatment. These findings demonstrate reciprocal regulation of Sirt1 and Sirt2 consistent with their respective roles in proliferation and differentiation after white matter damage caused by Hx.

**Disclosures: B. Jablonska:** None. **L. Chew:** None. **V. Gallo:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.06/X6

**Topic:** C.09. Brain Injury and Trauma

**Support:** NHRI-EX106- EX105-10206NI

**Title:** Identification of genes that promote axonal regeneration of injured cortical neurons

**Authors: *C.-Y. CHANG**, L. CHEN
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**Abstract:** Neurons receive and propagate signals to coordinate behavior. Upon brain injury, one would suffer from impaired sensory, motor or cognitive functions. However, unlike the peripheral nervous system, the central nervous system exhibits poor recovery due to the non-permissive extrinsic environment as well as the low intrinsic regenerative capacity. Evidence shows that diminishing extrinsic inhibitor molecules confers limited neurite sprouting and is insufficient for improving distal axonal regeneration. We thus set out to explore the intrinsic growth capacity within brain neurons. In response to injury, a number of regeneration-associated genes have been identified and show promising potential for facilitating axonal regeneration of cortical and hippocampal neurons. This work is supported by National Health Research Institutes, Taiwan (NHRI-EX106- EX105-10206NI).

**Disclosures: C. Chang:** None. **L. Chen:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.07/X7

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH grant 1 F32 NS096895-01A1

NIH grant R01NS052568-07

**Title:** Cyclin-dependent kinase inhibitors attenuate mitochondrial injury in neuronal apoptosis

**Authors: *T. G. AUBRECHT**[1], B. SABIRZHANOV[2], B. ROELOFS[4], B. M. POLSTER[5], B. A. STOICA[3], A. I. FADEN[6]
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**Abstract:** Traumatic brain injury (TBI) remains a major public health problem, effecting more than 1.7 million people in the United States. In 2010, the center for disease control estimated the direct and indirect costs of TBI were $76.5 billion. Patients with TBI have impaired cognition, learning and memory deficits are also observed in animal models of TBI. In addition to direct, immediate primary injury, delayed molecular and cellular changes contribute to cell death and neurological dysfunction caused by TBI. A key secondary injury mechanism in experimental TBI is cell cycle activation (CCA). TBI induced CCA results in apoptosis of post-mitotic cells such as neurons and oligodendroglial cells as well as microglia proliferation, causing neuroinflammation and secondary neurotoxicity. Pharmacological inhibition of CCA with selective cyclin-dependent kinase (CDK) inhibitors prevents activation of microglia and astrocytes and improves cognitive function. We further investigated the beneficial effects of CDK inhibitors in a neuronal cell death model *in vitro.* After 6h of etoposide treatment, basal mitochondrial function of primary rat cortical neurons (RCN) is not affected, however, the ability of the neurons to respond to maximal energetic demand is impaired. Treatment of RCN with CDK inhibitors prevents impairments in the ability of the neurons to respond to maximal energetic demand on the mitochondria. Additionally, RCN treatment with CDK inhibitors prior to etoposide prevents induction of pro-apoptotic BCL2 family member's expression downstream of p53 phosphorylation/activation. CDK inhibitors prevent induction in Bim and Cdkn1a (p21), as well as cleavage of Caspase-3, Fodrin, Parp-1. Thus, our data indicate the robust protective effect of CDK inhibitors in response to neuronal injury is due to attenuation of p53 dependent apoptosis.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 577.08/X8

**Topic:** C.09. Brain Injury and Trauma

**Support:** DOD Grant W81XWH-13-1-0384

NIH Grant R01ES024233

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**Title:** ABCA1 deficiency affects brain transcriptome following traumatic brain injury in APOE3 and APOE4 mice

**Authors: *E. L. CASTRANIO**[1], C. M. WOLFE[1], K. NAM[1], F. LETRONNE[1], A. MOUNIER[1], J. SCHUG[2], N. F. FITZ[1], R. KOLDAMOVA[1], I. LEFTEROV[1]
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**Abstract:** Traumatic brain injury (TBI) is a leading cause of death and disability in army personnel and general population. Apolipoprotein E (APOE) is the major cholesterol carrier and lipid transporter in the CNS. Recent studies suggest a role for APOE in determining outcome severity following TBI, with inheritance of the APOE4 allele conferring worse outcome. APOE lipidation and stability is modulated by ATP-binding cassette transporter A1 (ABCA1), a transmembrane protein that transports lipids and cholesterol onto APOE. It is unknown how deficiency of ABCA1 would influence outcome following TBI. We hypothesized that inheritance of APOE4 combined with a deficiency of ABCA1 would lead to worse cognitive outcome and gene expression changes following TBI. This study uses mice expressing human APOE3 and APOE4 isoforms that are haplodeficient for the *Abca1* gene (APOE3/*Abca1*$^{+/-}$; APOE4/*Abca1*$^{+/-}$). Young adult, 3 month old mice received either a controlled cortical impact (CCI) brain injury or a craniotomy in the left hemisphere. *Abca1*$^{+/-}$ TBI mice had increased risk-taking behavior demonstrated by significantly more time in the open arms of the Elevated plus maze compared to sham ($p$<0.01). Additionally, *Abca1*$^{+/-}$ TBI mice performed worse than their sham counterparts ($p$<0.001) in Morris Water Maze (MWM), regardless of APOE isoform. To investigate the molecular mechanisms underlying this effect, we performed mRNA-seq using samples from cortices and hippocampi followed by genome-wide differential gene expression analysis. The transcriptomic profiles were compared to our published results for APOE mice

expressing both functional copies of *Abca1*. Using Network Analysis, we identified co-expressed-networks correlated to TBI, APOE isoform and Abca1 expression. We have identifiifed gene networks specific to each phenotype demonstrating roles for injury, APOE isoform and ABCA1 in modulating gene expression in the brain and after TBI.

**Disclosures:** **E.L. Castranio:** None. **C.M. Wolfe:** None. **K. Nam:** None. **F. Letronne:** None. **A. Mounier:** None. **J. Schug:** None. **N.F. Fitz:** None. **R. Koldamova:** None. **I. Lefterov:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.09/X9

**Topic:** C.09. Brain Injury and Trauma

**Support:** Boston University Start-up Funding

**Title:** Microglial activation and hyperexcitability in piriform cortex after repeated TBI

**Authors: *E. WITKOWSKI**, I. G. DAVISON, 02215, G. DEWALT, B. ELDRED
Biol., Boston Univ., Boston, MA

**Abstract:** Despite the growing prevalence of traumatic brain injury (TBI) in athletics and combat, we still know very little about the pathophysiological changes that occur in the brain following injury. Mild, closed-skull forms of injury are the most common form of TBI, but remain particularly poorly understood. To probe the cellular and synaptic changes in cortical circuits arising from mild TBI, we used a closed-skull weight-drop model in mice and assessed affected areas using histology and cellular electrophysiology. We found that 2 days after repetitive injury, there was strong microglial activation in entorhinal and piriform cortices, indicated by altered morphology visualized with Iba-1 immunostaining. Focusing on piriform cortex, we used whole cell patch clamp recordings in acute brain slices to test for changes in both excitatory and inhibitory synaptic inputs onto superficial pyramidal cells. Injury tended to increase the frequency of spontaneous excitatory input without affecting amplitude, while inhibitory inputs were largely unaffected. Direct comparison of excitatory and inhibitory components of electrically evoked synaptic responses also indicated a shift towards greater excitation. These data are consistent with findings in other TBI paradigms, but identify piriform cortex as a potential locus of sensitivity to mild injury. Piriform and associated regions are also susceptible to damage in epilepsy and are strongly involved in propagating temporal lobe seizures, suggesting that injury-induced hyperexcitability in these areas may contribute to the established links between TBI and post-traumatic epilepsy.

**Disclosures:** **E. Witkowski:** None. **I.G. Davison:** None. **G. DeWalt:** None. **B. Eldred:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.10/X10

**Topic:** C.09. Brain Injury and Trauma

**Support:** VA Merit Review

NIH Grant HD061963

**Title:** Effect of treadmill exercise on cognitive and behavioral outcomes following repeated mild traumatic brain injury

**Authors: M. ZEMEL**, M. BRANHAM, L. PLYLER, *R. RAGHUPATHI
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**Abstract:** Youth and young adults participating in contact sports such as football, hockey, lacrosse and soccer are apt to sustain multiple concussions (mild traumatic brain injury, mTBI) throughout their playing career. The long-term consequences of these repetitive injuries include altered cognitive function, increased incidence of headaches, and development of depression, anxiety and impulsivity. A number of preclinical models of repetitive mTBI have been developed in both rats and mice but few, if any, have addressed whether physical exercise, either prior to or after the injury, has the ability to influence the development of post-injury behavioral deficits. In the present study, anesthetized male mice (6-7 weeks of age) were subjected to 3 impacts (one every 48 hours) to the intact skull on the midline centered between bregma and lambda; sham-injured mice were anesthetized and surgically prepared but did not receive impacts. Repetitive mTBI resulted in a spatial learning deficit when mice were tested in the Morris water maze on days 1-3 following injury, an increase in open arm time in the elevated plus maze (EPM) at 4 weeks (suggestive of impulsivity) and increased facial allodynia at 5 weeks post-injury (suggestive of post-traumatic headache); there was no change in immobility time in forced swim test for depression. When mice were subjected to treadmill exercise (10m/min) for an hour each day for 3 weeks prior to repetitive TBI, the spatial learning deficit observed on the first 3 days after the last injury was significantly ameliorated. Pre-injury exercise did not affect injury-induced increases in open arm time but appeared to reduce the incidence of facial allodynia. Current guidelines for the treatment of concussed athletes are divided between a period of rest and continued physical activity prior to return to play. To test this concept, a subset of mice that received exercise prior to injury were subjected to treadmill exercise beginning on day 5 following the last injury and lasting for 4 weeks. When tested in the EPM, post-injury exercise decreased the open arm time of brain-injured mice when compared to non-exercised or

pre-injury exercised mice. However, the incidence of facial allodynia was similar in unexercised mice and in mice that received exercise post-injury suggesting that continued exercise may reverse the effect of pre-injury exercise. Together, these data establish a clinically-relevant model of repetitive mTBI in young adult mice and underscore the complexity in the ongoing characterization of the long-term functional deficits following multiple concussions.

**Disclosures:** **M. Zemel:** None. **M. Branham:** None. **L. Plyler:** None. **R. Raghupathi:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.11/X11

**Topic:** C.09. Brain Injury and Trauma

**Support:** Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT) 14-12A

NIH R01 NS072302-02S1

R01 NS0072302

T32 NS077889

P30 NS051220

**Title:** Insulin-like growth factor-1 overexpression enhances neurogenesis and activates the mTOR pathway after moderate TBI

**Authors:** ***E. LITTLEJOHN**, D. SCOTT, A. DESANA, J. JURAS, K. SAATMAN
Univ. of Kentucky, Lexington, KY

**Abstract:** Nearly 5 million people in the United States are living with TBI related disabilities, in part because of the brain's limited capacity to replace lost and damaged neurons. Immature neurons in the hippocampus are highly vulnerable to trauma, but can be replaced through proliferation and differentiation of neural stem cells in the subgranular zone. The extent of injury-induced neurogenesis, however, may be injury severity dependent. Insulin-like Growth Factor 1 (IGF1) modulates basal and injury-induced hippocampal neurogenesis. Using a transgenic mouse model with IGF1 overexpression restricted to astrocytes (IGF Tg) to raise brain levels of IGF1 by means of injury-induced astrogliosis, we previously showed that IGF1 enhances recovery of the immature neuron population and morphology after severe TBI. Mammalian target of rapamycin (mTOR), a signaling molecule downstream of IGF1, has been identified as a potential target for TBI interventions because of its regulatory role in plasticity and cell survival. We hypothesized that increased IGF1 would stimulate mTOR activity

following moderate injury, resulting in improved neurogenesis. To this end three cohorts of IGF Tg and wild-type (WT) mice received moderate controlled cortical impact (CCI, n=8-11/genotype) and survived 1, 3 or 10d or received sham injury (n= 3/genotype; 72h survival) At 1 and 3d following moderate injury, immunohistochemical labeling of pS6, a well characterized downstream effector of mTOR, was quantified in the granule cell layer, molecular layer, and the hilus of the dentate gyrus. Analysis of pS6 at the injury epicenter suggests that IGF1 stimulates activity of the mTOR pathway following moderate TBI in a region-specific manner. At 10d after moderate injury, IGF1 overexpression enhances recovery of immature neurons.

**Disclosures: E. Littlejohn:** None. **D. Scott:** None. **A. DeSana:** None. **J. Juras:** None. **K. Saatman:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.12/X12

**Topic:** C.09. Brain Injury and Trauma

**Support:** PIA Grant ACT1411

**Title:** Adolescent binge alcohol consumption affects hippocampal function through the impairment of mitochondrial dynamics and bioenergetics in the adulthood

**Authors: *C. TAPIA-ROJAS**[1], A. TORRES[1], F. CARVAJAL[2], R. MIRA[2], C. ARCE[2], W. CERPA[2], R. QUINTANILLA[1]
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**Abstract:** In the adolescent population, “binge drinking” is a common pattern of alcohol consumption, which is characterized by a short period of heavy alcohol use followed by a period of abstinence. Adolescents are highly susceptible to alcohol and their immature brains are more vulnerable to the effects of binge drinking. However, relatively little is known about the effects of adolescent binge drinking on mitochondrial structure and their effects on brain function. Male Sprague-Dawley rats, postnatal day 25 (PND25), were exposed to binge-like ethanol protocol; rats were treated by i.p. injections of ethanol (3,0g/Kg, 25% v/v) or saline solution during two consecutive days, with gaps of 2 days without injections, during 2 weeks. Then 1, 3 or 7 weeks after treatment rats were exposed to cognitive and social test. Finally, the animals were euthanized and the hippocampus tissue was analyzed through electrophysiological, biochemical and histochemical assays.
Our results show that binge-like alcohol consumption alters the levels of key proteins involved in mitochondrial dynamics such as Drp1, Fis1, Mfn1, Mfn2 and Opa1, disrupting the balance between fission and fusion events. Ethanol treated rats also showed changes in oxidative stress,

impaired synaptic plasticity and recognition and social memory loss. Some of these effects were compensated possibly through a mechanism that involves the activation of the Nrf-2 signaling pathway. The alterations in the regulation of mitochondrial dynamics continue overtime and importantly a delayed inflammatory response appearin the adult hippocampus. Additionally, we observed changes associated with mitochondrial dysfunction that persist until adulthood as is indicated by a deficient ATP production, reduced expression of mitochondrial respiratory complexes, increased ROS generation, decreased mitochondrial membrane potential, and increased mitochondrial calcium. Altogether, these results suggest that adolescent binge-like alcohol consumption, negatively affects mitochondrial dynamics regulation inducing mitochondria-mediated oxidative stress and triggering synaptic, cognitive and mitochondrial function defects that persist in the adult brain.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.13/X13

**Topic:** C.09. Brain Injury and Trauma

**Support:** EY011261

**Title:** GluN2B mediates activity-driven circuit recovery following injury

**Authors: *A. C. GAMBRILL**[1], C. MCKEOWN[1], R. L. FAULKNER[2], H. T. CLINE[1]
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**Abstract:** Sensory activity is instructive for the development and refinement of sensory circuits, such as the visual circuit of the optic tectum in Xenopus laevis, which receives information from the retina and sends projections to motor regions. However, how sensory activity might exert an effect in a circuit following a traumatic brain injury is unknown. We have previously developed a penetrative brain injury model and shown that injury to the Xenopus tectum results in loss of visual avoidance behavior. Recovery of behavior is dependent on cell proliferation, and takes 4-7 days. Here, we test whether increased sensory activity can increase the speed with which the animal recovers from injury. We determined sensory activity significantly increased the ability of the animal to perform our behavior assay at two days following injury (behavior index, with activity: 0.57 +/- 0.03 vs ambient control: 0.38 +/- 0.05). Animals which received additional sensory activity also reached baseline levels of behavior more quickly. This behavioral recovery was correlated with changes in the frequency of spontaneous events recorded in neurons born post-injury, and with the frequency with which these neurons received barrages of organized

circuit activity, suggesting behavioral recovery is correlated with the successful incorporation of new neurons into the existing tectal circuit. Behavioral recovery was impeded by pharmacological blockade of the GluN2B subunit of the NMDA receptor. Loss of GluN2B subunit also decreased activity, as measured electrophysiologically. In conclusion, these data show enhanced sensory activity as mediated by NMDA receptors contributes to the repair of the post-injury tectal circuit. This work was supported by EY011261 to HTC.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.14/X14

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH grant

**Title:** Gene expression analysis reveals how Abcd1 alters tight junction, cell cycle and extracellular matrix function in human brain endothelium

**Authors: *Y. SUBBURAJ**, N. SASIDHARAN, A. BERENSON, Y. GONG, P. MUSOLINO, F. EICHLER
Neurol., MGH,Harvard Med. Sch., Boston, MA

**Abstract:** X-linked adrenoleukodystrophy (ALD) is a debilitating neurological disorder caused by mutations in the peroxisomal half transporter, *ABCD1*. Blood brain barrier (BBB) disruption with migration of leukocytes to the brain, as indicated by histopathology has for a long time been implicated in the cerebral inflammatory form of the disease. To assess how *ABCD1* alters brain endothelial barrier function we performed a gene expression analysis by direct RNA sequencing in human brain microvascular endothelial cells(HBMECs). Bioinformatic analyses identified upregulation of 1527 and downregulation of 1706 genes upon *ABCD1* silencing. Preliminary pathway analysis on these data showed that TGF-β pathway is upregualted while NF-κβ, cell cycle and extracellular matrix pathways are significantly downregulated in HBMECs lacking *ABCD1*. These molecular changes also correspond with our experimental findings demonstrating that lack of *ABCD1* in brain microvascular endothelium increases their permeability to monocytes and alters angiogenesis by upregulation of the TGFBeta1 pathway. Our analysis provides novel insights on molecular mechanisms and potential therapeutic targets for cerebral ALD.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.15/X15

**Topic:** C.09. Brain Injury and Trauma

**Title:** Lipid nanoparticles delivery nucleic acids, including CRISPR components into primary neurons

**Authors: *G. T. THARMARAJAH**[1], A. THOMAS[1], R. DESOUZA[1], I. BACKSTORM[1], A. BROWN[1], E. OUELLET[1], S. GARG[1], K. MARSHALL[1], S. CHANG[1], T. LEAVER[1], A. WILD[1], P. DENG[2], K. FINK[3], J. TAYLOR[1], E. RAMSAY[1]
[1]Precision Nanosystems Inc., Vancouver, BC, Canada; [2]Genome Ctr., UC Davis, Davis, CA; [3]Dept. of Neurol. and Stem Cell Program, UC Davis Med. Ctr., Sacramento, CA

**Abstract:** Advances in the gene editing arena, specifically with CRISPR-Cas9, has pushed the demand for efficiently delivering payloads even further. Of the tools available, developments in the field of lipid nanoparticles (LNPs) has allowed for the reliable and efficient delivery of CRISPR components, both in research and clinical settings. Here, we bridge that gap by describing the development of an LNP delivery system for CRISPR components, robustly manufactured with clinical-grade materials using microfluidic technology at scales for *in vitro* applications, with the potential to move into animal models. We describe the use of lipid-based nanoparticles for highly efficient encapsulation and delivery of payloads, such as siRNA, mRNA and plasmid. We show that representative small RNAs, mRNAs and plasmids can be successfully delivered to primary neurons. LNPs manufactured to encapsulate various nucleic acids can do so with high efficiency, encapulating more than 95% of the payload, minimizing payload loss. Transfection efficiency of the LNPs is variable based on payload. The biological endpoint assays used to determine the accessibility of the payloads delivered varies for siRNA, mRNA and plasmid. Using doses of 1μg per mL of media, we achieved >90% knockdown with siRNA delivery, >90% of the primary neurons are GFP+ with GFP mRNA delivery and 60% of the primary neurons are GFP+ with GFP plasmid delivery. The LNPs are well tolerated, such that 5x the required doses have no observable cytoxicity. We can deliver gRNAs to Cas9-expressing cells to achieve up to 80% editing. We also can co-deliver Cas9 mRNA and gRNAs in separate nanoparticles to achieve editing in wildtype cells. We show that the LNPs can also be used to deliver payloads into various regions of the animal brain. These validation studies provide suitable insights in establishing strategies for efficiently delivering CRISPR components into primary cultures.

**Disclosures:** **G.T. Tharmarajah:** None. **A. Thomas:** None. **R. DeSouza:** None. **I. Backstorm:** None. **A. Brown:** None. **E. Ouellet:** None. **S. Garg:** None. **K. Marshall:** None. **S. Chang:** None. **T. Leaver:** None. **A. Wild:** None. **P. Deng:** None. **K. Fink:** None. **J. Taylor:** None. **E. Ramsay:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.16/X16

**Topic:** C.09. Brain Injury and Trauma

**Support:** Combat Casualty Care Research ProgramsCCCRP

**Title:** MiRNA expression profiling and systems biology indicate BACE-1 upregulation and APP loss during severe TBI progression

**Authors: *A. M. BOUTTE**[1], D. JOHNSON[2], B. WILFRED[1], S. GRANT[1], B. ABBATIELLO[1], B.-A. STEPHEN[1], J. GILSDORF[1], D. SHEAR[1]
[1]Neurosci. and Psychiatry, Walter Reed Army Inst. For Res., Silver Spring, MD; [2]Dept. of Pathology & Area Lab. Services, Landstuhl Regional Med. Center, US Army, Landstuhl, Germany

**Abstract:** Severe traumatic brain injury (TBI) is a risk factor for neurodegenerative diseases, such as Alzheimer's disease (AD), yet mechanistic links remain elusive. Micro (mi) RNAs, small stable RNAs that regulate translational degradation or gene repression, are proposed to be central to both TBI and AD progression. Therefore, this study determined miRNA gene expression patterns, mRNA transcript levels, and resulting protein/peptide levels in brain tissue after 10% penetrating ballistic-like brain injury (PBBI) compared to sham (craniotomy) controls. Microarray analysis was conducted using ipsilateral coronal brain tissues collected 1, 3, or 7d post-injury. Differentially expressed miRNAs (PBBI vs. sham fold change) were mapped to the APP processing pathway defined by Pathway Studio Gene Set Enrichment Analysis. Messenger (m) RNA of select targets was determined with quantitative (Q) PCR Taqman Assays. APP content was confirmed with western blotting and amyloid beta (Aβ) peptide measurements were conducted using electrochemilluminescent ELISAs. Eight (5 up / 3 down), 7 (2 up / 5 down), and 46 (31 up / 12 down) miRNAs were differentially abundant 1, 3, and 7d, respectively, after PBBI. Suppression of miR-135A at 1d (0.59-fold), miR-328 at 3d (0.63-fold) and 7d (0.83-fold), as well as mir-29 at 7d (0.91-fold) displayed inhibitory relationships with BACE1. MiR-21, also negatively associated with BACE-1, was upregulated (1.5-fold) after 7d. BACE-1 mRNA increased at 1d (2.7-fold), peaked at 3d (5.3-fold), and returned to near sham levels at 7d following PBBI. APP mRNA also increased at 1d (1.7-fold) and 3d (3.9-fold). Western blots indicated that full-length APP levels progressively decreased at 3d (0.13-fold) and 7d (0.05-

fold). However, neither Aβ-40 nor -42 peptide quantities were significantly greater after PBBI compared to sham controls. The relationship between select miRNAs, BACE-1 and APP transcript upregulation, coupled to post-translational APP loss may be key indicators of AD-like mechanisms in brain tissues during TBI progression. These relationships imply that PBBI leads to APP loss due to the reduced miRNA suppressive regulation of BACE-1. Interestingly, the lack Aβ peptide upregulation may indicate that APP is degradation is not dependent upon further proteolytic cleavage. The regulatory role of select miRNAs upon APP cleaving enzymes and generation of toxic peptides in TBI mediated neurodegeneration warrants further investigation.

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**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

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**Program#/Poster#:** 577.17/X17

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH Grant NS083544

**Title:** Critical role of acid sphingomyelinase in mitochondrial dysfunction due to glutamate/cystine antiporter-dependent ferroptosis in oligodendrocytes

**Authors: S. A. NOVGORODOV**[1], J. A. VOLTIN[1], M. A. GOOZ[2], J. J. LEMASTERS[2], *T. I. GUDZ[1]
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**Abstract:** The glutamate/cystine antiporter system $x_c^-$ plays an important role in regulating the extracellular glutamate levels and in maintaining the intracellular cysteine/glutathione (GSH)-dependent antioxidant defense mechanisms in the brain. In tumor cells, blocking system $x_c^-$ triggers a novel form of programmed necrosis, ferroptosis, which is characterized by increased ROS generation and lipid peroxidation. Therefore, drugs that can inhibit system $x_c^-$ are considered as potential treatments for cancer. Yet, the mechanisms of system $x_c^-$-dependent ferroptosis in the neural cells remain unexplored. Primary oligodendrocytes (OLs) were treated with glutamate to provoke the system $x_c^-$-mediated cell death. Pharmacological analysis revealed ferroptosis as a major contributing factor to glutamate-initiated OL death. Although RIP1 kinase inhibitor necrostatin-1 slightly protected OLs from glutamate toxicity, there was no activation of the RIP1 and/or RIP3 kinase-mediated necroptosis signaling pathway, suggesting an off-target effect of necrostatin-1. Quantitative lipidomics analysis showed a significant elevation of sphingolipids (ceramide and sphingosine) that was prevented by inhibitors of acid sphingomyelinase (ASM). Notably, blocking ASM activity or knocking down ASM protected

OLs against glutamate toxicity suggesting the critical role of ASM in OL death. Glutamate-induced ASM activation seems to involve post-transcriptional mechanisms and is associated with decreased GSH levels. Further investigation of the mechanisms of OL response to glutamate revealed enhanced mitochondrial ROS production, augmented lipid peroxidation and dissipation of the mitochondrial transmembrane potential that was attenuated by hindering ASM. The results of these studies highlight a novel mechanism of ASM involvement in governing mitochondrial ROS production and suggest an important role of ASM in system $x_c^-$- dependent ferroptosis in the brain. Supported by NIH grant NS083544.

**Disclosures: S.A. Novgorodov:** None. **J.A. Voltin:** None. **M.A. Gooz:** None. **J.J. Lemasters:** None. **T.I. Gudz:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.18/X18

**Topic:** C.09. Brain Injury and Trauma

**Title:** Alternate strategies for ablation of microglia during brain injury in adult zebrafish

**Authors: *K. SKAGGS**
Biol., Univ. of Findlay, Findlay, OH

**Abstract:** Zebrafish are increasingly used for the study of neurogenesis following injury to the central nervous system in vertebrates because, unlike mammals, they regenerate damaged neurons. After stab lesioning, initial cell death is followed by a robust inflammatory response 1-2 days post-injury, a marked increase in proliferation of radial glia-like progenitors that peaks 3-4 days post-injury, and ultimately the generation of new neurons that migrate to the site of lesion and seem to repair the damage. In order to study the role of the early inflammatory response on neurogenesis, we ablated microglia that responded to injury by injection of Clodronate-containing liposomes at the time of stab lesion. In the absence of microglia, proliferation and neurogenesis were markedly reduced, resulting in incomplete repair of damage in Clodronate-treated lesioned brains. These results suggested that the early inflammatory response may be an important signaling event that stimulates neurogenesis and repair after brain injury in adult zebrafish. A significant limitation of Clodronate treatment is that the critical time period during which microglia crucially affect regeneration cannot be determined. Therefore, we employed a small molecule inhibitor of colony-stimulating factor receptor 1 (CSFR1) to determine the effects of non-invasive microglial ablation on regeneration following brain injury and to further determine with increased precision the time period during which microglia are required to stimulate the neurogenic response following injury. The small molecule can be administered in

fish water, allowing us to investigate how differential timing of microglial ablation affects regeneration following injury.

**Disclosures: K. Skaggs:** None.

**Poster**

**577. Brain and Neuronal Injury: Cellular and Molecular Mechanisms**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 577.19/X19

**Topic:** C.09. Brain Injury and Trauma

**Title:** Polycomb protein family member cbx7 regulates intrinsic axon growth and regeneration

**Authors: *C. LIU**[1], R.-S. DUAN[1], G.-B. TANG[1], H.-Z. DU[1], Y.-W. HU[2], R.-Y. WANG[2], Z.-Q. TENG[1]
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**Abstract:** Neurons in the central nervous system (CNS) lose their intrinsic ability and fail to regenerate, but the underlying mechanisms are largely unknown. Polycomb group (PcG) proteins, which include PRC1 and PRC2 complexes, function as gene repressors and are involved in the regulation of many biological processes. Here we report that one of PRC1 components polycomb chromobox (Cbx) 2, 7 and 8 are novel regulators of axon growth and regeneration. Especially, Knockdown of Cbx7 in embryonic cortical neurons and adult dorsal root ganglion neurons enhances their axon growth ability. GATA4 and SOX11 are functional downstream targets of Cbx7 in adult sensory neurons for controlling axon regeneration. Moreover, knockdown of GATA4 or SOX11 in cultured DRG neurons prevents axon regeneration. Finally, downregulation of GATA4 and SOX11 could rescue the phenotype in the Cbx7 lacking DRG neurons. Taken together, these data suggest that Cbx7 is a key intrinsic regulator of axon growth and regeneration.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.01/X20

**Topic:** C.09. Brain Injury and Trauma

**Support:** Pilot Grant from the Craig H. Neilsen Foundation

New York State Empire Clinical Research Investigator Program

**Title:** Using the international spinal cord injury data sets to assess pain and thermosensory dysfunction in persons with chronic sci

**Authors: *O. BLOOM**[1,2], A. BEAUFORT[1], K. GIBBS[1,2], R. MONAHAN[1], A. STEIN[1,2]
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**Abstract:** In addition to alterations in motor function, traumatic spinal cord injury (SCI) may also cause changes in sensory, autonomic and other organ systems. These changes lead to medical consequences of SCI, such as pain and thermoregulatory dysfunction (Handrakis et al 2016, Bryce et al 2000). Unfortunately, pain symptoms are often refractory to treatment and along with pain medications, negatively impact quality of life (Finnerup et al 2012). Thermoregulatory dysfunction is less well studied, but is more common after higher level injuries and has also been shown to negatively impact abilities to perform activities of daily living (Handrakis et al 2016). Here, we characterized symptoms of pain and thermoregulatory dysfunction in adults with chronic SCI (≥ 1 year from initial injury), using the recently developed ISCoS/NINDS International SCI Pain Basic Data Set and Skin and Thermoregulation Function Basic Data Set (Widerstrom-Noga et al, Spinal Cord 2014, Karlsson et al, Spinal Cord 2012). To understand symptom variability in chronic SCI, data was obtained at 2 study visits at 6 months apart. Participants (N=31, 81% males) had an average time from SCI of 15.7±2.3 years (mean±SEM). Most injuries were above the T3 level (65%) and were AIS grade A. Participants' age was 55.0±2.8 (mean±SEM). Most participants reported pain symptoms within 7 days prior to a study visit (62% & 76%, visit 1 & 2). The most common pain subtypes were nociceptive (32%) and neuropathic (25%) (visit 1). Most participants (60%) received pharmacological pain management: Gabapentin and Pregabalin (43%), acetaminophen (28%), NSAIDS (20%) and opioids (9%). Most participants reported no interference of pain in daily activities, sleep or mood. Participants who described their worst pain problem as neuropathic reported significantly higher pain intensity. Most participants reported symptoms of altered thermoregulatory function within 3 months prior to a study visit, including sudomotor (64%) and hypo- or hyper-thermia (36%). A lower oral temperature obtained at the study visit was recorded for participants who

reported symptoms of altered thermoregulation function. In the future, these data will be used to explore potential correlations between thermosensory symptoms and biochemical changes.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.02/X21

**Topic:** C.09. Brain Injury and Trauma

**Support:** Ontario Institute for Regenerative Medicine

Wings for Life Fundation

Krembil Research Fundation

**Title:** Transplantation of human spinal oligodendrogenic neural progenitor cells enhances remyelination and functional recovery after traumatic spinal cord injury

**Authors: *M. KHAZAEI**[1], C. S. AHUJA[1,2,3], H. NAKASHIMA[1], N. NAGOSHI[1], J. WANG[1], M. G. FEHLINGS[1,2,3,4]
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**Abstract:** The harsh microenvironment generated after traumatic spinal cord injury (SCI) results in significant early necrotic and apoptotic cell death. Oligodendrocytes are particularly susceptible to the death after injury and are one of the first cell types which die resulting in widespread demyelination of both injured and spared axons. Cell replacement therapy with neural progenitor cells (NPCs) represents a promising therapeutic potential for SCI, however, the proportion of NPCs differentiated to oligodendrocytes after transplantation is very low. This is more dramatic for human cells. We have developed a unique method to bias the differentiation potential of tripotent human NPCs towards more oligodendrogenic fate (oligodendrogenic NPCs; oNPCs) while preserving their potential to generate neurons and astrocytes. In clinically-relevant models of rodent cervical and thoracic clip-contusion SCI, we studied the effects of these novel cells on lesional area, graft-host integration, and functional recovery. Transplanted oNPCs migrated rostrocaudally along spinal cord and differentiated into $NeuN^+$/$Tuj1^+$ neurons and $GFAP^+$ astrocytes as well as $Olig2^+$ immature and GST-$pi^+$ mature oligodendrocytes. Mature human oligodendrocytes also expressed MBP and integrated with rodent NF $200^+$ neuronal axons, indicating the potential of transplanted oNPCs to remyelinate host axons in both the cervical and thoracic spinal cord. Furthermore, oNPC transplanted rats demonstrated

significantly reduced lesion volumes and enhanced tissue preservation, white matter sparing and motor functional recovery. This work provides evidence that partially biasing NPCs towards an oligodendrogenic fate can improve recovery via remyelination and highlights oNPCs as a promising cell-based approach for CNS-repair.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.03/X22

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH Grant R01NS083983

Bryon Riesch Paralysis Foundation

**Title:** Corticospinal regeneration and optogenetic verification of synapse formation after combined gene therapy and stem cell grafting

**Authors: *N. JAYAPRAKASH**, N. KRUEGER, D. NOWAK, M. BLACKMORE
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**Abstract:** Axon regeneration following spinal cord injury (SCI) is critical to restore lost motor function. Axon regeneration is prevented both by cell-extrinsic cues, and by a cell-intrinsic failure of many injured neurons to express key pro-regenerative genes. To address neuron-intrinsic constraints, we have shown previously that forced overexpression of pro-regenerative transcription factors (TFs), including KLF6, promotes regeneration of the corticospinal tract (CST). However, KLF6-stimulated axons regenerate only through spared tissue and avoid lesion sites, suggesting that environmental cues continue to constrain successful regrowth. Hence, an integrated approach to simultaneously boost intrinsic capacity along with negating inhibitory extrinsic cues could be a promising strategy going forward. To address this, we adopted a system of stem cell grafting shown recently to provide a permissive environment for CST regeneration. Here we tested the ability of KLF6-stimulated axons to regenerate through the stem cells matrix and used an optogenetics-based approach to assess their ability to form functional synapses. Adult mice received midline cervical (C4-C5) 1mm deep wire-knife transections followed by transplantation of embryonic neural progenitor cells (NPCs) expressing Td-tomato. Cortical neurons were transduced with AAV-KLF6 or AAV-EBFP control, along with AAV-Channelrhodopsin (rAAV9/CamKII-ChR2-EYFP). Consistent with prior reports, by 8-10 weeks CST axons extended into the stem cell transplants. KLF6 expression significantly increased the

number and distance of CST axon growth into the graft and, importantly, into host tissue beyond the graft. Optical stimulation of CST axons evoked clear post synaptic responses in spinal neurons above the injury site in both controls and KLF6-treated animals. More than half of KLF6 animals, but no controls, also showed light-evoked responses as far as 1mm below the injury site, indicating successful synapse formation by regenerated CST axons. However, KLF6-treated animals showed no improvements over control in a horizontal ladder task, hinting that axon growth remained insufficient or potentially mistargeted. Ongoing experiments are testing additional genetic manipulations and alternative retrograde gene deliveries to further improve axon growth. Overall our results show that an integrated approach of elevating intrinsic growth capacity of injured CST axons, while also providing cut axons with growth-permissive stem cell grafts, is a promising approach to maximize axon regeneration after SCI.

**Disclosures: N. Jayaprakash:** None. **N. Krueger:** None. **D. Nowak:** None. **M. Blackmore:** None.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.04/X23

**Topic:** C.09. Brain Injury and Trauma

**Support:** NIH Grant P20GM103444

SC-SCIRF Grant 2014 I-02

**Title:** Rolipram-loaded polymeric nanoparticle reduces secondary injury after rat compression spinal cord injury

**Authors: *C. MACKS**[1], S.-J. GWAK[2], M. LYNN[3], J. LEE[2]
[1]Bioengineering, Clemson Univ., Duncan, SC; [2]Bioengineering, Clemson Univ., Clemson, SC; [3]Southeastern Neurosurgical and Spine Inst., Greenville Hosp. Syst., Greenville, SC

**Abstract:** Spinal cord injury (SCI) disrupts axonal pathways, leading to permanent motor, sensory, and autonomic dysfunction, as well as chronic pain, respiratory impairment, and loss of bowel or bladder control. Several complex pathophysiological mechanisms limit spontaneous recovery following SCI. The ultimate goal of our work is to develop cationic, amphiphilic polymeric nanoparticles for simultaneous delivery of drugs and therapeutic nucleic acids (TNAs) to promote axonal regeneration and plasticity. We recently reported the synthesis and characterization of poly (lactide-co-glycolide)-graft-polyethylenimine (PgP) and its ability to efficiently transfect TNAs in various neural cell lines and primary chick forebrain neurons *in vitro,* as well as in the normal rat spinal cord [1]. We have also shown that PgP can deliver

siRNA targeting RhoA, a critical signaling pathway activated by multiple extracellular inhibitors of axonal regeneration, to the injured spinal cord and maintain RhoA knockdown for up to 4 weeks post-injection, reduce astrogliosis and cavitation, and increase axonal regeneration [2]. In this study, we investigated the ability of amphiphilic copolymers (PgP) carrying rolipram (Rm) to restore cAMP level and reduce secondary injury following SCI. Rm was loaded in the PgP solution by solvent evaporation method and the amount of Rm loaded in PgP was measured by HPLC. Compression injuries were created by application of a micro vascular clip (70 gm pressure, Roboz Surgical Store) for 10 minutes at the T9 spinal cord in rats. To evaluate Rm loaded PgP (Rm-PgP) effect on cAMP level, 10 µl Rm-PgP (10 µg Rm) was injected at the injury lesion immediately after SCI. At 1, 2, 3, and 7 days post-injury, rats were sacrificed and cAMP level was evaluated using ELISA Assay. TUNEL and ED1 staining was also performed to evaluate Rm-PgP effect on apoptosis and inflammatory response. We found that single injection of Rm-PgP (10 µg Rm) in rat spinal cord lesion site restores cAMP levels to sham animal level up to 2 days and reduces apoptosis and the inflammatory response in rat SCI compression injury. In conclusion, we demonstrated that cationic amphiphilic polymeric nanoparticle, PgP is a promising carrier for Rm to for the SCI repair. In the future, we will assess the expression of key inflammatory cytokines and also the functional recovery after Rm-PgP treatment in rat SCI model.
Reference: 1. Gwak *et al*., (2016) *Acta Biomater.* 35:98-108. 2. Gwak et al., Biomaterials, 121(2017), 155-166.

**Disclosures: C. Macks:** None. **S. Gwak:** None. **M. Lynn:** None. **J. Lee:** None.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 578.05/X24

**Topic:** C.09. Brain Injury and Trauma

**Support:** ISRT

**Title:** Combined ChIP and RNAseq analysis identifies a Cited2-H3K27/H3K9ac-dependent regenerative network at the core of opposed axonal regenerative ability between the injured peripheral and central nervous system

**Authors: *E. MCLACHLAN**[1], I. PALMISANO[1], M. C. DANZI[2], L. ZHOU[4], G. KONG[4], A. HERVERA ABAD[1], T. H. HUTSON[1], F. DE VIRGILIIS[1], J. L. BIXBY[5], V. LEMMON[3], S. DI GIOVANNI[1]
[1]Imperial Col. London, London, United Kingdom; [3]Neurolog. Surgery, [2]Univ. of Miami, Miami, FL; [4]Neuroregeneration and Repair, Hertie Inst. For Clin. Brain Res., Tuebingen, Germany; [5]Miami Proj to Cure Paralysis, Univ. Miami, Miller Sch. Med., Miami, FL

**Abstract:** Regenerative failure is responsible for lack of functional recovery following spinal cord injury (SCI). To date, there are no effective treatments that overcome regenerative failure after SCI. Clarification of the molecular mechanisms underlying regenerative failure therefore remains a priority. In contrast to central nervous system axons, axons in the peripheral nervous system are able to mount a regenerative response. A key model to assess differences in this regenerative capacity are the L4-L6 dorsal root ganglia (DRG). These neurons extend one axonal branch towards the periphery that regenerates after injury and one branch towards the dorsal columns of the spinal cord that does not. In contrast to central branch injury, following injury to the peripheral branch, the neuron cell body is able to mount a profound transcriptional response that drives the expression of key genes that direct axonal outgrowth and regeneration. Given that histone acetylation facilitates gene expression, we hypothesised that injury-induced signalling converges on the epigenome to enhance histone acetylation and regenerative gene expression after sciatic nerve but not central branch injury. To address this hypothesis, we performed a systematic investigation, integrating RNA and ChIPseq for H3K9ac and H3K27ac after sciatic nerve (SNA) vs dorsal column axotomy (DCA). We identified that *(i)* increased histone acetylation is associated with an enhanced expression of key transcriptional and signalling dependent networks after SNA, which are not found following DCA. *(ii)* Bioinformatics analysis revealed that central to several signalling networks, is Cited2, an interacting transactivator of the histone acetyltransferases p300 and CBP. We found that Cited2 recruitment enhances the activation of p300 and CBP to promote increased H3K27ac and H3K9ac whilst also bridging upstream regenerative signalling through recruiting various transcription factors to the acetylated promoters. Next, (iii) we investigated FDA approved compounds that would trigger the expression of Cited2, histone acetylation and the Cited2 signalling network as a means to exploit the regenerative translational potential of Cited2 network modulation. We identified the FDA approved HDAC inhibitor Panobinostat, which we found enhances H3K27ac and H3K9ac after SCI, driving the expression of Cited2 and a number of members of the Cited2 associated transcriptional/signalling dependent networks. Lastly, we demonstrated that systemic administration of Panobinostat can promote axonal regeneration, sprouting and functional recovery following a thoracic spinal cord dorsal hemisection.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.09. Brain Injury and Trauma

**Support:** the Krembil Foundation, Phillip and Peggy DeZwirek

the University of Toronto Spine Program

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**Title:** Neural stem cell mediated recovery is enhanced by chondroitinase ABC pretreatment in chronic cervical spinal cord injury

**Authors: *H. SUZUKI**[1,2], A. G. CHRISTOPHER[2], R. P. SALEWSKI[2], L. LI[2], K. SATKUNENDRARAJAH[3], N. NAGOSHI[4], T. TAGUCHI[5], M. G. FEHLINGS[6]
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**Abstract:** [Introduction]Traumatic spinal cord injury (SCI) interrupts sensory and motor tracts resulting in severe, lifelong functional impairments for patients. Despite the need, one of the greatest challenges in developing an effective therapy for chronic SCI has been the inhibitory microenvironment of the injured spinal cord. Aspects of chronic spinal cord injury (SCI) environment such as formation of glial scars and Chondroitin sulfate proteoglycans (CSPGs) act as barrier to repair and regeneration. To address this environment Chondroitinase ABC (ChABC) is used to breakdown CSPGs and facilitate a permissive environment for the transplantation neural stem cells (NSCs) derived from induced pluripotent stem (iPS) cell in cervical SCI mouse model. [Materials and Methods]Six weeks after cervical SCI we had continuously injected ChABC into subarachnoid space for a week using an osmotic pump. After which NSCs derived from iPS cells (iPSC-NSC) are intraspinally transplanted rostral and caudal to the injury site. We examined neurobehavioral tests in BMS score, grip strength meter, inclining test and CatWalk analysis. In addition 8 weeks after transplantation, we performed histological and electrophysiological analysis. [Results]The administration of ChABC reduces elements of the glial scar and result in greater iPSC-NSC survival and engraftment. Fig.1 is the Schematic representation of experimental design. The combinatory treatment of iPSC-NSCs and ChABC significantly promoted forelimbs neurobehavioral recovery in grip strength meter and CatWalk analysis. The iPSC-NSCs integrate into the chronically injured spinal cord (Fig.2) and differentiated into neurons, astrocyte and oligodendrocyte without evidence of tumorigenesis. There is evidence that exogenous cells that differentiate to oligodendrocytes contributing for remyelination, while other exogenous cells become motor neurons. These motor neuron make new functional synaptic connections between host and grafted neurons via glutamate and acetylcholine receptors in patch clump analysis and electron microscope. [Conclusion]By altering the glial scar in cervical SCI prior to delivering iPSC-NSC, we demonstrate that even the chronic injury environment remained therapeutic relevant for iPSC-based treatments. This is the first report that we obtained the functional recovery in chronic SCI with solid scientific evidence. This results suggested that we can expect a good results in clinical trials in the patients with chronic SCI.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.09. Brain Injury and Trauma

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Projeto Estratégico – LA 26 – 2011-2012 and Projeto Estratégico – LA 26 – 2013-2014

PEst-C/SAU/LA0026/2011; PEst-C/SAU/LA0026/2013

**Title:** The beneficial effect of systemic IL-4 treatment after spinal cord injury

**Authors: *N. SILVA**, R. LIMA, S. MONTEIRO, E. GOMES, R. SILVA, MSc, F. TEIXEIRA, M. MORAIS, N. SOUSA, A. SALGADO
Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal

**Abstract:** Traumatic spinal cord injury (SCI) provokes dramatic disability and dysfunction in the motor, sensory and autonomic systems. The potent inflammatory reaction that occurs after SCI is strongly associated with further tissue damage. As such, immunomodulatory strategies have been developed aiming at reducing inflammation but also at shaping the immune response in order to protect spared neural tissue, to repair and to promote regeneration. One of those promising strategies is the intraspinal administration of the cytokine IL-4 that was shown to promote a phenotype on specific immune cells associated with neuroprotection and repair.
In this work we evaluated if a systemic delivery of IL-4 for a 7-days period was also capable to

promote neuroprotection after SCI by analyzing different neural cells populations and motor recovery.
IL-4 treatment promoted an elevation of the anti-inflammatory cytokine IL-10 in the serum both at 24h and 7 days after injury. Locally, treatment with IL-4 led to a reduction on cells expressing markers associated with inflammation CD11b/c and iNOS. Importantly, IL-4 treatment increased the neuronal markers βIII-tubulin and NeuN, and the oligodendrocyte marker O4, suggesting a neuroprotective effect.
Moreover, 100% of the animals treated with IL-4 were able to recovery a clinically relevant feature of locomotion (weight support) against only 33% of saline treated animals.
Overall, these results show that systemic administration of IL-4 positively impacts different aspects of spinal cord injury, creating a more favorable environment for recovery to take place.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.08/X27

**Topic:** C.09. Brain Injury and Trauma

**Support:** VA

NIH

**Title:** Characterization of forelimb or hindlimb corticospinal tract regeneration into neural progenitor cell graft after upper cervical spinal cord injury

**Authors: D. DANIEL KULINICH**[1], J. CONNER[1], D. GIBBS[1], M. TUSZYNSKI[1,2], *P. P. LU[1,3]
[1]UCSD, La Jolla, CA; [2]VA San Diego Healthcare Syst., San Diego, CA; [3]VA-San Diego Healthcare Syst., San Diego, CA

**Abstract:** Recently, our lab demonstrated robust regeneration of corticospinal tract (CST) axons into neural progenitor cell (NPC) grafts placed in mid-cervical spinal cord injury (SCI) sites (Kadoya et al., Nat Med 2016). We hypothesized that forelimb and hindlimb CST axons regenerate into NPC grafts placed in the mid-cervical SCI sites, since both systems are axotomized. To test this hypothesis, we grafted rat embryonic day 14 spinal cord-derived NPCs expressing green florescent protein (GFP) into a C3 dorsal column lesion site, and then injected AAV-expressing red florescent protein (RFP) into only the forelimb or hindlimb regions of the motor cortex. To our surprise, we found that few forelimb CST axons regenerate into NPC grafts

one month later, whereas hindlimb CST axons robustly regenerate into these grafts at C3. To verify this unexpected result, we utilized highly specific, Cre-mediated tracing of CST axons projecting to either C6 or L3. We first injected AAV9 viral vectors expressing Cre-dependent Td-tomato into forelimb and hindlimb motor cortex, and then injected AAV8-Cre (which undergoes retrograde transport) into C6 (N=4 animals) or L3 (N=4 different animals), respectively. This approach specifically enabled Td-tomato expression exclusively in forelimb- or hindlimb-projecting CST axons. Preliminary studies confirm rare regeneration of forelimb CST axons into NPC grafts, whereas regeneration of hindlimb CST axons is robust. An examination of mechanisms underlying this selective regeneration is underway, and will contribute to efforts to optimize corticospinal regeneration after SCI.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Topic:** C.09. Brain Injury and Trauma

**Support:** GAUK 414216

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GACR P304/12/G069

GACR 17-11140S

**Title:** Structural dynamics in the injured spinal cord

**Authors: *B. SVOBODOVA**[1], O. ZELENKA[2], P. JENDELOVA[1]
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**Abstract:** Spinal cord injury (SCI) is a devastating trauma that seriously impacts a patient's physical as well as psychological health. Elucidation of the dynamic process of degeneration of injured axons is very important for the development of therapeutic strategies for the treatment and investigating the therapeutic time window. In past, the pathological changes after SCI were mostly exanimated in isolated tissue by immunohistochemistry and anterograde tracing which provide information about the pathology at the given time points in different animals. We used a method for time-lapse observation of injured axons in living mice. This approach enables optical imaging of the dorsal spinal cord for a prolonged period of time up to several months without the

need of repeated surgical procedures. Briefly, we expose the vertebrae T11-L1 and construct custom-made chamber around exposed vertebral column using cyanoacrylate. A laminectomy of Th12 vertebra was carried out with dental drill and compression spinal cord injury was performed. Then a thin layer of silicone elastomer was applied to the surface of spinal cord and 3 mm round coverslip was positioned over the centre of the imaging window. Cyanoacrylate was used to seal the chamber. We investigated the role of endogenous individual cell types (neurons, reactive astrocytes, microglial cells and oligodendrocytes) in secondary injury processes using several transgenic mice, which express fluorescence in different cells. The morphological changes of cells were explored by two-photon microscopy which can observe deep structures of tissues including spinal cord axons. We observed fragmentation of axons and tracked retraction and degeneration of injured axons. We saw infiltration of microglial cells which exhibit changes in morphology, motility and phagocytic activity. This method may help clarification of pathophysiology of SCI and development of therapeutic modules for the treatment.

**Disclosures: B. Svobodova:** None. **O. Zelenka:** None. **P. Jendelova:** None.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** C.09. Brain Injury and Trauma

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NSF Career 1150125 (RJG)

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**Title:** Nanosphere delivery of neurotrophins mobilizes oligodendrocyte progenitor cells after spinal cord injury

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**Abstract:** The glial scar formed after a spinal cord injury acts as a physical and chemical barrier to nerve regeneration and functional recovery. The scar is composed of chondroitin sulfate proteoglycans (CSPGs), which strongly inhibit axonal regrowth. Previous work by Osterhout and

Siebert suggests that CSPGs may also inhibit remyelination, by influencing the behavior of endogenous oligodendrocyte progenitor cells (OPCs) near the injury. Migration and differentiation of OPCs is inhibited the presence of CSPGs. When OPCs come in contact with CSPG deposits, their processes retract and turn away. Treatment with chondroitinase ABC (cABC), which removes the glycosaminoglycan (GAG) side chains from CSPGs, creates a permissive environment for both axonal sprouting and OPC migration *in vivo*. Moreover, *in vitro* screening of neurotrophins that stimulate axonal growth revealed that several of these factors can also promote OPC migration and differentiation in the presence of CSPGs. Using a nanoparticle delivery system, we examined the effects of neurotrophins GDNF and NT3 on endogenous OPCs *in vivo* after spinal contusion injury. The total number of OPCs surrounding the lesion site was significantly increased after growth factor treatments as compared to control. This is due to enhanced migration of OPCs and is evident at early times post injury in the presence of growth factors. However, the OPCs accumulated at the immediate edges of the lesion and did not readily move into the lesion center. Axonal sprouting is also evident at earlier times with neurotrophin treatment, as compared to control. Collectively, these data demonstrate that nanospheres can effectively deliver neurotrophins to a spinal cord lesion, which stimulates early axonal sprouting as well as rapid OPC migration to the lesion site. This treatment may not only speed axonal regeneration, but stimulate remyelination and maximize recovery of function after SCI.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 578.11/X30

**Topic:** C.09. Brain Injury and Trauma

**Title:** Longitudinal assessment of cerebrovascular dysfunction after Traumatic Brain Injury (TBI) using functional near-infrared spectroscopy (FNIRS)

**Authors: *M. A. SANGOBOWALE**[1], F. AMYOT[2], R. R. DIAZ-ARRASTIA[3], H. AYAZ[5], Y. REDDY[6], E. SILVERMAN[4], T. MEREDITH-DULIBA[4], M. MOYER[4], D. SANDSMARK[4]
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**Abstract:** Traumatic cerebrovascular injury (TCVI) is common after TBI is responsible for a significant portion of TBI-related disability. Development of therapies targeting TCVI will require reliable and inexpensive biomarkers to measure vascular dysfunction and document

target engagement. Cerebrovascular reactivity (CVR) is impaired following TBI and methods which reliably and non-invasively measure CVR are available, making CVR an attractive candidate predictive and pharmacodynamic biomarker for TCVI-directed therapies. This study was designed to assess CVR longitudinally after TBI in humans using functional Near Infrared Spectroscopy (fNIRS) from the acute to the subacute and chronic stages. We also studied the effect of treatment with a phosphodiesterase 5 inhibitor, sildenafil citrate, in order to assess the utility of fNIRS as a pharmacodynamic biomarker in future clinical trials. 7 participants with complicated mild TBI were studied in the acute (within 72 after injury) and subacute (14 days after injury) stages, in addition to 4 age- matched healthy controls (HC), who were studied once. CVR was assessed by measuring the changes in oxygenated hemoglobin (ΔHbO) and deoxygenated hemoglobin (ΔHbR) concentration produced by mild hypercapnia (5% $CO_2$). The change in CVR before and one hour after the administration of single dose of sildenafil citrate (60 mg orally) was also assessed. Mean (± SEM) CVR was lower in TBI patients compared to HC (HC: 0.100 ± 0.028%/mmHg; and TBI: CVR 0.057 ± 0.011%/mmHg, p=0.22). Sildenafil administration did not result in an increase in CVR in HC (t=0.1463 df=3, p=.89) whereas TBI patients show a clear potentiation of the CVR response (p<.08). These findings support the hypothesis that vascular injury represents a distinct endophenotype following TBI and PDE5 inhibition as a potential therapy for TCVI.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Program#/Poster#:** 578.12/X31

**Topic:** C.09. Brain Injury and Trauma

**Support:** BPi

Fondation d'Entreprise Michelin

**Title:** Thrombospondin repeat-derived peptide (NX210) induces axonal regrowth and functional recovery in a spinal cord injury rat model

**Authors: *S. GOBRON**[1], N. DELÉTAGE[1], L. SAKKA[2]
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**Abstract:** In adult mammals the repair capacities of the central nervous system (CNS) white matter are poor. It follows that patients with spinal cord injury (SCI) usually evolve toward permanent disability. This can be partly explained by the action of components of the extracellular matrix (ECM). NX210 is a peptide derived from SCO-spondin, a protein of the ECM early expressed during the development of vertebrate CNS. SCO-spondin is a multi-domain protein containing several consensus domains, especially domains called thrombospondin type 1 repeats (TSRs), which are potential sites of protein-protein interactions. These TSR domains are shared by developmental proteins of the CNS and involved in crucial biological functions including axonal growth and pathfinding, and synaptogenesis. NX210 properties to increase neuronal survival, neurite outgrowth and fasciculation, and to inhibit oxidative stress and cell death have suggested its potential interest as a neuroprotective agent after SCI. The mechanism of its multi-functional activity was previously demonstrated to be partly related the cell surface receptor β1 integrin. We firstly assessed regenerative properties of NX210 in a rat model of SCI using a section of dorsal funiculi. Ten days after injury a sharp fiber regrowth in 4/5 NX210-treated rats *versus* a minor regrowth in 1/5 control rats were observed using neurofilament immunostaining. We secondly assessed functional recovery after treatment with NX210 in a contusion model of rat SCI using the NYU impactor and using the BBB score and reflex testing. Single intrathecal injection of NX210 (100 and 30 µg/kg) determined functional recovery with a dose-dependent effect as compared to the vehicle. BBB score was significantly higher in the NX210-group from D14 to the end of the study (D28); 60% (6/8) and 25% (2/8) of rats had a BBB score≥14 with a 100µg/kg and 30µg/kg of NX210 respectively compared to 17% (1/6) with vehicle. Reflex testing included paw placement and toe-spread reflexes. At D28 post-injury normal response was observed in 50% (4/8) and 38% (3/8) of rats to paw placement testing, and in 100% (8/8) and 50% (4/8) of rats to toe-spread reflex after 100µg/kg and 30µg/kg NX210 treatment, respectively. By contrast normal response was observed in only 33% (2/6) of rats to both reflex testing in the vehicle group. NX210 safety has been subsequently demonstrated in rats and dogs. A first-in man clinical trial on acute SCI is expected to start soon.

**Disclosures: S. Gobron:** A. Employment/Salary (full or part-time):; Neuronax. **N. Delétage:** A. Employment/Salary (full or part-time):; Neuronax. **L. Sakka:** 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.; Neuronax.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.13/X32

**Topic:** C.09. Brain Injury and Trauma

**Support:** Wings for Life foundation (RES0025323)

**Title:** TLR4 activation restores window of opportunity for rehabilitative training in rats with chronic cervical spinal cord injury

**Authors: *A. TORRES ESPÍN**[1], J. FORERO[2], K. K. FENRICH[2], A. M. LUCAS-OSMA[2], E. SCHMIDT[2], D. J. BENNETT[2], P. G. POPOVICH[3], K. FOUAD[2]
[1]Physical Therapy, [2]Univ. of Alberta, Edmonton, AB, Canada; [3]Dept. of Neurosci., The Ohio State Univ., Columbus, OH

**Abstract:** Rehabilitative training is currently one of the most successful therapies to promote recovery after spinal cord injury (SCI). A significant challenge for rehabilitative training following SCI is the timing of the training onset. Starting training in the early stages following injury is much more effective in promoting recovery than starting it weeks thereafter. The challenge is that SCIs are often accompanied by multiple traumas, making rehabilitative training of motor tasks early after injury difficult. Here we investigated the possibility to reopen the window of opportunity for effective motor training in rats with chronic SCI by reintroducing inflammation with systemic lipopolysaccaride (LPS), administration. 8 weeks after a cervical spinal cord injury, animals were trained in a grasping task for 2 months in combination with LPS or Saline injection. 3 different experiments were performed using 3 training intensities. We found that rehabilitative training efficacy in a single pellet reaching and grasping task can be increased by systemic LPS administration. A loss-of-function experiment using a TLR4 antagonist prevented the LPS-increased training efficacy, suggesting that this effect is mediated by TLR4 activation. However, a beneficial effect was achieved only when high-intensive training was provided. Importantly, in contrast to training alone, the combination of systemic LPS and high-intensive training promoted functional improvement by increasing reparative rather than compensatory mechanisms, which was observed in functional and anatomical outcome measures. Thus, we propose that neuroinflammation plays a key role in determining the window of opportunity for rehabilitative training and reparative neuroplasticity after SCI. Modulation of post SCI neuroinflammation could thus become an approach to reopen the window for effective rehabilitative training even weeks after SCI.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** Department of Defense #SC140033

Kentucky Spinal Cord and Head Injury Research Trust #14-5

Paralyzed Veterans of America

**Title:** Effects of activity-based training on upper and lower urinary tract function following spinal cord injury

**Authors: *L. R. MONTGOMERY**, C. YANG, J. E. ARMSTRONG, C. H. HUBSCHER
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**Abstract:** Urinary complications are common following spinal cord injury (SCI) which severely impacts quality of life. Improving urinary function is one of the top priorities identified by SCI individuals. Our group has previously shown that activity-based training in human SCI research participants as well as in a rodent SCI model leads to improvements in urinary function, although the mechanisms are unknown. We have shown using metabolic cages in our animal studies that SCI-induced polyuria (increase in urine output) benefits from both locomotor training (LT; quadrupedal stepping on a treadmill with all limbs) as well as forelimb training (FT; non-weight-bearing exercise on a treadmill without hind-limb engagement). In this study, a cohort of adult male Wistar rats was given a T8 spinal contusion (215 kD) and randomly assigned to trained (daily for one hour/seven days a week beginning two weeks post-SCI) and non-trained groups. To elucidate possible mechanisms that may be driving the training effect on polyuria, vasopressin (AVP) levels were periodically measured. Terminal study assessments after two months of training targeted both the lower (terminal cystometrogram with external urethral sphincter EMG) and upper (Western blot analysis of kidney tissue) urinary tract. As we have shown previously, 24 hour urine output increased after SCI, with non-trained animal groups being higher by the end of training. AVP levels in all animals were decreased post-SCI and decreased further with training. Western blot analysis of kidney tissue however, showed differences in vasopressin (V2) receptor numbers between the groups, with LT/FT animals showing similar receptor levels to sham controls and non-trained animals showing significantly decreased receptor levels. Kidney aquaporin 2 (AQ2) receptor numbers were also significantly decreased in non-trained animals compared to sham and LT/FT animals. All animals exhibited a decrease in the inter-burst period during voids compared to sham, although the inter-burst duration was shorter for the non-trained compared to LT/FT animals, indicating increased sphincter activity in the non-trained SCI animals. Thus, activity-based training has a positive effect on urinary tract function in SCI animals. The decreased urine output is thought to occur in part to the ability of training to limit the SCI-induced loss of V2 and AQ2 receptors in the kidney. In addition, training appears to have a beneficial effect on external urethral sphincter activity allowing for longer inter-burst intervals during voiding compared to non-trained animals.

**Disclosures: L.R. Montgomery:** None. **C. Yang:** None. **J.E. Armstrong:** None. **C.H. Hubscher:** None.

**Poster**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** Department of Defense #SC140033

Kentucky Spinal Cord and Head Injury Research Trust #14-5

**Title:** Locomotor training alters penile reflex responses in a rat model of spinal cord injury

**Authors: *C. J. STEADMAN**, R. F. HOEY, L. R. MONTGOMERY, C. H. HUBSCHER
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**Abstract:** Sexual dysfunction is rated by the spinal cord injury (SCI) population as a top priority issue that significantly impacts quality of life. In SCI males, erectile function, ejaculation, and fertility are severely impaired. The current examination of erectile function in a clinically relevant male rat contusion model was prompted by a recent report showing improvements in sexual function by male SCI research participants undergoing 60-minute daily sessions of locomotor training (LT) on a treadmill (harnessed with weight support). The present study utilized a behavioral reflex measure of penile dorsiflexion and terminal electromyography (EMG) assessments of the bulbospongiosus muscle (BSM) in an *in vivo* anesthetized preparation using uni- and bi-lateral electrical stimulation of the dorsal nerve of the penis (DNP) to examine any potential effects of daily one-hour LT (quadrupedal-trained) on erectile function. Other animal groups examined included a non-weight bearing exercise SCI group (forelimb-trained), a non-trained SCI control group harnessed for an equivalent one-hour time frame, and a home-cage injured control group that received limited handling. By the end of 8 weeks of daily task-specific training, latency to reach dorsiflexion reflex in the LT rats was shifted closer toward normal from pre-training values which was not found for the SCI groups that received either no training or forelimb-only daily training. Maximum and average amplitude of BSM response to DNP stimulation was significantly higher in LT animals as compared with forelimb-only trained animals and non-trained controls, and BSM response latency in LT animals was significantly decreased compared to non-trained controls. Initial histological examination of the lesion epicenter revealed no correlates associated with any of the changes in penile responses. Initial decreases in latency of dorsiflexion reflex after SCI is likely due to disruption of bilateral, descending reticulo-spinal projections in the spinal cord. Increases in dorsiflexion reflex latency after LT may indicate the strengthening of some residual connections through the lesion as the small but significant change could be indicative of a partial return of supra-spinal depression of this DNP-pudendal motoneuron reflex. An alteration in BSM response latency and amplitude after LT indicates an increase in the excitability of the circuitry below the level of the lesion. A

lack of effect with exercise alone suggests that sensory input through task-specific stepping and/or hind-limb loading may increase the excitability of the lumbosacral cord.

**Disclosures: C.J. Steadman:** None. **R.F. Hoey:** None. **L.R. Montgomery:** None. **C.H. Hubscher:** None.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Program#/Poster#:** 578.16/Y2

**Topic:** C.09. Brain Injury and Trauma

**Support:** Department of Defense #SC140033

Kentucky Spinal Cord and Head Injury Research Trust #14-5

**Title:** Task-specific training effects on at-level allodynia in a rat model of spinal cord injury

**Authors: *J. GUMBEL**, J. D. FELL, C. H. HUBSCHER
Anatom. sciences and Neurobio., Univ. of Louisville, Louisville, KY

**Abstract:** At-level allodynia is a common complaint among the spinal cord injury (SCI) population as a quality of life issue. This type of hypersensitivity to touching the dermatomal zone just rostral to the level of injury also develops in male rats receiving a severe T8 spinal cord contusion (C.H. Hubscher and R.D. Johnson, J Neurotrauma, 16(6):533-541, 1999). Locomotor training (LT) after SCI is used as a rehabilitative therapy to improve quality of life factors such as bladder, bowel, and sexual function, but also has benefits of reducing SCI-related pain (P.J. Ward et al., J Neurotrauma, 31:819-833, 2014). In the present study, contusion injuries were made to test for the efficacy of activity-based training without hind-limb engagement, as such training was found to improve urinary tract function in addition to LT (Hubscher et al., AJP Renal, 310:F1258-F1269,2016). Using Semmes-Weinstein monofilaments at the level of injury, rats were scored weekly post-SCI using a 10-point scale for allodynia based upon pain-like behavioral responses previously defined (B.J. Hall et al., J Pain, 11(9):864-876, 2010). Rats in the current study underwent 60 minutes of activity-based training on a treadmill daily for eight weeks beginning at two-week's post-injury. Both the quadrupedal LT-trained and forelimb-only trained (non-weight bearing) groups scored significantly lower (indicative of reduced sensitivity) than the non-trained/home-cage group, starting around four weeks after the initiation of activity-based training. This result suggests that multiple forms of exercise/rehabilitative therapies, including LT, could benefit the SCI population with SCI-related pain. Mechanisms underlying these effects are currently unknown but likely include multiple factors that modulate interactions amongst neuronal, endocrine and immune systems.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** Craig H. Neilsen Foundation

Departemtn of Veterans Affairs Merit Award

New York State Department of Health SCIRB

**Title:** AAV vector mediated delivery of NG2 function neutralizing antibody and neurotrophin NT-3 improves synaptic transmission, locomotion and urinary tract function after mild spinal cord injury (SCI) but not in severe injury model of adult rats

**Authors: *H. A. PETROSYAN**[1,2], M. LENZI[2], S. GUMUDAVELLI[1], R. MUFFALETTO[1], K. LASEK[1], V. ALESSI[1], N. PHAGU[1], J. LEVINE[1], W. COLLINS[1], V. ARVANIAN[1,2]
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**Abstract:** The role of NG2 proteoglycan in the function of spinal circuitry is dual. NG2 is considered one of the key inhibitory factors restricting axonal growth and blocking axonal conduction following SCI. Some studies, however, indicate the importance of NG2 in formation of synaptic contacts. We hypothesized that the optimal treatment would be neutralization of inhibitory function of NG2 without its physical removal. For prolonged delivery of NG2 function neutralizing antibody we (together with Dr. Levine) developed a novel gene therapy tool: an AAV construct expressing recombinant single chain variable fragment (scFv) anti-NG2 antibody (AAV-NG2Ab). We examined effects of AAV-NG2Ab alone or in combination with AAV-NT3 in adult rats with thoracic T10 contusion injuries. Single cell electrophysiology was used to evaluate synaptic function. Bladder function was assessed during the survival period and terminal cystometry with simultaneous acquisition of external urethral sphincter EMG activity. Battery of behavioral tests was used to evaluate sensory and motor function recovery. AAV-NG2Ab treated groups demonstrated significant improvements in synaptic transmission, locomotor and bladder function in mild contusion injury model. The best recovery was observed in the group that received AAV-NG2Ab combined with AAV-NT3. In severe spinal cord injury model, however, AAV-NG2Ab did not induce significant improvements of function. We hypothesize that AAV-NG2Ab mediated attenuation of the NG2-induced block of axonal conduction in spared fibers spanning the injury epicenter maybe a critical mechanism underlying

beneficial effects of AAV-NG2Ab in the mild contusion model. In the case of severe injuries, however, the portion of the anatomically intact fibers spanning injury epicenter is limited and improvement of conduction through these few fibers, if any, cannot mediate better recovery. In order to understand better how NG2 may block axonal conduction and how AAV-NG2Ab may attenuate this inhibitory action of NG2 in the model of mild contusion, we have also examined the presence of NG2 in the vicinity of the nodes of Ranvier. Comparison of sections double stained for Caspr (to identify the nodes) and NG2 revealed a significantly higher number of nodes with close contacts with NG2-positive processes in injured animals. Examination of sections from AAV-NG2Ab treated animals is on-going, with results to be analyzed. The results will demonstrate if treatment with AAV-NG2-Ab will result in a decreased number of nodes with close proximity to NG2 positive processes or will attenuate NG2 function to block conduction without physically removing NG2 processes from the nodes.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Topic:** C.09. Brain Injury and Trauma

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W.M. Keck Foundation

**Title:** Non-invasive bioluminescence-activated optogenetic stimulation for rehabilitation following traumatic spinal cord injury

**Authors: *E. D. PETERSEN**, A. PAL, E. D. SHARKEY, J. R. ZENCHAK, A. J. PENA, P. OTERO, W. E. MEDENDORP, T. BROWN, B. PALMATEER, M. PRAKASH, U. HOCHGESCHWENDER
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**Abstract:** Current methods used to treat spinal cord injury with stimulation include transcutaneous electrical stimulation at the muscle and intrathecal electrical stimulation. Both of these methods can offer quality of life improvements for patients but are not entirely effective at restoring function. Optogenetic stimulation provides a method of stimulation that can be done at the neuronal level and does not result in the rapid fatigue which occurs as a result of electrical

stimulation at the neuronal level. The current drawback of traditional optogenetic stimulation is the invasiveness, as this approach requires an implanted light source. Here we describe a method for the activation of optogenetic constructs in a spinal cord injury model where genetically expressed opsins are activated by a light-producing luciferase that is fused to the opsin (luminopsin). Using this approach, cells expressing the construct are activated by the injection of the luciferase substrate allowing for light to be produced by the luciferase. The light sensitive opsin then opens, allowing for non-selective cation flow, exciting the neuron. We found activation of neurons below the level of injury to significantly improve locomotor recovery following spinal cord injury. This new approach can be applied to distinct populations of endogenous neurons. This is the first example of non-invasive activation of an optogenetic component as a potential therapy following spinal cord injury. This approach can likely be applied to other neurological conditions and may prove to be a useful tool in future therapies for patients.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** Craig H Neilsen Foundation

Rick Hansen Foundation

**Title:** Ketogenic diet improves mitochondrial function and reduces inflammation after spinal cord injury in rodents

**Authors: *W. TETZLAFF**[1], K. KOLEHMAINEN[2], O. SEIRA[1], W. PLUNET[1], R. BOUSHEL[3]
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**Abstract:** Introduction: Recent findings in our lab demonstrated that the ketogenic diet (KD) may be a promising SCI treatment as rats fed with KD acutely after cervical SCI showed behavioural improvement in forelimb function. KD is a high fat, low carbohydrate diet and is clinically used in drug-resistant epilepsy in children. Here we tested the hypothesis that KD improves recovery by reducing inflammation and improving mitochondrial function. Methods:

Male Sprague-Dawley rats had a C5 hemicontusion injury and were subsequently fed with a standard diet (SD) or KD beginning 4 hours after injury. Spinal cords were then extracted and assayed for cytokine levels and mitochondrial function. At 48 hours post-injury, pro-inflammatory (TNFα, IL-6, and IL-1β) and anti-inflammatory (IL-10 and IL-4) cytokine production was assessed using the MesoScale Diagnostics (MSD) multi-spot assay system. At 7 days post-injury, mitochondrial function was assayed using the Oroboros Oxygraph-2K high-resolution respirometry system. Results: Pro-inflammatory cytokine production was upregulated after SCI while anti-inflammatory cytokine production was reduced. Although KD did not change pro-inflammatory cytokine levels at the 48hr time point, it did significantly increase the anti-inflammatory cytokine IL-10 suggesting that KD does have anti-inflammatory effects. Mitochondrial function was reduced after SCI. However, administration of KD partially rescued function of Complex II after SCI and showed an overall improvement in mitochondrial function that approached non-injury levels. Conclusions: KD can improve recovery from spinal cord injury, in part by promoting production of anti-inflammatory cytokines and rescuing mitochondrial function.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 578.20/Y6

**Topic:** C.09. Brain Injury and Trauma

**Support:** RO1NS083983

**Title:** Retrograde AAV delivery of pro-regenerative genes for spinal cord injury

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**Abstract:** Axon regeneration in the central nervous system is limited in part by neuron-intrinsic patterns of gene expression that preclude effective axon extension. Thus, some form of gene manipulation in injured neurons may be needed to restore connectivity after CNS injury. Indeed, we and others have achieved improvements in axon regeneration by using viral vectors to manipulate gene expression in injured neurons after spinal injury. The current practice of direct injection to brain targets is likely impractical for clinical translation, however, due to the size and wide distribution of relevant supraspinal populations, and concerns about unwanted growth in off-target neurons. A potential solution is retrograde delivery of viral vectors. In principle, focal injection of retrograde vectors to the spinal cord could transduce broadly distributed cell bodies

that project axons to the vicinity of the injury, while avoiding transduction of non-supraspinal populations. Prior retrograde vectors have shown insufficient transduction efficiency, however, particularly in corticospinal tract (CST) neurons. A newly developed vector, termed rAAV-Retro, (Tervo et al., 2016) shows promising retrograde properties. Here we tested the timecourse and efficiency of rAAV-Retro in a mouse model of spinal cord injury. rAAV2-Retro-tdTomato was co-injected with retrograde tracer CTB-488 to C4/5 cervical spinal cord of adult mice in the presence or absence of cervical hemisection injury. Retrogradely transduced neurons were detected in diverse supraspinal populations including reticulopsinal, rubrospinal, and corticospinal neurons as early as three days post-injection. Expression was robust by one week post-injection, and unaffected by injury. Remarkably, transduction efficiency, as quantified by CTB-488/tdTomato co-expression, exceeded 90%. Transduction is likely synapse-independent, because spinal injection of rAAV at early postnatal timepoints, prior to the onset of widespread synaptogenesis, produced similarly efficient retrograde CST transduction. In contrast, retrograde transduction was minimal in ascending sensory neurons. Ongoing work is testing retrograde delivery of pro-regenerative genes including KLF6 and STAT3. Overall these data indicate that rAAV offers a simple and highly effective tool for widespread retrograde transduction of descending motor systems.

**Disclosures:** **Z. Wang:** None. **B. Maunze:** None. **M. Blackmore:** None.

**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Topic:** C.09. Brain Injury and Trauma

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ALARME Foundation

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**Title:** Identifying factors that stimulate or improve propriospinal axon regrowth after severe spinal cord injury

**Authors: *M. A. ANDERSON**[1], T. M. O'SHEA[2], J. E. BURDA[2], S. BARLATEY[1], Y. AO[2], A. BERNSTEIN[2], A. WOLLENBERG[3], R. KAWAGUCHI[4], G. COPPOLA[4], C. WANG[5], Z. HE[5], T. J. DEMING[3], G. COURTINE[1], M. V. SOFRONIEW[2]
[1]Swiss Federal Inst. of Technology, Lausanne, Geneve, Switzerland; [2]Neurobio., [3]Bioengineering, Chem. and Biochem., [4]Psychiatry and Neurol., UCLA, Los Angeles, CA; [5]Children's Hosp Boston, Boston, MA

**Abstract:** Propriospinal neurons can relay functional information past incomplete spinal cord injuries (SCI) and are good candidates to target for restoring neural connectivity across anatomically complete SCI. Propriospinal neurons do not regrow spontaneously across complete SCI lesions and the molecular requirements to stimulate or improve such regrowth are not characterized. In this study we are examining, individually and in combination, various mechanisms with the potential to stimulate or improve propriospinal axon regrowth, including (i) biomaterial depots that locally deliver axon-specific chemoattraction in the form of glial cell-derived growth factor (GDNF) that has previously been reported to stimulate propriospinal axon growth after SCI; (ii) inducing growth supportive extracellular matrix by increasing the local expression of laminin; and (iii) activating propriospinal neuron intrinsic growth programs by using adeno-associated viral vectors (AAV) to deliver manipulations previously reported to activate intrinsic growth programs of other central nervous system neurons. Supported by NIH-NINDS NS084030 and F32NS096858, the ALARME Foundation, the International Foundation for Research in Paraplegia, the Roland and Fabièn Loos PhD fellowship award, the Craig H. Neilsen Foundation #381357, the Dr. Miriam and Sheldon G. Adelson Medical Foundation, and Wings for Life.

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**Poster**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** 1R43NS084489-01A1

**Title:** Self-deliverable siRNA compounds to evaluate PTEN as a therapeutic target to promote axon regeneration after central nervous system injury

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**Abstract:** Phosphatase and tensin homologue (PTEN) is a critical neuron-intrinsic suppressor of axon regeneration in the injured central nervous system (CNS). Neuron-specific PTEN gene silencing, for instance by Cre-Lox recombination or by viral introduction of PTEN shRNA sequences, increases axon regeneration after optic nerve and spinal cord injury in rodents. While these approaches validated neuronal PTEN as a potent target to augment regeneration of injured mammalian CNS axons, drugs that target PTEN gene expression in a therapeutically relevant fashion have not been developed. Therefore, we created self-deliverable small interfering RNAs (sdRNAs) against PTEN sequences conserved in rat and human to evaluate PTEN gene silencing as a therapeutic strategy to promote axon regeneration after CNS trauma. sdRNAs are a novel class of chemically modified small interfering RNAs (siRNAs), which are suitable for therapeutic use due to their high gene silencing efficacy, nuclease resistance and cell penetration without specific delivery vehicles or formulations. We screened a library of multiple in silico-predicted PTEN sdRNA sequences for their mRNA-targeting efficacy using a luciferase reporter assay, which revealed a lead candidate sdRNA, termed BA-434. BA-434 strongly reduced PTEN mRNA levels in both rat PC12 and human HeLa cells and furthermore, induced long-lasting, yet reversible PTEN protein knockdown in rat primary neurons. After intravitreal injection of BA-434 in the eye of adult rats, PTEN protein levels were significantly reduced in the retina for at least 2 weeks as revealed by immunoblotting and immunohistochemistry. Strikingly, PTEN reduction in the retina was accompanied by increased activity of the phosphatidylinositol 3-kinase (PI3-K) signaling axis as demonstrated by increased phosphorylation of AKT and its downstream effectors glycogen synthase kinase (GSK) 3β and ribosomal subunit S6. Finally, we demonstrated that BA-434 promotes neurite outgrowth in cultured PC12 cells and enhances axon regeneration after optic nerve crush in adult rats when delivered intravitreally at post-injury time points. These results suggest, that PTEN gene silencing by the self-deliverable siRNA BA-434 is a promising therapeutic strategy to foster CNS repair after injury.

**Disclosures:** **J. Ruschel:** A. Employment/Salary (full or part-time):; Bioaxone Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bioaxone Biosciences. **T. Shmushkovich:** A. Employment/Salary (full or part-time):; Advirna LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Advirna LLC. **M.D. Abbinanti:** A. Employment/Salary (full or part-time):; Bioaxone Biosciences Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bioaxone Biosciences Inc. **L. Guo:** A. Employment/Salary (full or part-time):; Bioaxone Biosciences Inc. **F. Yang:** A. Employment/Salary (full or part-time):; Bioaxone Biosciences Inc. **E. Niederst:** A. Employment/Salary (full or part-time):; Bioaxone Biosciences Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bioaxone Biosciences Inc. **M. Betancur-Boissel:** A. Employment/Salary (full or

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 578.23/Y9

**Topic:** C.09. Brain Injury and Trauma

**Support:** Dr. Miriam and Sheldon Adelson Medical Research Foundation (AMRF)

**Title:** Complement proteins and receptor are required for optic nerve regeneration induced by several pro-regenerative treatments

**Authors: *S. L. PETERSON**, Y. LI, T. KURIMOTO, K. YUKI, B. STEVENS, L. BENOWITZ
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**Abstract:** The failure of axons to regenerate in the mature central nervous system (CNS) underlies the permanent functional deficit observed after clinical CNS damage such as spinal cord injury, traumatic brain injury, and optic nerve injury, as well as diseases such as Alzheimer’s and glaucoma. Several recent, promising strategies for improving CNS axon regrowth have been discovered using the optic nerve crush model, including inflammatory zymosan[1], the growth factor oncomodulin[2], PTEN deletion[3], combinatorial treatments[4], and chelation of free zinc using TPEN[5]. The inflammatory response to optic nerve crush, similar to other CNS injuries and diseases, involves the complement cascade, which normally functions in response to pathogen threat by recruiting inflammatory cells, marking pathogens for removal, and directly initiating cell lysis. While the role for complement proteins and their receptors in inflammatory host defense to pathogens is well characterized, recent findings have also suggested various non-traditional roles for complement proteins in CNS development, injury, and disease, including myelin clearance, neuroprotection, neurotoxicity, neuronal migration, synaptic engulfment, and axon guidance[6]. However, the potential involvement of complement in axon regeneration after optic nerve injury has not been investigated. We report that genetic removal of complement proteins C1q or C3 or complement receptor CR3 blocked treatment-induced RGC axon regeneration following optic nerve injury in mice. The number of GAP43-

labeled, regenerating axons beyond the crush site following treatment with zymosan plus cAMP, AAV2-shPTEN plus oncomodulin plus cAMP, or TPEN was significantly reduced in several lines of complement knockout mice in comparison to wild-type littermates 14 days post-injury. However, neither C1q, C3, nor CR3 knockout consistently affected RGC survival. These data suggest that the complement system is required for axon growth in the mature central nervous system, adding to the mounting evidence for non-traditional roles for inflammatory complement proteins in the nervous system.

[1]Yin Y et al. (2003) J Neurosci 23:2284–93.
[2]Yin Y et al. (2006) Nature Neuroscience 9:843–852.
[3]Park KK et al. (2008) Science 322:963–6.
[4]de Lima S et al. (2012) PNAS 109:9149–54.
[5]Li Y et al. (2017). PNAS 114:E209–E218.
[6]Peterson S & Anderson A. (2014) Exp Neurol 258:35-47.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Program#/Poster#:** 578.24/Y10

**Topic:** C.09. Brain Injury and Trauma

**Support:** NINDS R01NS076976

**Title:** Olfactory ensheathing cell transplantation combined with epidural stiumation and climb training as a treatment for severe spinal cord injury

**Authors: *K. L. INGRAHAM**[1,2], M. A. THORNTON[1], A. K. YEUNG[1], M. D. MEHTA[1], P. AKKARA[1], K. AGGARWAL[1], T. T. MORAD[1], E. A. DALE[1], H. ZHONG[1], V. R. EDGERTON[1], P. E. PHELPS[1]
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**Abstract:** Known challenges to promoting functional axon regeneration across the injury site after a severe spinal cord injury (SCI) are: 1) the limited growth potential of adult neurons, 2) the growth inhibitory factors in the injury site, and 3) and the formation of functional circuits. We tested a combination of two promising therapies for SCI that theoretically act through different mechanisms. We asked if transplantation of olfactory ensheathing cells (OECs) combined with epidural stimulation (ES) and voluntary climb training would improve functional and anatomical recovery in a long-term model of severe rodent SCI. Because our previous study found that

transplanted OECs died between 4 and 8 weeks post-injury in outbred Sprague-Dawley rats, this study used an inbred Fischer 344 rat model to prevent transplant rejection. Fischer rats received a severe SCI at T8-T9 followed by a 2-week delayed transplantation of either OECs or media. One month post-injury the spinal rats received ES while climbing on a grid 3 times a week, 20 min/day for 5 months. Rats were tested and videotaped monthly on the climbing apparatus and perfused 5.5 months post-injury. Initially, we found that all of the OEC-transplanted rats had cell survival in both the lesion core and spinal stumps out to 5 months post-transplantation and 50% of them had OEC bridges spanning the lesion. Additional analyses of the injury site showed that OEC-transplanted rats had a greater volume of spared tissue compared to controls. We also assessed regeneration of descending serotonergic axons and neuron survival near the injury site. Lastly, we compared recovery of spinal rat climbing behavior between 2 and 5 months post-injury using a qualitative assessment of hindlimb locomotor features typical of pre-injury climbing. These findings show that OEC transplantation, combined with ES and climb training, can facilitate recovery when compared to media-transplanted controls that received the same treatment.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

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**Program#/Poster#:** 578.25/Y11

**Topic:** C.09. Brain Injury and Trauma

**Title:** Effect of adelmidrol, a palmitoylethanolamide analogue, on overactive urinary bladder syndrome induced by SCI in mice

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**Abstract:** The disruption of coordinated control between the brain, spinal cord and peripheral nervous system caused by spinal cord injury (SCI) leads to several secondary pathological conditions, including lower urinary tract dysfunctions. In fact, a life-threatening disability after SCI is urinary dysfunction, which could be attributable to lack of neuroregeneration of supraspinal pathways that control the bladder function. As a consequence, individuals develop overactive urinary bladder (OAB), a syndrome characterized by exacerbated contractions of the urinary bladder during the filling phase, associated with detrusor sphincter dyssynergia (DSD) and inefficient voiding. The goal of the current study was to explore the effects of adelmidrol, an

analogue of the anti-inflammatory fatty acid amide signaling molecule palmitoylethanolamide, in OAB after SCI in mice. SCI was induced by an application of a vascular clip to the spinal cord dura at T5-T8 to provoke injury. Mice were treated with adelmidrol (10 mg/kg, intraperitoneally) daily for 48 h and 7 days after SCI. SCI was associated with a marked inflammatory response and functional changes in the urinary bladder. Adelmidrol reduced significantly mast cells degranulation and down regulated NFκB pathway in the bladder after SCI both at 48h and 7days. Moreover adelmidrol was able to reduce NGF expression, usually correlate with intravescical pressure caused by OAB, suggesting an association between neurotrophins and bladder pressure. Seven days after SCI, bladder was characterized by a considerable bacterial infection and proteinuria; surprisingly adelmidrol reduced significantly these two parameters. Therefore, taken together, these data show the protective roles of adelmidrol in OAB following SCI in mice, highlighting a potential therapeutic target for controlling OAB, making a significant impact on improving the function, wellness, and overall quality of life.

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**Topic:** C.09. Brain Injury and Trauma

**Support:** Field Neurosciences Institute

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**Title:** Treating spinal cord injury with the co-transplantation of mesenchymal stem cells that overexpress SDF-1 and neural stem cells

**Authors: *A. N. STEWART**[1], G. KENDZIORSKI[2], Z. M. DEAK[2], D. J. BROWN[2], M. N. FINI[2], K. L. COPELY[2], J. ROSSIGNOL[4], G. L. DUNBAR[3]
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**Abstract:** Genetic engineering of mesenchymal stem cells (MSCs) and neuronal stem cells (NSCs) has been used to treat spinal cord injuries (SCI). As a mechanism of therapy, MSCs secrete high amounts of anti-inflammatory cytokines and trophic factors, while NSCs can

differentiate into neuronal lineages and aid in tissue replacement. Additionally, the forced overexpression of secreted proteins can enhance the secretome of transplanted cells, which can increase therapeutic efficacy. This project was broken up into two studies that first utilized a combinational treatment strategy that forced the overexpression of the chemokine stromal-derived factor-1 (SDF-1) from MSCs (SDF-1-MSCs), to treat a rat model of SCI. The next study further expanded on these findings by performing a co-transplantation of these SDF-1-MSCs with NSCs using the same transplantation parameters. Transplants occurred at 9-days post-injury, and motor functions were evaluated for 7-weeks post-injury. The first study evaluated the effects of MSCs and SDF-1-MSCs on axonal growth and cavitation in longitudinally cut sections, while the next study evaluated white matter sparing and axon densities surrounding the lesions in cross-sections. Findings from these studies demonstrate that overexpressing SDF-1 from MSCs can reduce cavitation and enhance axonal growth into the lesion site, while co-transplanting SDF-1-MSCs with NSCs can improve motor functions and enhance axon densities surrounding the lesion. However, no white matter sparing was found in either study, but some tumors were observed in rats given co-transplantations with either SDF-1-MSCs and NSCs or unmodified-MSCs and NSCs. Collectively, these studies offer evidence that overexpressing SDF-1 from MSCs can improve the efficacy of stem-cell-based therapies for SCI, but ways to avoid tumors need to be developed.

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**Poster**

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Phillip and Peggy DeZwirek

**Title:** SMaRT neural stem cells to degrade scar and optimize regeneration of the traumatically injured cervical spinal cord injury

**Authors: *C. S. AHUJA**[1], M. KHAZAEI[3], P. CHAN[2], J. MERCHANT[2], S. BAIG[2], J. WANG[2], M. G. FEHLINGS[4]
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**Abstract: Introduction**
Human induced pluripotent stem cell-derived neural stem cell (hiPS-NSC) are an exciting therapeutic strategy for traumatic spinal cord injury (SCI) with the capacity to replace lost neural circuits, remyelinate denuded axons and provide trophic support. Unfortunately, most affected individuals are in the chronic phase of their injuries where dense deposition of perilesional chondroitin sulfate proteoglycan (CSPG) scar significantly hinders regenerative cell migration and neurite outgrowth. Scar-modifying enzymes have been shown to mitigate this effect, however, nonspecific delivery via intrathecal catheters risks off-target CNS effects. We aimed to generate a unique, genetically-engineered line of hiPS-NSCs, termed Spinal Microenvironment Modifying and Regenerative Therapeutic (SMaRT) cells, capable of locally expressing a scar-degrading enzyme to enhance functional recovery without the risks of untargeted administration.
**Materials/Methods**
Using non-viral techniques, a proprietary scar-degrading enzyme was genetically integrated into hiPS-NSCs under a human promoter and a monoclonal line was generated by FACS. Enzyme expression and activity was extensively characterized *in vitro* by biochemical assays, immunoblotting, and functional cell culture assay. To assess *in vivo* efficacy, T-cell deficient rats (N=60) with chronic (8 week) C6-7 clip-contusion SCIs were randomized to receive (1) human iPS-NSCs, (2) SMaRT Cells, (3) vehicle control, or (4) sham surgery. Behavioural assessments are ongoing; asynchronous rehabilitation will begin at 20 weeks post-injury with a study endpoint of 32 weeks. Graft survival, cell differentiation, extracellular matrix changes, and graft-host connectivity (trans-synaptic tracing/optogenetic stimulation) will be key outcome metrics in addition to sensorimotor recovery.
**Results**
The scar-degrading ENZYME is robustly expressed by the transgenic SMaRT cells. Importantly, SMaRT cells retain critical human NSC characteristics. The expressed ENZYME appropriately degrades human CSPGs and allows neurons to extend into CSPG-rich regions *in vitro.* Conditioned SMaRT cell media also degrades rodent CSPGs in *ex vivo* serial injured cord cryosections. While blinded behavioural assessments are ongoing, an interim histologic analysis of several animals shows that grafted human cells are extending remarkably long (>20,000um) axons along rodent white matter tracts.
**Conclusion**
This work provides exciting proof-of-concept data that genetically-engineered SMaRT cells can degrade CSPGs *in vitro* and that human NSC grafts can form long axonal processes in the chronic cervical SCI niche.

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**Poster**

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**Topic:** C.09. Brain Injury and Trauma

**Support:** R01NS079702

**Title:** Promoting targeted reinnervation of phrenic motor neurons with BDNF after cervical spinal cord injury to restore respiratory function

**Authors: *B. A. CHARSAR**[1], M. URBAN[1], B. GHOSH[1], G. M. SMITH[2], A. C. LEPORE[1]
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**Abstract:** We are testing a novel strategy to promote both regeneration of damaged descending bulbospinal respiratory axons and reinnervation of phrenic motor neurons (PhMN) after cervical spinal cord injury (SCI) in rats. More than half of all SCI cases occur in the cervical region, leading to respiratory dysfunction due to damage to respiratory-related neuronal soma and/or their axons. The C3-C6 mid-cervical spinal cord levels house the PhMNs, which are responsible for activation of the diaphragm via muscle innervation by the phrenic nerve. These PhMNs are predominately mono-synaptically innervated by supraspinal respiratory neurons located in a brainstem nucleus called the rostral Ventral Respiratory Group (rVRG). Our goal is to promote diaphragm recovery, and hence inspiratory breathing, by restoring respiratory neural circuitry after cervical SCI. To this end, we are selectively expressing the axon guidance molecule, brain-derived neurotrophic factor (BDNF), at the location of PhMNs to direct regenerating rVRG axons toward PhMN targets. In C2 hemisection rats, we intraspinally inject adeno-associated virus serotype 2 (AAV2)-BDNF specifically into the C3-C6 ventral horn at the location of the PhMN pool. AAV2 vector delivery results in efficient and selective neuronal transduction, as well as significantly increased BDNF expression in ventral horn of the C3-C6 spinal cord. In preliminary findings, AAV2-BDNF injection promotes partial recovery of ipsilateral hemi-diaphragm function (compared to AAV2-GFP control), as assessed by *in vivo* recording of electromyography amplitudes. We are currently elucidating the neuroanatomical mechanisms underlying this functional recovery by selectively labeling various descending rVRG axon populations after treatment. By combining retrograde CTB labeling of PhMNs from the diaphragm with stereotaxic injections of the anterograde/trans-synaptic tracer, AAV2-mCherry/wheat germ agglutinin (WGA), into either the ipsilateral or contralateral rVRG, we are assessing possible contribution of regrowth of damaged rVRG axons and sprouting of spared contralateral fibers, respectively.

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**Poster**

**578. Spinal Cord Injury: Therapeutic Strategies**

**Location:** Halls A-C

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**Program#/Poster#:** 578.29/Y15

**Topic:** C.09. Brain Injury and Trauma

**Support:** Craig H. Neilsen Grant 382387

**Title:** Direct osmotherapy for the reduction of edema following spinal cord injury

**Authors: *J. M. YONAN**[1], C. HALE[2], V. G. J. RODGERS[2], D. K. BINDER[1]
[1]Div. of Biomed. Sci., [2]Biomed. Engin., Univ. of California Riverside, Riverside, CA

**Abstract:** Spinal cord injury (SCI) is characterized by an initial injury due to trauma, which is followed by a cascade of cellular events, resulting in a greater degree of secondary damage. One of these secondary events is the accumulation of edema at the injury site, leading to expansion of the cord tissue within the spinal column. Current decompressive strategies do nothing to address this issue, leaving the expanding tissue to become compressed against the surrounding dura mater. This results in increased intraspinal pressure, reduced spinal perfusion and eventual tissue infarction. Our lab has developed an Osmotic Transport Device (OTD) to perform Direct Osmotherapy (DOT) following injury. This device is placed directly on the surface of the tissue and utilizes the creation of an osmotic gradient to actively remove water from the spinal cord after injury. We hypothesize that DOT following SCI will effectively remove water from the spinal cord and improve functional outcome. In this study, we first characterized the time course of edema following severe contusion SCI at thoracic level 8 in rats. At varying time points post injury, between 1 hour and 28 days, 15mm of spinal cord encompassing the lesion epicenter were freshly dissected. This tissue was further divided into 5mm segments at the lesion epicenter, as well as rostral and caudal to the lesion and analyzed for percent tissue water content. Results revealed increased water content at the lesion epicenter beginning at 1 hour after injury, which continued to increase until a peak was reached at 72hrs post injury. Water content levels decreased after this point, but remained significantly elevated compared to sham controls for at least 4 weeks post injury. While increases in water content were delayed in the rostral and caudal segments compared to the epicenter, they too peaked at 72 hours post injury and returned to control levels there after. This data provides insight into a critical period for treatment. Preliminary application of the device is underway to determine proper device parameters for effective removal of edema. Thus, DOT may prove to be a novel and minimally invasive method to effectively reduce edema formation following spinal cord injury.

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**Title:** Targeting towards early vascular responses following contusive SCI

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**Abstract:** In spinal cord injury (SCI), the initial mechanical damage immediately leads to two categories of effects: vascular effects and biochemical cellular alteration including excitotoxicity. In a conventional hypothesis, both vascular disruption and excitotoxicity-caused neuronal death, necrotic form of other cell death start to occur only in the injury epicenter, interacting with each other and push the boundary toward the adjacent area, so-called “transitional zone”, thus gradually expand the size of the lesion. However, in this study, we have observed vascular disruption in the “transitional zone” as early as 30 minutes after SCI, which was unexpected in the conventional hypothesis. In this “transitional zone”, our two-photon *in vivo* imaging data has indicated that vascular velocity, diameter and permeability were all affected by an acute SCI. Within the same “transitional zone”, histological evidence has also shown significantly increased number of leaked vessels at ventral gray matter. Meanwhile, the number of neurons and glial cells has been maintained in the acute phase of contusive SCI. These data suggest an alternative hypothesis that simultaneously with what happened in the epicenter, vascular damage has also occurred in the “transitional zone” while the cells have not responded to the injury yet. The data also indicates a time window between vascular disruption and neuronal loss at the transitional zone. In addition, targeting towards these early vascular responses and protecting vascular

integrity at an early phase of SCI could provide early neuronal protection and delay the expansion of damaged tissue in the spinal cord.

**Disclosures: C. Chen:** None. **X. Xu:** None.

**Poster**

**579. Headache and Migraine**

**Location:** Halls A-C

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**Topic:** D.03. Somatosensation: Pain

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University of Kansas Medical Center Chancellor's Doctoral Fellowship

**Title:** Investigating the effect of stress and exercise in models of elicited headache pain in mice

**Authors: *O. ELLER-SMITH**, X. YANG, J. A. CHRISTIANSON
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**Abstract:** Experiencing stressful events during the formative years is associated with an increased risk of developing comorbid chronic pain and mood disorders in adulthood. These include interstitial cystitis, chronic prostatitis, vulvodynia, migraine, fibromyalgia, and depression. The exact mechanism underlying the development of these disorders remains unknown, but it likely involves altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis regulates the stress response and alters one’s perception of pain. To study the consequences of early life stress, we utilize a mouse model of neonatal maternal separation (NMS)). In our model, NMS mice are separated from the dam for 3 hours/day beginning on postnatal (P) 1 through P21. Naïve mice remain undisturbed in their home cage until weaning on P22. Previously, we have shown that NMS mice display evidence of dysfunctional limbic regulation of the HPA axis as well as increased urogenital sensitivity. We are now expanding our studies into peripheral tissues involved in other chronic pain disorders and investigating if NMS mice display behavioral and molecular evidence of susceptibility to headache and fibromyalgia. We are also interested in researching the influence of exercise on chronic pain. At 4 weeks of age, NMS and naïve mice were divided into exercised (Ex) or sedentary (Sed) groups. Ex mice

were pair housed and received access to a running wheel in their home cage, while Sed mice did not receive running wheels. 8-12 weeks later, experiments were carried out. Different methods were used to elicit a migraine episode, including application of noxious solutions (inflammatory soup (IS) or 5% mustard oil (MO)) onto the dura mater via a modified cannula injection through the lambdoidal suture, or intraperitoneal injection of nitroglycerin (NG). Mice were subsequently evaluated for behaviors indicative of headache and widespread allodynia, including mouse grimace score (MGS), rearing episodes, evoked facial grooming, and forepaw or hind paw mechanical withdrawal threshold. Results indicate a significant impact of IS application on forepaw allodynia and MO application or NG injection on hind paw allodynia. We also see an impact of exercise on MGS in our mice. Finally, we found that NMS Sed mice weigh significantly more compared to NMS Ex mice and naïve mice. In summary, our results support the evidence of widespread allodynia experienced by individuals during a migraine attack, indicate that voluntary wheel running can influence the painful behaviors mice display during elicited migraine attacks, and suggest that NMS could have an influence on metabolic regulation that is attenuated by exercise.

**Disclosures: O. Eller-Smith:** None. **X. Yang:** None. **J.A. Christianson:** None.

**Poster**

**579. Headache and Migraine**

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DOD W81XWH-16-1-0211

**Title:** Vascular contributions of peripheral CGRP in migraine-like photophobia

**Authors: *B. N. MASON**[1], A. KUBURAS[2], W. J. KUTSCHKE[3], M. W. CHAPLEAU[4], A. F. RUSSO[2]
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**Abstract:** The neuropeptide calcitonin gene-related peptide (CGRP) is a key player in migraine. While migraine can be induced by peripherally administered CGRP (intravenous) and can be treated using CGRP antagonists that act peripherally, the relevant sites of CGRP action remain unknown. To address the role of CGRP both within and outside the central nervous system, we used a mouse model of photophobia. Photophobia is abnormal discomfort to non-noxious levels of light and is experienced by approximately 90% of migraine patients. We have previously shown that peripheral (intraperitoneal, IP) injection of CGRP resulted in light aversive behavior in wild-type CD1 mice similar to aversion previously seen following central (intracerebroventricular, ICV) injection. Importantly, two clinically effective migraine drugs, the 5-$HT_{1B/D}$ agonist sumatriptan and a CGRP-blocking monoclonal antibody, attenuated the peripheral CGRP-induced light aversion and motility behaviors. Our goal for this study, is to identify the mechanism of action of peripheral CGRP using light aversion. Previously we showed that ICV CGRP, but not IP CGRP, in nestin/hRAMP1 mice, mice that overexpress CGRP receptors in the nervous system, causes enhanced light aversion. We have now used transgenic CGRP-sensitized mice that have globally elevated levels of hRAMP1 (global *hRAMP1*) in all tissues. Interestingly, our preliminary data show sensitivity to low light after IP CGRP in these mice. This suggests that CGRP actions in the periphery play an important role in the induction of light aversion in this model. We have now begun investigating the role of the vasculature in peripheral CGRP-induced light aversion by using two approaches (1) genetic overexpression of the CGRP receptor in the vasculature (2) injection of phenylephrine to minimize vasodilation induced by CGRP. Our preliminary data show that IP CGRP in Tagln (SM22)/hRAMP1 mice, mice that overexpress RAMP1 on smooth muscle, display enhanced light aversion. These results suggest that CGRP can act in both the periphery and the brain by distinct, but possibly overlapping, mechanisms. This also suggests that peripheral CGRP actions may be transmitted to the CNS via indirect sensitization of peripheral nerves and likely not on CGRP receptors in the nervous system to cause migraine-like photophobia.

**Disclosures: B.N. Mason:** None. **A. Kuburas:** None. **W.J. Kutschke:** None. **M.W. Chapleau:** None. **A.F. Russo:** None.

**Poster**

**579. Headache and Migraine**

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**Program#/Poster#:** 579.03/Z1

**Topic:** D.03. Somatosensation: Pain

**Support:** Veterans Affairs RR&D. IK2 RX-002010-0

**Title:** Potential brain regions involved in light-aversive behavior

**Authors: *L. P. SOWERS**[1,2], B. REA[1], R. TAUGHER[1], Y. KIM[1], A. KUBURAS[1], J. WEMMIE[1,2], A. RUSSO[1,2]
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**Abstract:** Veterans returning from active duty are at an increased risk for post-traumatic headache (PTH) and migraine. Migraine alone affects 10% of men and 25% of women, with the lifetime risk increasing to 18% and 43% respectively. Sensory abnormalities are present in individuals with PTH and migraine including extreme light and sound sensitivity. Light sensitivity in patients with PTH or migraine can be debilitating and treatments are lacking. One reason interventions and treatments continue to fall short is a poor understanding of the relevant neuroanatomical correlates that underlie sensory changes in headache. Calcitonin gene-related peptide (CGRP) is a critical neuropeptide involved in pain signaling and has recently come to the forefront of migraine research where it contributes to headache and associated sensory abnormalities. In this study we attempt to identify anatomical regions where CGRP could act to induce light-aversive behavior in migraine and PTH. The posterior thalamus (Po), periaqueductal grey (PAG), and amygdala have been suggested to be regions that could contribute to light-aversive behavior. We hypothesized that CGRP could act as a neuromodulator in these regions to induce light aversive behavior. To test this hypothesis, we used targeted approaches to probe these areas of the brain thought to be important in the development of photophobia. The first approach was direct CGRP injection. The second was optical stimulation using channelrhodopsin driven by the CaMKII promoter. We found that CGRP injection and optical stimulation of the Po and the PAG resulted in different types of light-aversive behavior. These results may begin to shed light on the complex circuitry of light-aversive behaviors in mice.

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**Poster**

**579. Headache and Migraine**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 579.04/Z2

**Topic:** D.03. Somatosensation: Pain

**Title:** Neural basis for chronic headache and photophobia after mild traumatic brain injury

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**Abstract:** Mild traumatic brain injury (mTBI) accounts for the majority of head injuries, and posttraumatic headache (PTH) is the most common adverse effect. The most PTH has migraine-like phenotype (Hyperalgesia, Photophobia, Throbbing pain) and is difficult to resolve.

Headache pain is thought to require sensitization of the ophthalmic branch of the trigeminal nerve leading to long-term changes in the activity of neurons in the trigeminal brainstem nuclear complex (TBNC) and higher brain centers. Although the triggers that lead to sensitization of dural-responsive TBNC neurons are not well defined, considerable evidence suggests spatial summation from extradural inputs onto dural-responsive neurons in the brain plays an important role. Luminance-induced TBNC activity is directly relevant to headache pain since heightened light sensitivity is a recognized diagnostic feature of migraine. Neurovascular coupling within the eye likely mediates the discomfort and pain in photophobia that could trigger or exacerbate headache pain. Previously, we reported that the blight light-evoked increases in trigeminal subnucleus caudalis (Vc) neural activity depended on neurovascular link. This study used a rat model to examine the effects of laser-induced shock wave on the Vc neural activity. The parietal region of male rats was irradiated with laser-induced shock wave (diameter 3mm, 4J/cm$^2$) under barbiturate anesthesia. In awake male rats, ocular surface application of hypertonic saline (2.5 M NaCl; 40µl) evoked eye wipe behavior that was enhanced 3-14 days after shock wave irradiation (SWR). In separate rats, under isoflurane anesthesia, single cornea/dura responsive neurons were recorded at the Vc. Hypertonic saline (0.15-5M) and blight light (irradiance=50, 300, 500 W/m$^2$) selectively activated ocular surface and intraocular (neurovascular system), respectively. In SWR rats (seven-ten days after SWR), Vc units had enhanced responses to hypertonic saline and blight light compared to naïve rats. To determine if SWR caused bright light evoked intracranial blood flow increases, blood flow was monitored in arteries of the exposed cranial dura mater and the parietal cortex in naive and SWR rats. In SWR rat, bright light enhanced the magnitude of blood flow but not naive rat, and this blood flow increases evoked dura -responsive Vc units activities. It is concluded that mTBI produces a chronic state of hyperalgesia and light evoked vessels dilation that is reflected in the sensitization of trigeminal -parasympathetic circuit. This model may be suitable for future studies of migraine.

**Disclosures: A. Tashiro:** None. **H. Ohta:** None.

**Poster**

**579. Headache and Migraine**

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**Program#/Poster#:** 579.05/Z3

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH DE017805

**Title:** Neck muscle inflammation induces trigeminal central sensitization: a risk factor for episodic migraine

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**Abstract: Objective:** The goal of this study was to understand the cellular changes associated with neck muscle inflammation, a reported co-morbid condition with migraine, that promote a sensitized state of trigeminal nociceptors. **Background:** Many migraine patients report stress, which can manifest as tension/tenderness and inflammation in neck muscles, as a major risk factor. Projections from neurons emanating from neck muscles converge with primary sensory trigeminal ganglion (TG) neurons in the spinal trigeminal nucleus (STN). Although neck muscle inflammation is implicated in migraine pathology, the cellular mechanisms that mediate an enhanced state of trigeminal sensitization is not well understood. **Methods:** To induce muscle inflammation in adult male Sprague Dawley rats, complete Freund's adjuvant (CFA) was injected at ten different sites of the trapezius. TG and STN tissues collected from naïve animals, and on days 1, 4, and 8 post CFA injection were used for immunohistochemical analysis of Calcitonin Gene-Related Peptide (CGRP) and Protein Kinase A (PKA), GFAP and Iba1, and P-ERK. Nociception to mechanical stimuli of V1 (eyebrow) nociceptors was determined on days 0, 1, 4, and 8 post CFA injection utilizing von Frey filaments and Durham animal holder. To act as an odorant trigger, some animals were exposed to an extracted oil from leaves of the California Bay tree 8 days post CFA injection and nociception assessed 2 hours post exposure. **Results:** Immunoreactive levels of CGRP were transiently upregulated in STN tissues 1 day post CFA as compared to naïve levels, while PKA expression exhibited a temporal increase, reaching significance by day 8 post CFA. Elevated levels of GFAP in the STN were detected at day 1 post CFA, but decreased to naïve levels by day 4. However, Iba1 expression was significantly elevated at days 1, 4, and 8 post CFA. No significant temporal changes in TG levels of CGRP, PKA, or P-ERK levels were detected in CFA alone treated animals. In agreement, mechanical nocifensive sensitivity was not different from naïve at any timepoint post CFA. However, animals with ongoing neck muscle inflammation exposed to the odorant trigger exhibited significantly higher levels of nociception as compared to control animals. **Conclusion:** Results from our study provide evidence that neck muscle inflammation promotes development of central sensitization via upregulation of the CGRP/PKA pathways, acute activation of astrocytes, and sustained microglial activation. We propose that neck muscle inflammation functions as a migraine risk factor by lowering the activation threshold of trigeminal nociceptors to a pungent odor reported as a migraine trigger.

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**Poster**

**579. Headache and Migraine**

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**Topic:** D.03. Somatosensation: Pain

**Support:** Allergan Canada Inc.

**Title:** Intrinsic brain network abnormalities in chronic migraine are reversed following Onabotulinum toxin-A

**Authors: *A. K. KANUNGO**[1], D. TURCOTTE[2], J. KORNELSON[2], T. KOLESAR[2], B. MANSOURI[1]
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**Abstract:** *Objective:* The objective of this study was to compare functional connectivity patterns across cortical brain regions utilizing resting state functional magnetic resonance imaging (rsfMRI) in patients with chronic migraine (CM) before and after treatment with onabotulinun toxin-A (BoNT-A).
*Methods:* An open-label, prospective interventional pilot study was conducted involving a total of 12 patients with confirmed CM. Patients followed a structured timeline consisting of follow-up with headache logbooks, well-validated migraine significance assessments and psychological inventories along with two cycles of BoNT-A injections on Day 0 and 84. Patients underwent rsfMRI scanning protocol at baseline and on Days 56, and 140 to monitor for differences in brain activity. Psychological and pain changes were analyzed by ANOVA, and rsfMRI data via independent component analysis.
*Results:* After the first treatment of BoTN-A, a change in brain connectivity was observed in the default mode network (DFN), right central executive network (RCEN) and salience network (SN), that most prominently involved the left superior parietal gyrus and the bilateral extrastriate gyri, which correspond to components of the lateral pain system and the visual association areas, respectively. Changes to the pattern of functional connectivity within these brain regions was correlated with clinical improvement of migraine frequency and severity (i.e. transformation of CM into episodic migraine (EM)), as well as associated anxiety. Additionally, we observed at baseline and following BoTN-A administration, no significant functional brain connectivity within the left central executive network in our cohort with CM.
*Conclusions:* CM patients demonstrate an abnormal pattern of functional connectivity in the DFN, RCEN and SN, corresponding to brain regions involved with higher cortical pain and visual processing. This pattern of connectivity is altered by BoTN-A administration. The functional activity differences between CM and EM have been described in subcortical and brainstem areas in the past. However, to the best of our knowledge, our study demonstrated cortical connectivity changes after transforming CM to EM for the first time. This novel finding may have significant application in the clinical approach for management of CM in future. Furthermore, the absence of a left executive network in CM patients, which did not change after BoTN-A treatment, was unexpected and may provide a mechanistic explanation for cognitive abnormalities seen in this disorder. To confirm these findings, further research with larger number of patients are needed, in conjunction with placebo control.

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**Poster**

**579. Headache and Migraine**

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**Program#/Poster#:** 579.07/Z5

**Topic:** D.03. Somatosensation: Pain

**Title:** Association between migraine-related disability and co-morbid depressions among migraineurs having follow up at two neurology referral clinics in Addis Ababa, Ethiopia

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**Abstract: Background**: Migraine headache is one of commonest neurologic disorder to cause great disability among its sufferers, migraine not only cause pain, but also cause significant disability, economic loss, low productivity and reduced quality of life of individuals. According to recently published population based study in Ethiopia, the prevalence of Migraine headache in both urban and rural population is estimated to be 16.7%. **Objectives**: The aim of this study is to assess the association between migraine-related disability and co-morbid depression among migraineurs **Methods:** Cross sectional, descriptive survey was conducted among migraine patients age ≥ 13 who attended clinics during study period were included. Correlation between variables where determined using spearman correlation coefficient .The study was conducted between June 1- October 30, 2016. **Results:** During five months of study periods total of seventy migraine patients were seen at both neurology referral clinics. Fifty-three (74.3%) of our study participants are female with commonest age group being 20-30 years. Twenty (28.6%) of the participants completed secondary education. Seventy (70%) of participants has migraine without aura subtype, while the mean headache frequency and headache intensity was found to be 23.4± 14.9 and 7.4 ± 1.2 respectively. More than Forty-one (41.4%) of participants fulfilled Major depressive disorder (MDD) criteria. Out of depressive symptoms participants were asked, 10% admitted they have suicidal thoughts in the past two weeks. The mean Migraine disability assessment score and Patient health questioner score in our study is 46.7±30 and 9.2±4.4 respectively. More than two third (74.3%) of our study participants has severe disability. The correlation between migraine -related disability and depression was found positive, with statistically significant P-value 0.007 and correlation coefficient (r= 0.318). **Conclusion**: Based on this study we can conclude, there is positive correlation between migraine-related disability and co-morbid depression among migraineurs having followed up at both neurology referral clinics. In addition there is high prevalence of depression and severe disability among these study participants, indicating high disease burden. So we recommend having wider population based study in order to understand this association more deeply and to understand the wider social and economic impact of this most disabling neurologic disorder, yet ignored.

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**Poster**

**579. Headache and Migraine**

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**Support:** NIH Intramural Research Program, Clinical Center

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**Title:** RNA-Seq investigations of human post-mortem trigeminal ganglia: A transcriptomic perspective on migraine genetics

**Authors: *M. J. IADAROLA**[1], M. R. SAPIO[1], D. M. LAPAGLIA[1], J. THIERRY-MIEG[2], D. THIERRY-MIEG[2], S. J. RAITHEL[1], P. D. BURBELO[3], C. E. RAMSDEN[4], A. J. MANNES[1]
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**Abstract: Background:** The trigeminal ganglion contains neurons that relay sensations of pain, touch, pressure, and many other somatosensory modalities from the body to the central nervous system. The ganglion is also a reservoir for latent herpes virus 1 infection. To gain a better understanding of molecular factors contributing to migraine and headache, transcriptome analyses were performed on postmortem human trigeminal ganglia.
**Methods:** RNA-Seq measurements of gene expression were conducted on small subregions of 16 human trigeminal ganglia. The samples were also characterized for transcripts derived from viral and bacterial genomes. Herpes simplex virus 1 (HSV-1) antibodies in blood were measured using the luciferase immunoprecipitation assay.
**Results:** Molecular heterogeneity across the samples was prominent and could be explained by sampling of anatomically distinct sub-regions of the excised ganglia consistent with neurally-enriched and non-neural, i.e. Schwann cell, enriched regions. The levels of HSV-1 transcripts detected in trigeminal ganglia correlated with blood levels of HSV-1 antibodies. Multiple migraine susceptibility genes are strongly expressed in neurally-enriched trigeminal samples and a separate group in blood vessels. Further profiling across species and in single cell DRG datasets suggested cell-specific expression of specific genes.
**Conclusions:** These data provide a comprehensive human trigeminal transcriptome and a framework for evaluation of inhomogeneous post-mortem tissues through extensive quality

control and refined downstream analyses for RNA-Seq methodologies. Expression profiling of migraine susceptibility genes identified by genetic association appears to emphasize the blood vessel component of the trigeminovascular system. Other genes displayed enriched expression in the trigeminal compared to dorsal root ganglion and in depth transcriptomic analyses of the *KCNK18* gene underlying familial migraine shows selective neural expression within two specific populations of ganglionic neurons. These data suggest that expression profiling of migraine-associated genes can extend and amplify the underlying neurobiology obtained from genetic association studies.

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**Poster**

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**Topic:** D.03. Somatosensation: Pain

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**Title:** Conditioned place aversion prolongs craniofacial cutaneous allodynia induced by acute nitroglycerin (NTG) administration: Novel animal model of chronic migraine

**Authors: *S. S. KOKANE**[1], A. NGO[1], R. BROWN[1], J. GULLACE[1], T. DUONG[1], E. KALAFCHI[1], F. TAO[2], Q. LIN[3]
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**Abstract:** Several clinical and preclinical studies have demonstrated induction of a migraine-like headache upon acute administration of nitric oxide donor NTG. Cutaneous mechanical and thermal allodynia in the cranial receptive field of the ophthalmic branch of the trigeminal nerve has been observed as a result of NTG administration. However, the effects of NTG are acute and last for a few hours after administration. Repeated administration of NTG has been shown to induce chronic migraine-like symptoms which remain as long as the NTG continues to be administered. Upon withdrawal from NTG administration, these effects are reversible.In the present study, we have used conditioned place aversion paradigm to prolong the migraine-like effects induced by a single administration of NTG to last for more than 10 consecutive days.

After conditioning, C57BL/6 mice were administered with a single, 10 mg/kg dose of NTG intraperitoneally. Following single NTG administration, these animals were merely exposed to the pain-paired environment every day for 30 minutes for up to 12 days. Von Frey filaments were used to test cutaneous allodynia in the craniofacial region after exposure.We observed that craniofacial cutaneous allodynia induced by NTG administration persisted for all 12 days so long as the animals were exposed to the pain-paired environment. Upon cessation of exposure to the pain-paired environment, cutaneous allodynia was gradually extinguished. However, a single exposure to the pain-paired environment was sufficient to reinstate the craniofacial cutaneous allodynia. In another group of animals, topiramate was administered after NTG administration and exposure to the pain-paired environment. Topiramate prevented the development of craniofacial cutaneous allodynia. Topiramate is a clinically approved drug prescribed to migraine patients and was used to verify the development of migraine in the animal model.Taken together these results demonstrated that NTG administration coupled with exposure to the pain-paired environment led to prolongation of the craniofacial cutaneous allodynia induced by NTG for as long as the animal was exposed to the pain-paired environment. The development and prolongation of cutaneous allodynia was deferred in animals that were treated with topiramate. These results indicate that the negative affective component associated with headache pain persists for much longer and could possibly underlie the development towards chronicity in migraine patients.

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**Poster**

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**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant NS098660

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**Title:** Medullary pain-modulating neurons show enhanced responses to light in rodent model of migraine headache

**Authors: Y. ZHANG**[1], M. E. MARTENSON[2], A. P. LASSETER[3], *M. M. HEINRICHER[4]
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**Abstract:** Despite the high prevalence of migraine in the general population, our knowledge of the underlying mechanisms remains incomplete. A variety of theories have focused on peripheral neural or neurovascular mechanisms. More recently, the importance of sensitization of *central* trigeminal pathways has been recognized. None of these theories has so far provided a complete explanation for migraine headache pain. We have suggested that pain-facilitating neurons in the rostral ventromedial medulla (RVM) contribute to pain in migraine headache, amplifying abnormal afferent transmission. In addition, we recently reported that a subset of pain-facilitating "ON-cells" and pain-inhibiting "OFF-cells" in the RVM respond to light stimulation. This light-evoked response could be relevant to photophobia in migraine. Moreover, a shift in the balance of activity between these two classes of cells can lead to enhanced or diminished nociception. The objective of this study was to test the hypothesis that the photoresponsiveness of RVM ON- and OFF-cells is altered in a rat model of migraine headache induced by systemically administered nitroglycerine (NTG). We recorded RVM ON- and OFF-cells in lightly anesthetized Sprague-Dawley (albino) and Long-Evans (pigmented) rats. We analyzed both initial (30 min post-NTG) and latent (90 min post NTG) effects of NTG (1 mg/kg) on the responses of these cells to white light of different intensities (30 to 16k lux). In the initial phase, NTG produced a significant enhancement of light-evoked responses in both ON- and OFF-cells, and an increase in ongoing activity of ON-cells. Ongoing firing of OFF-cells was not changed significantly. During the latent phase, the ongoing activities of both ON- and OFF-cells were comparable to baseline, while the stimulus-response curves of both populations were shifted to the left. By contrast, NEUTRAL-cells, RVM neurons without a known role in pain-modulation, did not respond to light before or after NTG. Saline administration had no effect on basal activity or light-evoked responses. These data suggest that dysfunction of intrinsic pain-modulating systems could act in concert with activation of sensory pathways to produce pain and photophobia during migraine headache.

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**Poster**

**579. Headache and Migraine**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 579.11/Z9

**Topic:** D.03. Somatosensation: Pain

**Support:** BFU2015-66941R (MINECO/FEDER)

**Title:** The greater occipital nerve: An anatomical, immunohistochemical and tract-tracing study

**Authors: *N. GARCÍA MAGRO**[1,2], Y. B. MARTIN[2], M. GARCÍA-AMADO[1], C. AVENDAÑO[1], P. NEGREDO[1]

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**Abstract:** Craniofacial painful syndromes include a number of conditions that constitute a major public health problem. Only migraine ranks among the top three neurological disorders in Europe and the US. An increasingly popular approach to treat some of these conditions is the electrical neuromodulation of a cephalic peripheral nerve, such as the greater occipital nerve (GON), which has proved its therapeutic effectiveness particularly for chronic migraine. Only recently have rodent models been developed in an attempt to better understand the neurobiological bases of such therapies. Relevant data, however, are still scanty, and this is particularly so concerning the anatomy of the GON. With the aim of providing a thorough anatomical analysis of this nerve, we have recently started a comprehensive descriptive and morphometric study of the GON in young adult rats that includes the following: 1, A macroscopic anatomical study through microdissection; 2, a global retrograde labeling of the neurons in the cervical ganglia C2 and C3 that send their peripheral branches through the GON; 3, a characterization of these neurons by means of specific retrograde tracers, immunocytochemistry of the ganglia, and stereology; 4, an ultrastructural morphometric evaluation of the GON; and 5, a qualitative and quantitative analysis of the distribution in the cervical and medullary dorsal horn of the primary afferents transganglionically labeled from the GON. Our preliminary results show that, 1, the GON shows diverse patterns of peripheral branching; 2, neurons innervating the skin through the GON are located mainly in C2 and to a lesser extent in C3 ganglia; 3, quite likely, many peripheral axons originating in these ganglia divide before the GON reaches the subcutaneous tissue; and 4, there is a degree of topographical overlap between the central projections of C2 and C3 neurons and trigeminal primary afferents in upper spinal dorsal horn.

**Disclosures: N. García Magro:** None. **Y.B. Martin:** None. **M. García-Amado:** None. **C. Avendaño:** None. **P. Negredo:** None.

**Poster**

**579. Headache and Migraine**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 579.12/Z10

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant NS087321

NINDS Grant NS083698

**Title:** Targeted deletion of TRESK $K^+$ channel preferentially enhances cephalic pain in mice

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**Abstract:** TRESK (TWIK-related spinal cord $K^+$ channel, encoded by KCNK18) channel belongs to the two-pore-domain $K^+$ channel family and is abundantly expressed in all primary afferent neurons (PANs) in trigeminal ganglion (TG) and dorsal root ganglion (DRG). It is well established that TRESK is one of the major background $K^+$ channels in DRG neurons. Changes of TRESK activity in adult DRG neurons alter neuronal excitability and the transmission of extracephalic (body) pain. However, TRESK channel mutations in humans cause migraine headache but not extracephalic pain. This calls for a new model system to study the effects of TRESK mutations on the generation of cephalic pain, especially headache.
The first and best-studied TRESK mutation results in truncation of the TRESK protein. Mutant TRESK subunits do not form functional channels per se. When expressed in wild-type TG neurons, mutant TRESK reduces the endogenous TRESK current by 70%, exerting a strong dominant-negative effect. This suggests that targeted deletion of TRESK provides a good model to study the effects of mutation-induced de novo reduction of TRESK activity in humans.
In this study, we characterized the physiological properties of PANs and nociceptive responses of wild-type and TRESK knockout (KO) mice. Despite the ubiquitous loss of TRESK currents in all KO PANs, total background current was reduced in TG neurons but not DRG neurons from KO mice. Consequently, the excitability of small-diameter TG neurons that do not bind to isolectin B4 was significantly increased in TRESK KO mice; whereas the excitability of wild-type and KO DRG neurons were comparable. Moreover, the percentage of capsaicin-responsive neurons was significantly increased in TG but not DRG of TRESK KO mice. Quantitative PCR showed that the relative TRPV1 mRNA level was higher in KO TG tissues. We further investigated whether *de novo* loss of TRESK alter the responses to noxious stimuli at cephalic (cheek) and extracephalic (hindpaw) regions. Compared with wild-type mice, TRESK KO mice exhibited stronger responses to capsaicin, heat and cold stimuli on the cheek. Conversely, wild-type and KO mice responded similarly to capsaicin, heat, cold and mechanical stimuli to the hindpaw. In a mouse model of headache, dural application of inflammatory mediators elicited more robust head-directed nocifensive behavior in TRESK KO mice.
Taken together, our results indicate that *de novo* loss of TRESK in mice selectively increases the intrinsic excitability and capsaicin-responsiveness of TG neurons. Consequently, TRESK KO mice recapitulate the pathophysiology of TRESK mutations in humans, exhibiting enhanced cephalic pain but normal extracephalic pain.

**Disclosures: Z. Guo:** None. **C. Qiu:** None. **Y. Cao:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.01/Z11

**Topic:** D.03. Somatosensation: Pain

**Support:** P20GM103643 (NIGMS)

R01EY026145 (NEI-NIGMS)

**Title:** Lacrimal gland excision causes sex-specific corneal damage and alterations in corneal sensitivity

**Authors: *N. E. MECUM**[1,4], D. W. DEMERS[2,1], W. C. BUSHEY[1], T. E. DENIS[1], C. E. SULLIVAN[5], I. D. MENG[1,3]
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**Abstract:** Dry eye disease (DED), caused by insufficient production or quality of tears, leads to persistent corneal irritation and pain, and altered properties of corneal afferent neurons. Alterations in corneal nerve morphology, including reduced nerve fiber density in the sub-basal nerve plexus, have been reported in individuals with DED and may be correlated with the occurrence of corneal hypoesthesia; however, other studies have observed an increase in corneal sensitivity in DED. Currently, little is known regarding the progression of DED with respect to nerve damage and the subsequent changes in corneal sensitivity. The aim of this study was to examine corneal nerve morphology and to correlate this with changes in corneal sensitivity following lacrimal gland excision (LGE)-induced dry eye in female and male mice. To produce a graded severity of dry eye, unilateral excision of the extraorbital lacrimal gland (single LGE), or excision of both the intraorbital and extraorbital lacrimal glands (double LGE) were performed. Tears were evaluated using phenol red threads, and fluorescein was used to examine the severity of corneal damage. Eye closure (squinting) was quantified as a measure of ongoing irritation. Nav1.8Cre-tdTomato mice were used to observe the morphology of small diameter afferent neurons in whole mount corneas 1-4 weeks post surgery. Terminal density was imaged from corneal whole mounts using a confocal microscope and analyzed with FIJI. Trigeminal ganglion ATF3 expression was quantified using immunohistochemistry in 12µm cryostat sections. 0.1% capsaicin was applied to the cornea and nocifensive swipes were recorded. Single and double LGE caused a significant reduction in tears, damage to the corneal epithelium, and an increase in eye closure with graded severity. Overall, greater corneal damage and eye closure was observed in female single LGE and double LGE as compared to male mice. Nerve terminal density was lower in both male and female double LGE mice compared to sham and single LGE treatment

groups. ATF3 expression was significantly increased in both male and female mice after double LGE 2-weeks post surgery. At this same time point capsaicin application elicited a greater nocifensive swiping response in female mice after single LGE when compared to sham and double LGE. No difference in eye swiping behavior was observed in male mice. These results indicate sex-specific differences in corneal epithelial cell damage, nerve morphology, and corneal sensitivity after LGE and are consistent with epidemiological reports of a higher incidence of DED in women.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.02/Z12

**Topic:** D.03. Somatosensation: Pain

**Support:** This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea [grant No.: HI15C2589010015].

**Title:** Spinal D-serine modulates nNOS-PSD95 protein-protein interactions leading to the development of mechanical allodynia in a mouse model of neuropathic pain

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**Abstract:** D-serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptors (NMDARs) and has been reported to play an important role in the processing of nociceptive transmission in the spinal cord dorsal horn. However, the cellular mechanisms underlying the action of D-serine in this process have not been investigated. Here we examine the possible role of neuronal nitric oxide synthase (nNOS) in the D-serine-induced potentiation of NMDAR function using animal models of NMDA-induced nociception and neuropathic pain following chronic constriction injury (CCI) of the sciatic nerve. Intrathecal administration of D-serine facilitates NMDA-induced nociception and increases in both total nitric oxide (NO) levels and PKC-dependent (Ser896) phosphorylation of the NMDAR GluN1 subunit (pGluN1), which were attenuated by pretreatment with the selective nNOS inhibitor, 7-nitroindazole. In CCI mice, intrathecal administration of the serine racemase inhibitor, LSOS or the D-serine degrading enzyme, DAAO suppressed NO production and nNOS activation, which was evidenced by a

significant increase in both the membrane expression of nNOS and the PSD95-PDZ domain binding form. Intrathecal administration of the inhibitor of PSD95-nNOS protein-protein interactions, IC87201 suppressed NO production, mechanical allodynia (MA), and PKC-dependent pGluN1 expression. Furthermore, intrathecal administration of 7-nitroindazole, the soluble guanylyl cyclase (sGC) inhibitor, ODQ or the PKC inhibitor, chelerythrine attenuated CCI-induced MA and pGluN1 expression. By contrast, D-serine and nNOS/sGC signaling had no effect on CCI-induced thermal hyperalgesia or GluN1 expression. Collectively spinal D-serine increases nNOS activity in CCI mice and modulates NO-induced increase in PKC-dependent pGluN1 expression, and ultimately contributes to the induction of MA following peripheral nerve injury.

**Disclosures:** **S. Choi:** None. **H. Choi:** None. **M. Lee:** None. **H. Han:** None. **A.J. Beitz:** None. **J. Lee:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.03/Z13

**Topic:** D.03. Somatosensation: Pain

**Support:** Australian Government Research Training Program Scholarship

**Title:** $Na_V1.6$ in peripheral sensory neurons: An emerging role in pain

**Authors: *M. R. ISRAEL**[1], J. R. DEUIS[1], T. DUREK[1], I. VETTER[1,2]
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**Abstract:** Voltage-gated sodium channels ($Na_Vs$) are critical for the upstroke of the action potential in peripheral sensory neurons. They are therefore crucial for peripheral somatosensation including pain sensing. Indeed, loss and gain of function mutations in a range of peripherally restricted isoforms (including $Na_V1.7$, 1.8 and 1.9) have been linked to painful and painless neuropathies. However, a recent study identified a mutation in the isoform $Na_V1.6$ in a patient with trigeminal neuralgia (Tanaka *et al*, Mol Med 2016;3(22)). Evidence from animal studies including oxaliplatin-induced pain and ciguatoxin-induced pain (Deuis et al. Neuro-oncology 2014;16(10):1324-1332; Inserra et al. Sci Rep. 2017;7:42810). In order to study $Na_V1.6$ in detail, selective pharmacological probes are required. Animal venoms are a rich source of biologically active peptides many of which selectively target ion channels such as $Na_Vs$. This work utilises *in vitro* and *ex vivo* electrophysiology along with animal behavioural studies in order to assess the function of $Na_V1.6$ in peripheral sensory neurons. Here we report that synthetically produced β-scorpion toxin peptide Cn2 is a potent and selective probe for $Na_V1.6$ *in vitro*. Cn2 causes

significant shift in voltage-dependence of activation cells that overexpress $Na_V1.6$. Functional contribution of $Na_V1.6$ to action potential generation in peripheral nerve terminals was assessed using skin-saphenous nerve recordings. There is little effect on C-fibre firing in response to $Na_V1.6$ activation by Cn2. Two distinct responses to Cn2 emerge in A-fibres. One group responds to threshold mechanical stimulus by firing high frequency, continuous, repetitive bursts of action potentials. The other group responds to mechanical stimulus with both increased firing frequency and number of action potentials per stimulus. Variation in response correlates with conduction velocity. This is in line with previous findings outlining a function for $Na_V1.6$ in mechanosensation. Furthermore, intra-plantar injection of $Na_V1.6$ selective agonists *in vivo* causes both spontaneous pain behaviours and mechanical but not thermal allodynia. Taken together, we find activation of A-fibres drives spontaneous and mechanically-induced pain behaviours. This works highlights the use of toxins in elucidating pain pathways moreover the importance of non-peripherally restricted $Na_V$ isoforms in pain generation. Furthermore, it adds to the growing body of evidence supporting a clear role for A-fibres in pain.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.04/Z14

**Topic:** D.03. Somatosensation: Pain

**Title:** CRISPR/Cas9 editing of Nf1 gene identifies CRMP2 as a therapeutic target in neurofibromatosis type 1 (NF1)-related pain that is reversed by (S)-Lacosamide

**Authors: *A. MOUTAL**[1], X. YANG[1], W. LI[1], K. GILBRAITH[1], S. YEON[2], S. LUO[1], C. QU[1], J. Y. XIE[1], M. IBRAHIM[1], K. PARK[2], F. PORRECA[1], R. KHANNA[1]
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**Abstract:** Neurofibromatosis type 1 (NF1) is a rare autosomal dominant disease linked to mutations of the *Nf1* gene. NF1 patients commonly experience severe ongoing pain. Studies on mice with *Nf1* haploinsufficiency have been instructive in identifying sensitization of ion channels as a possible cause underlying the heightened pain suffered by NF1 patients. Data on pain predisposition in $Nf1^{+/-}$ mice have led to uncertain conclusions about the role of *Nf1* in pain. We used the clustered regularly interspaced short palindromic repeats/(CRISPR)-associated 9 (CRISPR/Cas9) genome editing system to create and characterize a novel rat model of NF1-related pain. Targeted intrathecal delivery of guide RNA/Cas9 nuclease plasmid in combination with a cationic polymer was used to generate allele-specific C-terminal truncation of neurofibromin, the protein encoded by the *Nf1* gene. Rats with truncation of neurofibromin,

showed increases in voltage-gated calcium (specifically N-type or CaV2.2) and voltage-gated sodium (particularly tetrodotoxin-sensitive) currents in sensory neurons. These gains-of-function were sufficient to underlie increased nociceptor excitability as well as behavioral hyperalgesia. The cytosolic regulatory protein collapsin response mediator protein 2 (CRMP2) regulates activity of these channels, and also binds to the targeted C-terminus of neurofibromin in a tripartite complex, suggesting a possible mechanism underlying NF1 pain. Prevention of CRMP2 phosphorylation with (*S*)-lacosamide resulted in normalization of channel current densities, excitability, as well as of hyperalgesia following CRISPR/Cas9 truncation of neurofibromin. These studies reveal the protein partners that drive NF1 pain and suggest that CRMP2 as a key target for therapeutic intervention.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.05/Z15

**Topic:** D.03. Somatosensation: Pain

**Support:** Dokkyo Med Univ Endowment Fund

**Title:** Increased N-methyl-D-aspartate receptor-mediated synaptic transmission and nociceptive behavior in serine racemase knockout mice

**Authors: *E. KATO**, T. FUKUSHIMA, M. MAEKAWA, R. KONNO, Y. HORI
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**Abstract:** D-serine is considered to be an endogenous NMDA receptor ligand. The NMDA receptors play key roles in synaptic plasticity including not only learning and memory but also in persistent pain. D-serine is synthesized by glial or neuronal enzyme serine racemase (SR), and is degraded by an enzyme, D-amino acid oxidase (DAO). We previously showed that peripheral nerve injury-induced neuropathic pain is associated with an increased spinal content of D-serine (Kato et al, 2017). However, it still remains to elucidate how D-serine and its related enzymes are involved in neuropathic pain. To realize the contribution of D-serine and SR to synaptic transmission in the spinal dorsal horn, we generated SR knockout (KO) mice. Immunohistochemical staining revealed that SR was expressed in the superficial dorsal horn (SDH) in wild-type (WT) mice, while there was little SR expression in SR-KO mice. Additionally, liquid chromatography-mass spectrometry showed that the D-serine content in the SDH of SR KO mice was less than 10% of that of WT mice. Tight-seal whole-cell recordings

were made from neurons in the SDH in lumbar spinal slices. Non-NMDA and NMDA receptor-mediated excitatory postsynaptic currents (non-NMDA-EPSCs and NMDA-EPSCs) were pharmacologically isolated. The analysis showed the following: 1) The NMDA/non-NMDA ratio was significantly smaller in SR KO mice than in WT mice. 2) The time constant of decay time course of NMDA-EPSCs was significantly longer in SR KO mice than in WT mice. 3) The synaptic charge transferred during NMDA-EPSC was significantly larger in SR KO mice than in WT mice. Real-time quantitative RT-PCR analysis revealed that the expression level of NMDA receptor 2B subunit in the SDH was significantly larger in SR KO mice than in WT mice. Further, we analyzed the effects of loose sciatic nerve ligation (Bennett model), spinal nerve ligation (Chung model), and formalin injection. Behavioral observations showed the following results: 1) Mechanical allodynia in the Chung model and Bennett model was significantly augmented in SR KO mice compared to WT mice. 2) The second phase of the formalin-induced licking response was also augmented in the KO mice. Additionally, characterization of NMDA-EPSCs in SR-KO mice subjected to nerve ligation is currently under experiment. The present results suggest that SR-KO mice have a different NMDA receptor subunit composition compared to WT mice. Balance of function between SR and DAO regulates D-serine content, which may lead to alterations in NMDA receptor subunit composition. This might partly explain the differences in synaptic transmission and nociceptive behaviors between the SR-KO and WT mice.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.06/Z16

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH RO1 NS073939

NIH RO1 NS074999

**Title:** Pi16 secretion by fibroblasts as a novel regulator of neuropathic pain

**Authors: *P. SINGHMAR**, J. MA, F. BAAMEUR, X. HUO, B. PENG, C. J. HEIJNEN, A. KAVELAARS
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**Abstract:** Understanding of cellular and molecular mechanisms that induce and maintain chronic pain is central to the development of new therapeutics. G-protein-coupled Receptor

Kinase 2 (GRK2) and Exchange protein directly activated by cAMP (Epac) play an active role in the pathogenesis of pain. Previous work by our group showed that GRK2 deficiency in nociceptors promotes the transition to chronic pain via a biased cAMP-induced Epac1-to-Rap1 signaling. In this study using RNA sequencing, we compared the transcriptome of the dorsal root ganglia (DRG) of WT mice that develop transient pain and of GRK2-deficient mice showing persistent pain in response to prostaglandin E2 (PGE2). On average, more than 50 million reads were identified in each sample and the sequencing results were analyzed by the EdgeR and Cufflinks software. Analysis of differentially expressed mRNAs associated with PGE2 treatment identified Peptidase inhibitor 16 (Pi16) as the most significantly upregulated transcript in GRK2-deficient mice. Pi16 is a poorly characterized member of the CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1) superfamily of proteins. The role of pi16 in pain is unknown. The aim of this study was to determine a possible contribution of Pi16 to chronic pain. We show that Pi16 levels increase specifically in the ipsilateral lumbar DRGs and sciatic nerve in the spared nerve injury model of neuropathic pain. Pi16 knockout mice are protected against mechanical allodynia and spontaneous pain in this model. Interestingly, Pi16 is a non-neuronal protein secreted by fibroblasts in the epineurium and perineurium. Our present data supports a key role of fibroblasts in the pathogenesis of pain through secretion of Pi16. We propose that fibroblast-derived Pi16 may represent an important novel non-neuronal target for the treatment of chronic pain. Understanding how this fibroblast-derived protein regulates neuropathic pain will advance our insight into the cellular and molecular mechanisms underlying chronic pain.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.07/Z17

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant F32 NS100404-01

**Title:** Functional and transcriptional changes in somatosensory neurons after peripheral nerve injury

**Authors: *I. TOCHITSKY**[1,4], W. RENTHAL[2], B. SINGH[5], I. CHIU[1], M. E. GREENBERG[3], C. J. WOOLF[6]
[1]Harvard Med. Sch., Boston, MA; [2]Neurobio., Harvard Med. Sch., Brookline, MA; [3]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [4]Neurobio., Boston Children's Hosp., Boston, MA;

[5]FM Kirby Neurobio. center, Boston Children's Hosp. Harvard Med. Sch., Boston, MA; [6]Neurobio., Children's Hosp. Boston, Boston, MA

**Abstract:** Neuropathic pain is a common disorder typically caused by disease of or damage to the peripheral nervous system. Spontaneous pain is a major feature of neuropathic pain disorders and is thought to be generated by the ectopic activity of peripheral somatosensory neurons. Unfortunately, it is not known which subtypes of peripheral sensory neurons become spontaneously active after nerve injury or what changes in gene expression cause their hyperexcitability. Here, we present functional and transcriptional data exploring the changes that take place in injured somatosensory neurons and contribute to their hyperexcitability. We used the sciatic nerve transection and spared nerve injury animal models of neuropathic pain. Sciatic nerve injury leads to pain behaviors such as heat and mechanical hyperalgesia, and mechanical allodynia, which are also present in patients with neuropathic pain. At various time points after nerve injury, we dissected and cultured the injured and naive lumbar L3-L5 dorsal root ganglia (DRG) neurons in vitro and then performed a range of functional and transcriptional analyses on these cultured neurons. We observed a higher incidence of spontaneous activity in the ipsilateral injured DRG neurons as compared to the naive control DRG neurons using both patch clamp electrophysiology and calcium imaging methods. Additionally, the injured DRGs were less excitable to a variety of electrical stimuli. We also observed a lower current density of voltage gated potassium currents in the ipsilateral DRG neurons, which would be consistent with their hyperexcitability. Finally, we performed a single cell transcriptional analysis on injured vs naive DRG neurons and found a number of differences in gene expression that are associated with injury as well as consistent with injury-induced hyperexcitability. We believe that a combined functional and transcriptional analysis may help identify changes that take place in defined subpopulations of somatosensory neurons in neuropathic pain and potentially identify new drug targets for the treatment thereof.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.08/Z18

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** NIH R01NS038253

**Title:** The role of intrinsic excitability in peripheral sensory neuropathy

**Authors: *N. WIMALASENA**[1], Y.-C. CHENG[2], J. SHIM[2], C. J. WOOLF[2]
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**Abstract:** Peripheral sensory neurons innervating the skin, muscle and viscera relay crucial information about thermal, mechanical, and chemical stimuli to the central nervous system. Small fiber neuropathy (SFN) is a chronic polyneuropathy, characterized by slow, length-dependent, bilateral degeneration of multiple nerve fibers. In SFN, small diameter axons and terminals of sensory Aδ and C sensory fibers selectively 'die back'. Recently, the disorder has been linked to gain-of-function mutations in voltage-gated sodium channels: $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$, raising questions as to whether hyperexcitability causes or increases susceptibility to peripheral degeneration and neuropathy. We utilize CRISPR/Cas9 to introduce human SFN-linked $Na_v1.7$ mutations into mice as well as human induced pluripotent stem cells (hiPSC). We are then interrogating mutant mouse primary DRG neurons and human sensory neurons derived from the mutant hiPSC for altered excitability and defects in neurite length and innervation pattern, as well as other signs of cellular stress, to better understand whether the hyperexcitability caused by these mutations is causally linked to dying back sensory neuropathy.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.09/Z19

**Topic:** D.03. Somatosensation: Pain

**Title:** Slow changes in neuronal excitability in the spinal superficial dorsal horn evoked by the activation of unmyelinated primary afferents

**Authors: *K. KANEKO**, T. SAOTOME, Y. NUMATA, T. TERASHIMA, S. YAMAGUCHI, Y. HORI
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**Abstract:** Synaptic response in the spinal dorsal horn evoked by the stimulation of myelinated primary afferent fibers (Aβ and Aδ fibers) are well characterized. We investigated how the activation of unmyelinated primary afferent fibers (C fibers) affects synaptic activity and neuronal excitability in the spinal dorsal horn by means of voltage-sensitive dye imaging. Under halothane anesthesia, three-week-old male ICR mice were subjected to partial ligation of the sciatic nerve (Seltzer model). Transverse slices of 500 μm thickness with the dorsal root attached at the lumber enlargement level were obtained seven days after the nerve ligation. The slices were stained with the voltage-sensitive dye Di-4-ANEPPS. Di-4-ANEPPS fluorescence was measured using a MiCAM2 optical imaging system (Brain Vision Inc., Japan). A decrease or

increase in the fluorescence of the dye indicates membrane hyper polarization or depolarization, respectively. We analyzed fluorescence changes in the superficial dorsal horn (SDH) in response to electrical stimulation of the dorsal root at C-fiber strength. Mechanical allodynia was observed in the nerve-ligated mice. The stimulation of the dorsal root evoked fluorescence changes in the SDH that lasted more than one second. In the presence of the $GABA_A$ receptor antagonist bicuculline (Bic), the glycine receptor antagonist strychnine (Str), the NMDA receptor antagonist APV, and the non-NMDA receptor antagonist CNQX, a small but significant response remained. The remaining response was further reduced by the application of the NK-1 receptor antagonist CP99994. The CP99994-sensitive component was significantly smaller in mice that had been subjected to neonatal capsaicin applications. Thus, this long-lasting depolarizing response is mediated by Substance P (SP), which is released upon electrical stimulation of unmyelinated primary afferent fibers. After the SP-mediated response was blocked by CP99994, small but distinct fluorescence changes were still evoked by dorsal root stimulation. The magnitude of the CP99994-resistant component was larger in nerve-ligated mice than in control mice. Additionally, peripheral nerve injury is known to downregulate the expression of Na+/K+ ATPase. Thus, the observed long-lasting CP99994-resistant fluorescence changes might be attributed to the change in extracellular potassium concentrations elicited by the dorsal root stimulation. Finally, the voltage-sensitive dye imaging system could provide useful tools to observe slow changes in neuronal activity. In addition, we are currently investigating the effect of the glial cell inhibitor fluorocitrate on the CP99994-resistant component.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.10/Z20

**Topic:** D.03. Somatosensation: Pain

**Title:** GABAergic system in the spinal sensory ganglia

**Authors: *R. RAMLI**[1,2], J. DEUCHARS[1,3], X. DU[3], H. ZHANG[3], N. GAMPER[1]
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**Abstract:** Peripheral nerves convey to the brain versatile information about the body's environment and are responsible for somatosensory sensations, including pain. Healthy peripheral nerves conduct action potentials without interruption from their sites of origin (peripheral nerve endings), to dorsal spinal cord where synaptic communication first takes place. It is assumed that in the spinal cord, and subsequently in the central nervous system, the

peripheral somatosensory signals are integrated and analyzed. Surprisingly, cell bodies of sensory neurons express multiple receptors for classical neurotransmitters such as glutamate and GABA. Yet, the role of these somatic receptors and the source(s) of neurotransmitters that activate them is only beginning to emerge. Our recent data suggest that dorsal root ganglion (DRG) neuron cell bodies contain fully functional GABAergic communication system that can modulate nociceptive transmission (1). Here we aimed to establish the sensory modalities of DRG neurons that can release GABA. To this end, we performed immunohistochemical investigation of the vesicular GABA transporter (VGAT) expression within DRG of adult male Wistar rats. VGAT expression was found in subpopulations of DRG neurons of all sizes but was twice as frequent in larger- diameter neurons as compared with small ones (30% of large neurons vs. 15-20% of small and medium). VGAT immunofluorescence was found in 27.5% of TRPV1-positive, 16.67% of IB4-positive, 50% of Nf200-positive and in 78.62% of TrkC-positive neurons. There was a strong colocalization of VGAT with the synaptic vesicle marker, SV2. VGAT immunofluorescence was absent in satellite glia (as confirmed with the glial marker S100b). We then tested if VGAT in DRG localizes to the releasable vesicles. We incubated live cultured DRG neurons with the C-terminal VGAT antibody (VGAT-C) that binds to the luminal part of the transporter and, thus, can only bind to VGAT during the vesicle fusion event. Depolarization of DRG cultures resulted in robust uptake of VGAT-C, but not an N-terminal VGAT antibody; the uptake was significantly reduced by exclusion of $Ca^{2+}$ from the extracellular solution. These results describe functional GABA release machinery in a subset of DRG neurons that is likely to contribute to the modulation of nociceptive transmission. (1) Du X, Hao H, Yang Y, Huang S, Wang C, Gigout S, Ramli R, Li X, Jaworska E, Edwards I, Deuchars J, Yanagawa Y, Qi J, Guan B, Jaffe DB, Zhang H, Gamper N. (2017) Local GABAergic signaling within sensory ganglia controls peripheral nociceptive transmission. J Clin Invest,127:1741-1756

**Disclosures:** **R. Ramli:** None. **J. Deuchars:** None. **X. Du:** None. **H. Zhang:** None. **N. Gamper:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.11/Z21

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant GM103801

**Title:** Identifying key residues involved in α-conotoxin RgIA blockade of α9α10 nicotinic acetylcholine receptors: Insights into a long-lasting, non-opioid analgesia

**Authors: *P. HUYNH**[1], P. J. HARVEY[2], S. B. CHRISTENSEN[1], D. J. CRAIK[2], J. M. MCINTOSH[1]
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**Abstract:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common complication of cancer treatments. Oxaliplatin is a first line drug for colorectal cancer that often produces neuropathic pain, including severe cold allodynia, often limiting the dose or duration of drug. Treatment with the specific α9α10 nicotinic acetylcholine receptor (nAChR) antagonist, α-conotoxin RgIA4, attenuates the development of oxaliplatin-induced cold allodynia. Here, we show that coadministration of RgIA4 with oxaliplatin not only relieves cold allodoynia during treatment, but also prevents this symptom for at least 3 weeks beyond cessation of treatment, suggesting a possible disease modifying effect. The parent compound RgIA is a bicyclic peptide with two disulfide bridges. It is a competitive antagonist of acetylcholine, binding at the α9(-)α10(+) nAChR subunit interface. Previous structural studies demonstrated that residues in the first cysteine loop of RgIA are essential for activity. Here, we demonstrate that residues in the second cysteine loop and at the C-terminus allow for the fine tuning of potency and binding kinetics. Modification of the identified residues remedy the discrepancy in potency between rat and human α9α10 nAChRs, closing a ~300-fold affinity gap. Solution NMR, molecular modeling studies, and two-electrode voltage clamping assays provide mechanistic insight into the mode of action at α9α10 nAChRs.

**Disclosures: P. Huynh:** None. **P.J. Harvey:** None. **S.B. Christensen:** None. **D.J. Craik:** None. **J.M. McIntosh:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.12/Z22

**Topic:** D.03. Somatosensation: Pain

**Support:** R21-AA023051

R01-DA018156

T32-AA014127

P50-AA022534

**Title:** T cell sex differences unmasked by β2-integrin antagonist in neuropathic mice

**Authors: *M. S. SUN**[1], S. NOOR[1], A. G. VANDERWALL[1,2], M. A. HAVARD[2], J. E. SANCHEZ[1], N. W. HARRIS[1], J. J. SANCHEZ[1], M. V. NYSUS[3], T. ANDERSON[3], J. P. NORENBERG[3], E. D. MILLIGAN[1,2]
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**Abstract:** Recent evidence in rodent models of peripheral neuropathy suggests sex differences may exist in immune cell phenotype and function underlying neuropathic pain. BIRT377 (BIRT) is a small molecule inhibitor of lymphocyte function-associated antigen 1 (LFA-1). LFA-1 is an adhesion molecule expressed on several immune cell types (e.g. T cells and monocytes) important for migration into areas of tissue damage. Allodynia, which is clinically observed as pathological sensitivity to light touch, can be induced in rodents following sciatic nerve damage using the well-characterized model, chronic constriction injury (CCI). Recent reports revealed that immune T cell actions in female rodents, but not in males, play a greater role in generating allodynia. Therefore, we hypothesized that the pain therapeutic effect of BIRT in females occurs via its actions on T cells while BIRT efficacy in males is T cell-independent. Following baseline (BL) hindpaw response thresholds to light touch stimulation (von Frey fiber test), all male and female mice (C57BL/6; ~3.5 months; N= 6/gp) underwent either sham or CCI (sciatic loose ligation of three 4-0 vs. 5-0 chromic gut sutures) surgery with response thresholds reassessed every 3-5 days for 56 days. In a separate group of aged-matched male and female C57BL/6 mice (N=6/gp) following BL and post-CCI (using three 5-0 chromic gut sutures) hindpaw assessment to Day 10, intravenous BIRT (i.v., tail vein; 2.5µg in 50µL) or equivolume vehicle was injected with daily response threshold reassessments through Day 24. This experiment was repeated in a third group of mice (C57BL/6, N=6/gp) but was terminated at maximal i.v. BIRT reversal efficacy followed by tissue collection for mRNA analysis. Lastly, T cells collected from naïve male and female mice (C57BL/6 aged-matched), cultured for 5 days +/- BIRT, were analyzed using flow cytometry to identify T cell-subsets expressing cytokines indicative of pain-related inflammatory (interleukin [IL]-17, RORɣ) or pain-suppressive anti-inflammatory (IL-10, transforming growth factor [TGF]β) function. Results reveal male and female mice develop allodynia similarly at onset, degree and duration, and similarly respond to i.v. BIRT with allodynic reversal lasting ~5 days. Sex differences in naïve T cell responses to BIRT revealed decreased IL-17 and RORɣ and increased IL-10 and TGFβ measured only in female-derived T cells, while no alterations were observed in male-derived cells. RNA analysis is ongoing. These data indicate female T cells are a key target underlying pain therapeutic effects of BIRT while in males, efficacy occurs through T cell-independent mechanisms.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.13/Z23

**Topic:** D.03. Somatosensation: Pain

**Title:** Nicotinic acetylcholine receptor beta 2 may be involved in pain pathways: Pilot study from CCI model

**Authors: *S. OCHI**[1], T. NISHIHARA[2], Y. YOSHINO[1], K. YAMAZAKI[1], J. IGA[1], T. YOROZUYA[2], S.-I. UENO[1]
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**Abstract:** Introduction: Brain nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels composed of a number of subunits (α2-10 and β2-4). Recently, it is reported that nicotinic acetylcholinergic mechanisms play important roles in regulating process of pain pathways. Thus, we investigated mRNA expression of several nAChRs subunits in the brain tissues using a rat model of chronic constriction injury (CCI) to clarify the new role of nAChRs. Materials and Methods: Wistar male rats (8 weeks old) were used, and their left sciatic nerve was exposed at the mid-thigh level through a gluteal muscle-splitting incision under anesthesia. Three loosely constrictive chromic gut ligatures were applied to produce CCI rat model. Sham rats were only exposed left sciatic nerve. Sensitivity to mechanical stimulation was measured by von Frey test using up-and-down method until 3 weeks. For gene expression analysis, rats were sacrificed 3 weeks later, and brain tissues from four regions (frontal cortex, thalamus, hippocampus, and amygdala) were dissected bilaterally on an ice-cold stage. The total RNA fraction of each tissue was extracted and treated with reverse transcriptase to keep as cDNA. The mRNA expression levels of the nAChR subunits genes; α3 (*chrna3*), α4 (*chrna4*), α7 (*chrna7*), β2 (*chrnb2*), and β4 (*chrnb4*), were calculated by real time PCR method with Taqman probe after GAPDH normalization. The calibrator cDNA of a matched brain region was randomly selected from 6 naive rats. The comparative CT method provides a relative quantification ratio according to the calibrator, allowing statistical comparison of gene expression among samples. Values from CCI and sham groups represented averages of triplicate measurements for each sample (n = 6, each). Results: The hind paw withdrawal thresholds in left side of CCI treated rats to von Frey stimulation were significantly reduced than those of others. *Chrnb2* mRNA expression in right thalamus was significantly increased than in the left in CCI groups, but was not significantly different in sham groups. However, there was no c*hrnb2* expressional difference between right and left sides in the frontal cortices of CCI rats. No other subunits showed significant laterality. Conclusion: The *chrnb2* expression in the thalamus may be involved in pain pathways.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.14/Z24

**Topic:** D.03. Somatosensation: Pain

**Title:** Epigenetic regulation by sigma-1 receptor in dorsal root ganglion: Link to neuropathic pain

**Authors: *H.-E. WU**[1], T.-C. SU[1], D. HUNG[1], T.-P. SU[2]
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**Abstract:** Primary sensory neurons at the dorsal root ganglions (DRGs) causes neuropathic pains, when undergoing pathophysiologic changes that lead to neuronal hyperexcitability, and are important target for treating neuropathic pains. The ER chaperone sigma-1 receptor (Sig-1R) has been known to play a role in neuropathic pains and has been shown to exist in DRGs notably at the nuclear envelope. However, the molecular event that may link Sig-1R to the modulation of neuropathic pain at the DRG is largely unknown. Recently it was reported that inhibition of histone methylation could reduce neuropathic pain by restoring potassium channel function in the DRG. Thus, we examined in the present study if the Sig-1R may play a role in the epigenetic regulation in DRGs with a specific focus on the Sig-1R effect on enzymes that affect the histone methylation and acetylation. The protein levels of those enzymes and their resultant activities as seen in the modification of respective substrates were compared in DRGs taken from wild type and Sig-1R knockout mice. Immunoblotting in Sig-1R knockout DRGs, when compared to those seen from wild type samples, showed a decreased expression of the enhancer of zeste homolog 2 methyltransferase (EZH2), euchromatic histone-lysine N-methyltransferase-2 (G9a), and histone deacetylase 4 (HDAC4), whereas exhibited an increased expression of the mixed-lineage leukemia 1 methyltransferase (MLL1). Correspondingly, the resultant level of the substrate for EZH2 and G9a, i.e., H3K27me3 and H3K9me2 respectively, was reduced while that (H3K4me3) for MLL1 was increased. However, the level of the substrate for HDAC4 acetyl H3 did not differ. In addition, we found a co-immunoprecipitation between Sig-1R and EZH2 in HEK293T cells. Taken together, our results suggest that the endogenous Sig-1R may participate in the genesis of neuropathic pain through the epigenetic modification of histone in the DRG. (Supported by the IRP, NIDA, NIH).

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.15/Z25

**Topic:** D.03. Somatosensation: Pain

**Support:** Grant-in-Aid for Scientific Research (C) 16K09001

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**Title:** Search of BEGAIN binding protein in the brain

**Authors: *T. KATANO**, S. ITO
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**Abstract:** We had been identified many proteins in the spinal dorsal horn related to chronic pain. Recently, we reported about brain-enriched guanlylate kinase-associated protein (BEGAIN) as a new responsible protein to neuropathic pain after spared nerve injury (SNI) in the spinal dorsal horn using knock-in mouse of Tyr1472 to Phe of GluN2B (eNeuro 3(5), e0110-16, 2016). Neuronal plasticity observed in chronic pain and memory in the spinal cord and / or the brain is regulated by many synaptic molecules, some of which are shared in their plasticity. In our previous report, BEGAIN also expresses in the cerebral cortex and hippocampus higher than in the spinal dorsal horn, and modifies the activity of NMDA receptors. However, it is not clear how BEGAIN acts on NMDA receptor function as a synaptic molecule. In the present study, to clarify the molecular functions of BEGAIN, we searched the BEGAIN binding proteins from synaptic fraction in the cerebral cortex. An approximately 80-kDa protein (p80) was purified as BEGAIN-binding protein by using a BEGAIN immobilized column. P80 had been identified as an increased protein in the spinal dorsal horn after SNI in our previous research as well as BEGAIN. Analysis of the molecular function of BEGAIN through interaction with p80 is in progress.

**Disclosures: T. Katano:** None. **S. Ito:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.16/Z26

**Topic:** D.03. Somatosensation: Pain

**Support:** National Natural Science Foundation of China ( Grant no. 81300967)

**Title:** Amygdala NMDA receptor involvement in mediating sensory-discriminative and affective-motivational pain responses after chronic constriction nerve injury

**Authors: T. ZHANG**, H. XU, W. CHEN, W. HUANG, *C.-W. CHOU
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**Abstract:** Many studies and intervention on neuropathic pain have focused on the spinal cord level, but the efficacy of treating neuropathic pain is to date unsatisfactory. As many patients suffering from neuropathic pain usually have affective- motivational pain responses, the aim of this study is to investigate the relationship between the affective-motivational dimension of pain and the changes in the supraspinal region.Amygdala is a center of affective modulation in the central nervous system and it has emerged as an important brain region for pain modulation.NMDA (N-m ethyl-D-aspartate) receptor has been proven to be involved in the initiation and maintenance of neuropathic pain in the spinal cord level,but little is known about its role in amygdala. Therefore,here we hypothesize that the NMDA receptors in amygdala might be associated with the affective-motivational pain responses in neuropathic pain condition. To test this hypothesis, a rat model of chronic constriction injury (CCI) was used to induce neuropathic pain and a paradigm that reflecting the motivational-affective aspect of pain was established in this study(Place avoidance testing). After CCI, rats showed mechanical allodynia on postoperative day 14 and the time spent in light side of the chamber was also reduced significantly. Stereotaxic injection of NR1/NR2A antagonist (TCN 201)into amygdala (CeA) alleviated mechanical allodynia and the time spent in light side of the chamber was increased when compared with vehicle group.While injection of NR2B antagonist (Co 101244 hydrochloride) only altered the time spent in light side of the chamber, but did not alleviate mechanical allodynia. It suggests that different subtypes of NMDA receptors in amygdala may involve in different dimensions of pain process after injury.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.17/Z27

**Topic:** D.03. Somatosensation: Pain

**Support:** CA200263

H.E.B.Professorship in Cancer Research

**Title:** Blocking MCP-1/CCR2 signaling attenuates oxaliplatin-induced mechanical allodynia

**Authors: A. M. ILLIAS**[1], H. ZHANG[2], A. K. KOSTURAKIS[3], *P. M. DOUGHERTY[4]
[1]The Univ. of Texas M.D. Anderson Cancer Ctr., Houston, TX; [2]The Univ. of Texas Hlth. Sci. Ctr. Houston, Houston, TX; [3]The Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX; [4]Pain Med., Howard Hughes Med. Inst. - Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

**Abstract:** Chemotherapy-induced peripheral neuropathy (CIPN) is the most common dose-limiting toxicity for several anti-cancer agents. However, the mechanism behind CIPN is yet to be determined. The dorsal root ganglia (DRG) are the most susceptible target for neuroinflammation caused by the upregulation of cytokines and chemokines following chemotherapy. In this study, we first studied the potential effect of monocyte chemoattractant protein-1 (MCP-1), and its receptor CCR2 signaling within rat dorsal root ganglion (DRG) on single dose oxaliplatin-induced mechanical allodynia. After a single injection of 3mg/kg oxaliplatin, mechanical allodynia increased from the first day after oxaliplatin injection and persisted until day 15. Immunohistochemistry staining (IHC) showed that MCP-1 signaling tended to increase as early as 4hrs after oxaliplatin treatment, followed by a significant upregulation in MCP-1/CCR2 signaling on day 5, but both signals were normalized when tested on day15. These results suggested that with single dose oxaliplatin, MCP-1/CCR2 signaling contributed to the induction and severity of mechanical allodynia but not in the maintenance of this pain. In the same experiment, co-treatment with intrathecal anti-MCP-1 prevented oxaliplatin-induced mechanical allodynia but it was not able to reverse the pain when given 1 week after chemotherapy. Minocycline is a semi-synthetic tetracycline antibiotic with anti-inflammatory properties that has been used to attenuate neuropathic pain in several published studies. Here we found that rats treated with 2mg/kg oxaliplatin, to a accumulative dose of 8mg/kg, demonstrated less allodynia throughout the experiment (day 1, 7,and 14) if they were co-treated with minocycline. IHC staining on day 7 showed a marked decrease in MCP-1 level in rats co-treated with minocycline. This is the first study to demonstrate MCP-1/CCR2 signaling in two different models of oxliplatin related CIPN, and it further shows that blocking of this signal can attenuate oxaliplatin-induced mechanical allodynia.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.18/Z28

**Topic:** D.03. Somatosensation: Pain

**Title:** Injured dorsal root ganglion neuron-derived FLRT3 induces neuropathic pain via Unc5b

**Authors: *M. YAMADA**[1], Y. FUJITA[2], T. YAMASHITA[2]
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**Abstract:** Neuropathic pain is a type of chronic pain, frequently occurs after nerve injury, and induces hypersensitivity or allodynia resulting in aberrant neuronal excitability in the spinal cord dorsal horn. Our previous study revealed that Netrin-4 secreted from dorsal horn interneurons binds to Unc5B, and enhances the excitatory synaptic transmission, leading neuropathic pain. In addition to Netrin-4, Unc5B has been reported to bind several molecules including Fibronectin type III domain Leucine-rich repeat transmembrane protein 3 (FLRT3). FLRT3 is known as a modulator of neurite outgrowth, axon pathfinding and cell adhesion. Interestingly, FLRT3 is upregulated following axotomy of the sensory neurons, although the function of FLRT3 after peripheral nerve injury remains unknown. Here, we investigated whether spinal FLRT3 is involved in neuropathic pain. In the spinal cord dorsal horn, FLRT3 protein was increased at 7 and 14 days after sciatic nerve ligation, and its immunoreactivity was distributed in lamina II, particularly in the lesion of IB4-loss. The expression of FLRT3 mRNA was upregulated in the dorsal root ganglion (DRG), but not in the spinal cord after injury, suggesting that spinal FLRT3 was derived from DRG. In DRG, FLRT3 signal was observed in cell plasma of ATF3-positive injured sensory neurons. Intrathecal administration of FLRT3 protein to naïve rats induced mechanical allodynia and phosphorylated GluN2B in the spinal cord. In addition, DRG-specific FLRT3 overexpression also developed mechanical allodynia in rats by using adeno associated virus vector. Conversely, administration of an antibody, which inhibits FLRT3-Unc5B interaction, attenuated mechanical allodynia after partial sciatic nerve ligation. Our findings suggest that FLRT3, which is derived from injured DRG neuron, increases the excitability of the dorsal horn and induces mechanical allodynia via Unc5B receptor.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.19/Z29

**Topic:** D.03. Somatosensation: Pain

**Support:** NIAAA #AA023051

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**Title:** Endogenous IL-10 is not required for spinal non-viral IL-10 transgene expression to treat neuropathic pain resulting in anti-inflammatory factors in DRG and spinal cord

**Authors: *A. G. VANDERWALL**[1,2], S. NOOR[1], J. E. SANCHEZ[1], M. S. SUN[1], X. O. YANG[3], L. L. JANTZIE[4], N. MELLIOS[1], E. D. MILLIGAN[1,2]
[1]Dept. of Neurosciences, [2]Dept. of Anesthesiol. and Critical Care, [3]Dept. of Mol. Genet. and Microbiology, [4]Dept. of Pediatrics, Univ. of New Mexico, Albuquerque, NM

**Abstract:** **Background:** Anti-inflammatory cytokine interleukin-10 (IL-10) reverses neuropathic pain in animal models by inhibiting actions of proinflammatory cytokines (e.g. IL-1β and tumor necrosis factor α [TNFα]). D-mannose (DM) is a mannose receptor (MR; CD206) agonist that improves therapeutic efficacy of non-viral IL-10 gene therapy (pDNA-IL-10; naked IL-10 plasmid). MR is a scavenger receptor but activation may increase IL-10 signaling. However, we previously presented DM+pDNA-IL-10 is efficacious in IL-10 deficient (IL-10 KO) mice following nerve damage (Vanderwall *et al.*, SfN 2016). Therefore, we examined whether enduring DM+IL-10 gene therapy induces local anti-inflammatory factors that act to diminish pain-generating proinflammatory factors.
**Methods:** IL-10 KO mice underwent unilateral sciatic Chronic Constriction Injury (CCI) vs. Sham surgery. Light touch sensitivity (allodynia) was assessed before and after surgery to Day 5, at which time intrathecal (i.t.) DM+pDNA-IL-10 vs. various control treatments was given followed by repeated assessment for allodynia through Day 17. Thereafter, tissues were collected (bilateral lumbar [L3-5] dorsal root ganglia [DRG] and cauda equina [CE]) for qRT-PCR mRNA analyses. Astrocytes in lumbar spinal cord (LSC) from previously behaviorally verified mice (citation above) were analyzed by GFAP immunoreactivity.
**Results:** While sham mice remained stably non-allodynic, CCI/DM+pDNA-IL-10 mice showed complete bilateral behavioral reversal throughout the timecourse compared to CCI-treated i.t. gene therapy controls. However, CCI mice given DM without pDNA-IL-10 revealed transient reversal from allodynia. Significant IL-10 mRNA was detected in CCI/DM+pDNA-IL-10 mice in both CE and ipsilateral (ipsi) DRG. Curiously, Sham/DM+pDNA-IL-10 mice did not reveal elevated IL-10 mRNA levels. While IL-1β (*il1b*) levels did not differ between groups, decreased TNFα (*tnf*) occurred in ipsi DRG from CCI/DM+pDNA-IL-10 mice compared to allodynic controls. The anti-inflammatory cytokine TGF-β1 (*tgfb1*) increased. Lastly, MR (*mrc1*) is expressed in ipsi DRG under all conditions. LSC dorsal horn astrocyte activation was decreased in CCI/DM+pDNA-IL-10 mice compared to allodynic controls. Ongoing studies will investigate changes post-gene therapy in contralateral DRG & LSC mRNA and LSC dorsal horn microglia.
**Conclusions:** IL-10 transgene is identified only under pathological conditions in pain-relevant tissues following a safe and unique non-viral DM+pDNA-IL-10 therapy with consequent anti-inflammatory changes in the DRG & LSC. Targeting DRG by DM+IL-10 gene therapy may ameliorate pathological spinal processes.

**Disclosures:** **A.G. Vanderwall:** None. **S. Noor:** None. **J.E. Sanchez:** None. **M.S. Sun:** None. **X.O. Yang:** None. **L.L. Jantzie:** None. **N. Mellios:** None. **E.D. Milligan:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.20/Z30

**Topic:** D.03. Somatosensation: Pain

**Title:** Cellular census of dorsal root ganglia by Drop-seq reveals new molecular targets for chronic pain

**Authors: *R. TONELLO**[1], S. LEE[2], A. CHAMESSIAN[3], Y. QADRI[4], Y. KIM[5], G. CHUNG[6], T. BERTA[1]
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**Abstract:** Dorsal root ganglia (DRGs) are the first gateway to the somatosensory system containing the cell bodies of primary sensory neurons and other numerous non-neuronal cells such as satellite glial cells (SGCs), macrophages and few T cells. Anatomical and functional evidence suggests that primary sensory neurons rely on interplays with these non-neuronal cells for maintenance of homeostasis and proper somatosensory functions. Conversely, dysfunctions of primary sensory neurons and non-neuronal cells in DRGs associated with tissue or nerve injury can result in somatosensory disorders, including chronic pain. Although high-throughput single-cell RNA sequencing (RNA-seq) have emerged as powerful tools to study the biology of cells in the brain, this approach has not been used yet to provide a comprehensive pictures of neuronal and non-neuronal cells in the DRGs. We have now established the use of Drop-seq, a high-throughput microfluidic technique that combining nucleic acid barcoding and RNA-seq, to analyze the transcriptome of thousands of individual DRG cells. We have identified and visualized 13 clusters of cells in DRGs according to their transcriptional profiling and expression of distinct marker genes, such as Tac1 for peptidergic nociceptors, Il31ra and MrgprA3 for pruriceptors, and Kcnj10 (i.e. Kir4.1) for SGCs. We are currently exploring new candidates in neuronal-glial signaling in the setting of chronic pain. This work provide the first comprehensive cellular census of DRG tissue and most importantly the identification of new neuronal and non-neuronal molecular signatures that may result in alternative therapeutic targets for the prevention and resolution of chronic pain, debilitating condition that affect more than 100 million Americans and for which there are few treatment options.

**Disclosures:** **R. Tonello:** None. **S. Lee:** None. **A. Chamessian:** None. **Y. Qadri:** None. **Y. Kim:** None. **G. Chung:** None. **T. Berta:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.21/Z31

**Topic:** D.03. Somatosensation: Pain

**Support:** MRC New Investigator Reasearch Grant (Franziska Denk)

Senior Wellcome Trust Investigator Award (Stephen B McMahon, Douglas Lopes)

**Title:** Characterization of peripheral neuron enhancer profiles in a model of neuropathic pain

**Authors: *F. DENK**, D. LOPES, S. B. MCMAHON
King's Col. London, London, United Kingdom

**Abstract:** Chronic neuropathic pain is frequently intractable to treatment and has a devastating impact on many lives. Its mechanisms are not fully understood, but there is evidence to suggest that hyperexcitability of peripheral nerves plays a major role. We hypothesize that some of this long-lasting hyperexcitability might be caused by changes to neuronal enhancer profiles. Enhancers are regions of the genome that regulate gene expression. Their location is cell-type specific and largely fixed once development is complete, though there have been reports of stimulus-dependent alterations in adult immune cells (e.g. Ostuni et al. 2013, Kaikkonen et al. 2013, Denk et al. 2016). In neurons, many enhancers are known to be induced by activity (Kim et al. 2010), and the same gene can be regulated by different enhancer combinations in a stimulus-specific manner (Joo et al. 2016). It is therefore conceivable that neuropathic injury alters the enhancer function of peripheral neurons to more permanently affect their transcriptional profile and ultimately their excitability.
This study used an animal model of chronic neuropathic pain. Male mice underwent partial sciatic nerve ligation or sham surgery and were sacrificed eight days or 28 days later. Dorsal root ganglia (DRG) were extracted, and nociceptors purified with the help of magnetically activated cell sorting. Cell-type specific transcriptional changes were assessed by RNA sequencing (n = 4). Enhancers were examined using chromatin immunoprecipitation and sequencing (ChIP-seq, n = 4) to probe for known associated histone modifications (H3k4me1, H3k27ac).
Contrary to transcriptional studies on mixed tissue, nociceptor-specific changes eight days after nerve injury did not center on immune-related genes, but exposed a signature primarily related to the consequences of axotomy.
Basic enhancer profiles as measured by H3K4me1 did not appear to be substantially altered by the pain state, either at 8 or 28 days.
In summary, our data so far suggest that peripheral neurons are distinct from immune cells, in that they do not display stimulus-induced remodeling of H3K4me1 enhancer marks. In depth

analysis of H3k27ac profiles, and hence conclusions about the effects of nerve injury on enhancer activity, are still outstanding.

**Disclosures: F. Denk:** None. **D. Lopes:** None. **S.B. McMahon:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.22/Z32

**Topic:** D.03. Somatosensation: Pain

**Support:** Neural and Pain Science, University of Maryland Dental School

**Title:** Metabolic mechanisms of bortezomib-induced painful peripheral neuropathy

**Authors: *O. K. MELEMEDJIAN**[1], T. LUDMAN[2]
[1]Dept. of Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD; [2]Univ. of Maryland, Baltimore, MD

**Abstract:** Chemotherapy-induced painful peripheral neuropathy (CIPN) is the most common toxicity associated with widely used chemotherapeutics. CIPN is the major cause of dose reduction or discontinuation of otherwise life-saving treatment. Unfortunately, CIPN can persist in cancer-survivors which adversely affects their quality of life. Moreover, available treatments have limited efficacy which warrants a better understanding of the biochemical changes that occur in response to chemotherapeutics. We have determined that the chemotherapeutic, bortezomib, alters the metabolism of sensory neurons where the production of energy becomes predominately reliant on glycolysis and to a lesser extent on oxidative phosphorylation. This metabolic phenotype is known as aerobic glycolysis. Thus, we propose to test the hypothesis that bortezomib induces aerobic glycolysis in sensory neurons which leads to the development of CIPN. Using molecular, biochemical and metabolic assays we characterized the metabolic changes that occur in sensory neurons in response to bortezomib. Moreover, we determined the mechanisms through which bortezomib initiates the biochemical changes that alter the metabolism of sensory neurons. Lastly, we have determine that targeting the molecules that maintain aerobic glycolysis alleviate CIPN. These data suggest that the development of therapeutic strategies that target aerobic glycolysis might prevent the development and maintenance of CIPN.

**Disclosures: O.K. Melemedjian:** None. **T. Ludman:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.23/AA1

**Topic:** D.03. Somatosensation: Pain

**Support:** Ministry of Education, Science and Culture, Sports, Science and Technology of Japan 26293412

**Title:** Up-regulation of inflammatory cytokines in trigeminal ganglia after infraorbital nerve constriction

**Authors: T. IWASA**[1], R. ARAKAKI[2], M. OSHIMA[1], S. AFROZ[1], M. INOUE[1], N. GOTO[1], N. ISHIMARU[2], *Y. MATSUKA[1]
[1]Stomatognathic Function and Occlusal Reconstruction, [2]Oral Mol. Pathology, Tokushima University/ Biomed. Sci., Tokushima, Japan

**Abstract:** Neuropathic pain (NP) is one of the most severe kinds of pain and many patients suffer with this condition. However available therapies are largely ineffective and inadequate in treating patients. NP is reported to be caused by peripheral nerve injury which induces hyper-excitability of the neurons in the sensory ganglion. Despite the absence of synaptic contacts in adult sensory ganglia, somata of sensory neurons can be transiently depolarized and cross-excited by activation of neighboring neurons within the same ganglion. Our previous research had shown that neurotransmitters are released within the peripheral sensory ganglia, which are responsible for causing pain. Further we have shown that inhibition of the release of these neurotransmitters reduced pain behavior in animal NP models. Our present research is focused on exploring the role of glial cells of trigeminal ganglion (TG) in NP. We are checking the release of cytokines from the primary culture of satellite glial cells (SGC) or Schwann cells of TG. We also aim to explore the neuron-glial interaction due to release of such cytokines. In the present study, we analyzed the cytokine up-regulation in TG cells in an animal model of orofacial NP. This model was created by infraorbital nerve constriction (IONC) surgery in Sprague-Dawley male rat. On the 7th day of IONC surgery, TGs were dissected and snap frozen for the fluorescent immunohistochemistry. SGC's were identified by their expression of glutamine synthetase (GS). Glial fibrillary acidic protein (GFAP), a marker of activation of SGC's was also analyzed. Co-expression of GS and GFAP on the constricted side of TG indicated increased SGC activity after the IONC surgery. We observed an increased expression of IL-2 in neuronal somata of TG. This expression of IL-2 was higher on the constricted side as compared to the non constricted side. We hypothesize that in the NP condition, there is an increased production of IL-2 by SGC's which is released extracellular. Because of the close proximity of neuron-SGC and various other mechanisms, IL-2 is taken up or endocytosed by the

neurons which is seen to be expressed in our immunohistochemistry section within the somata of the neurons. Our future work focuses to explore such possibilities and mechanisms.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.24/AA2

**Topic:** D.03. Somatosensation: Pain

**Title:** Diode laser nociceptor-selective quantitative sensory test standardized for large-scale clinical trials

**Authors: M. I. NEMENOV**[1,4], D. D. DESOUZA[2], S. NAGPAL[2], M. KLUKINOV[1], *D. C. YEOMANS[3]
[1]Anesthesia, [2]Neurol., [3]Stanford Univ., Stanford, CA; [4]LasMed, LLC.., Mountain View, CA

**Abstract:** Neuropathic pain can be triggered, in part, by activity of diverse primary afferent pathways, including C and A delta nociceptors. To what extent these afferents contribute to neuropathic pain is not clear. We developed a method of selective activation of A delta or C fibers based on diode laser (DLss), the selectivity of which is documented in several small scale volunteer and clinical studies as well as in rodents and pigs. Because of this capacity, DLss can be used as a biomarker of A delta or C fiber status. DLss also allows for differentiation of C polymodal vs. C mechano-insensitive thermonociceptive fiber activation.
Here we examined the means of optimizing DLss protocols for large-scale clinical study requirements.
Safety: Because DLss does not require overheating of the epidermis for heat to reach dermal fibers, no adverse effects have been reported in over 100 volunteers and patients.
Training: Two days of intensive training were sufficient for 3 independent groups of experienced QST researchers that never used DLss before to become competent in the methods.
Protocol: Subjects were prepared as to insure reproducibility: tested areas were shaved, each site was marked with grid with cell sizes of 10 mm X 10 mm, 3 columns and 10 rows and baseline skin temperature was maintained with accuracy +/-0.5 oC. Stimulation began from 0 mA current, and the current was increased in 100 mA (about 0.1 W) increments until the stimulation was felt as painful. Pain threshold was obtained by applying:
a) method of levels, which consisted of a series of ascending and descending stimuli with 5 up and 5 down changes until the last painful sensation was given a pain rating 3 out 10 on the numeric rating scale and
b) method of limits, which consisted of a series of ascending stimuli with fixed step of 100 mA

with up to 10 changes until the last painful sensation was given a pain rating of 3 out 10.
Both methods were applied for each subject three times to compare their accuracy and to define the duration of each test. The stimulation protocol for activation for C nociceptors included the 5 mm in diameter area of stimulation, and duration of each stimulus was 2 sec; for A-delta nociceptors, spot diameter was 1 mm and stimulus duration was 60 ms. Each subject was tested before and after test or control treatment for each type of fiber.
Reproducibility: we found that both protocols provide excellent reproducibility in the frame of baseline physiological variability with a variability of measurements from 5% to 8%.
Robustness: Each test took between 10 min to 15 min to define each fiber type pain threshold.

**Disclosures:** **M.I. Nemenov:** A. Employment/Salary (full or part-time):; LazMed, Inc.. **D.D. DeSouza:** None. **S. Nagpal:** None. **M. Klukinov:** None. **D.C. Yeomans:** None.

**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.25/AA3

**Topic:** D.03. Somatosensation: Pain

**Title:** Alternative therapeutic approach to attenuate orthodontic pain -$CO_2$ laser therapy

**Authors: *T. TSUCHIYA**[1], N. HASEGAWA[1], N. SUDA[1], K. ADACHI[2]
[1]Orthodontology, [2]Parmacology, Meikai Univ. Sch. of Dent., Sakado, Japan

**Abstract:** The analgesic effect of laser irradiation is applied to many orofacial diseases and the efficacy of laser application on orthodontic force-induced pain has been investigated in both clinically and laboratory. However, the detail of laser action is not fully understood and the analgesic effect of laser irradiation is inconsistently reported. The aims of this study were 1) to determine whether the laser irradiation attenuate orthodontic pain by using jaw-opening reflex (JOR) model and 2) to evaluate the optimal irradiation protocol to obtain maximal analgesic effect on orthodontic pain. General anesthetized rats were applied continuous orthodontic force by Ni-Ti coil spring to only the right maxillary first molar. On the following day, rats were generally anesthetized and pair of electrodes was inserted bilateral anterior digastric muscle to record EMG activity and the maxillary first molar gingiva to electric stimulation. Passing current (200 μs) was applied to the right then the left maxillary first molar region to determine JOR threshold. After measuring JOR threshold, $CO_2$ laser irradiation (15, 30 or 600 s) was applied the right side maxillary first molar region then JOR threshold was determined again for subsequent 90 min with 30 min interval (D1 group). $CO_2$ laser irradiation (30 s) was also applied to the right maxillary first molar region immediately after orthodontic force application in other animals, and JOR excitability was evaluated on next day (D0 group). To confirm effect of laser irradiation on JOR excitability, another set of animals received only laser irradiation (30, 600 s, without

orthodontic treatment) to the right maxillary first molar region before JOR excitability evaluation (control group). Laser irradiation did not alter JOR threshold between right and left stimulations in the control group. Since JOR threshold was variable across animals, the right side threshold was standardized with that of left side in each animal. Application of orthodontic force in the D1 group significantly ($P < 0.05$) decreased right side JOR threshold (71.5 ± 5.7 %) compared with that on the left side. Laser irradiation (30 and 600 s) in D1 group significantly ($P < 0.05$) increased right side JOR threshold (30 s:97.5 ± 13.6 % @ 30 min, 93.3 ± 7.5 % @ 60 min, 111.1 ± 11.3 % @ 90 min;600 s: 92.5 ± 4.2 % @ 30 min, 91.1 ± 2.1 % @ 60 min). Interestingly, in D0 group, right side JOR threshold was also significantly ($P < 0.05$) increased (111.6 ± 7.4 %). Taken together, $CO_2$ laser irradiation is applicable for attenuating orthodontic pain and the analgesic effect of $CO_2$ laser irradiation is not transient.

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**Poster**

**580. Mechanisms of Peripheral Neuropathic Pain II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 580.26/AA4

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH DK105687

**Title:** Results of fiber specific quantitative sensory test (QST) explains pain sensory deficits in patients with spontaneous peripheral neuropathic pain

**Authors: *M. I. NEMENOV**[1,2], M. KLUKINOV[2], D. C. YEOMANS[3], M. SCHMELZ[4]
[1]Lasmed LLC, Mountain View, CA; [2]Anesthesia, Stanford Univ., Palo Alto, CA; [3]Stanford Univ., Stanford, CA; [4]Heidelberg Univ., Mannheim, Germany

**Abstract:** Patients with painful peripheral neuropathies (PPN) have spontaneous pain but typically have reduced heat pain sensitivity, which is currently explained by significant denervation of epidermal cutaneous nociceptor fibers. However, we have showed that loss of pain sensitivity in PPN patients is actually fiber specific, with increased pain thresholds for Adelta and normal thresholds for C fiber mediated pain (1). These novel findings can't be completely explained by denervation. Besides, we found that mechano-insensitive C fibers (CMi), but not C polymodal (CMH) fibers are sensitized to heat in PPN patients (2) despite higher activation thresholds of normal CMi fibers for both electricity or heat when compared to those of CMH nociceptors. Thus, PPN associated loss of pain sensitivity could be explained by denervation of CMH and A delta fibers while responses of sensitized CMi fibers were relatively unaffected. Here we hypothesize that relatively low pain thresholds in PPN patients when assessed using diode laser C fiber specific stimulation (C-fiber DLss) as opposed to typical heat

stimuli are the result of the capacity of DLss to activate CMi fibers below pain threshold. Therefore, the heat pain deficit that has been measured in PPN patients with spontaneous pain may be related to the method of stimulation rather than overall loss of heat pain sensitivity. 12 healthy subjects without history of peripheral neuropathy were tested. Laser pulses of 1 sec, 5 mm diameter @980 nm (LassM-10M, LasMed, CA) were used for stimulation. Activation of CMi fibers was confirmed by recording of perfusion (PeriCAM, Perimed, Sweden). Baseline and laser evoked skin temperature was monitored by thermal camera (SC 6000, Flir, USA). The presence of post stimulus sensitization was assessed using neurological pins (Neurotips, Owen Mumford, USA). Laser-induced CMi activation (neurogenic flare) was recorded below pain threshold in ten subjects. No difference was observed in mechanical sensitivity between irradiated and surrounding skin. *We documented, for the first time, that neurogenic flare response, and therefore activation of CMi nociceptors can occur below the temperature threshold for pain in healthy subjects.* In parallel studies in pigs and rats, C-fiber DLss also induced neurogenic flare below pain thresholds. *The ability to activate CMi fibers with DLss should allow better assessment of PPN in patients and animal models.* 1. Moeller et al. Sensory Small Fiber Function Differentially Assessed with Diode Laser QST in Painful Neuropathy. Pain Medicine 2013. 2. Nemenov et al. Heating of deeper skin layers might detect spontaneously active heat-sensitized nociceptors. SFN 2015.

**Disclosures: M.I. Nemenov:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LasMed LLC. **M. Klukinov:** None. **D.C. Yeomans:** None. **M. Schmelz:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.01/AA5

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant DK105687

**Title:** Comparison of diode laser and 8% capsaicin patch depletions of cutaneous nociceptors

**Authors: M. I. NEMENOV**[1], M. KLUKINOV[2], D. C. YEOMANS[3], *F. L. RICE[4]
[1]Lasmed LLC, Mountain View, CA; [2]Dept. of Anesthesiol., Stanford Univ., Palo Alto, CA; [3]Stanford Univ., Stanford, CA; [4]Integrated Tissue Dynamics LLC, Rensselaer, NY

**Abstract:** Painful peripheral neuropathy (PPN) has a variety of causes, including diabetes, HIV, herpes zoster, chemotherapy and postsurgery. Most therapeutics target purported pain mechanisms in CNS with fewer than 1/3$^{rd}$ of patients achieving no more than 50% relief but while most having debilitating side effects. Here we directly compare a new laser non-contact

and 8% capsaicin patch (Acorda Theraputics) treatments. Capsaicin and heat are both TRPV1 agonists. Capsaicin depletes epidermal sensory endings of C fibers that presumably express TRPV1 as sources of PPN but the half-life of ~ 24 hours and can lead to prolonged inflammatory pain with continued treatment. This limits the use of high dose capsaicin patches even for FDA approved treatment of some types of PPN. We have previously shown that pulses of Diode Laser (DL) induced-heat, presumably an agonist of TRPV1 channels, can selectively activate mechano-insensitive C fibers that are abnormally spontaneously active in patients with painful versus painless neuropathy (1). In this SBIR Phase I feasibility study, we assessed the innervation density in pig skin biopsies seven days after 1 hour 8% capsaicin patch treatment (Acorda) and four dosages of laser irradiations that induced peak temperature up to 55$^{o}$C. Pigs were habituated for 2-3 days and trained to perform pain mechanical (neurological pins (Neurotips, Owen Mumford, USA) and diode laser C fiber selective behavioral tests (DLss, LasMed, CA) (1,2). The behavioral tests were conducted one day before and 6 days after the treatment in one pig and showed a significant increase of response latency to C fiber laser stimulation in the infrared-treated versus untreated areas ($p<0.02$) and in the same area before and after treatment ($p<0.04$). The PGP9.5 quantification demonstrated a significant depletion of cutaneous epidermal innervation with 10 pulses comparable to depletion produced by 8% capsaicin patch. Examination of alternating sections stained for H&E reveal no obvious damage from capsaicin treatment and DL treatment with 10 and 20 pulses, but some minor damage from 40 pulses. Therefore, the impact of a one-hour capsaicin treatment efficacy could be achieved in less than 2 minutes for DL treatment. 1. Moeller-Bertram T, Schilling JM, Bačkonja MM, Nemenov MI. Pain Med. 2013 Mar;14(3):417-21. 2. Brown JD, Saeed M, Do L, Braz J, Basbaum AI, Iadarola MJ, Wilson DM, Dillon WP. Sci Transl Med. 2015 Sep 16;7(305).

**Disclosures: M.I. Nemenov:** None. **M. Klukinov:** None. **D.C. Yeomans:** None. **F.L. Rice:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.02/AA6

**Topic:** D.03. Somatosensation: Pain

**Title:** Human-like chronic pain pathologies induced in porcine skin innervation and epidermal keratinocytes by proximal sciatic nerve insult: A translational platform to develop therapeutics for neuropathic pain

**Authors: *P. J. ALBRECHT**[1], I. SABBAG[2], D. CASTEL[3], E. RUGGIERO[4], M. DOCKUM[4], S. B. MEILIN[6], F. L. RICE[5]
[1]Integrated Tissue Dynamics, LLC, Rensselaer, NY; [2]Lahav Res. Inst., Kibutz Lahav, Negev,

Israel; [3]The Neufeld Cardiac Res. Institute, Sheba Med. Centre, Sackler Sch. of Med., Tel-Aviv Univ., Tel-Aviv, Israel; [4]Integrated Tissue Dynamics LLC, Rennselaer, NY; [5]Integrated Tissue Dynamics LLC, Rensselaer, NY; [6]MD Biosci., Ness Ziona, Israel

**Abstract:** The translation of basic research findings to the development of safe and effective strategies for preventing and treating chronic pain remains a daunting health care challenge. Given the myriad shortcomings of typical rodent models, we have previously developed a peripheral neuritis trauma (PNT) model of chronic pain induced by a proximal sciatic nerve irritation in pigs which have a body size, metabolism, skin structure, and cutaneous innervation more relevant to that of humans. Three sutures pre-soaked in complete Freund's adjuvant (CFA), closely-spaced and loosely tied around the proximal sciatic nerve in pigs produces sustained pain behavior symptoms consistent with painful neuritis and/or neuropathic pain (NP) in humans. Here, we used multi-molecular immunolabeling and microscopic analyses to evaluate pig skin biopsies from various sciatic nerve injury models and demonstrate cutaneous innervation and epidermal keratinocyte pathologies similar to those reported in several human chronic NP afflictions. As seen previously in several human neuropathic pain afflictions such as postherpetic neuralgia, complex regional pain syndrome,and painful diabetic neuropathy, a reduction of small caliber innervation, including intraepidermal nerve fibers (IENF), was coupled with an increase in algesic mediators and a decrease in analgesic mediators among epidermal keratinocytes, which directly influence the level of excitability of remaining sensory endings within and near the epidermis. Of particular interest, small caliber innervation that expresses the neuropeptide CGRP, implicated in inflammatory pain, was preferentially spared especially in association with capillaries and precapillary arterioles in the upper dermis. Expression of the neurosignaling properties NaV1.6, CGRP, and endothelin-1 receptor A (ETRA), which are implicated in algesia, were increased on keratinocytes, whereas ETRB, implicated in analgesia, was decreased. The decrease in innervation occurred within the first week. Alterations in keratinocyte neurosignaling properties, induced by the proximal nerve injury, occurred during the second week and was recapitulated through at least 4 weeks, by which time all of the originally impacted keratinocytes should have been replaced. This model not only provides a relevant model for translational research, but also provides insights into potential novel therapeutic strategies.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

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**Program#/Poster#:** 581.03/AA7

**Topic:** D.03. Somatosensation: Pain

**Support:** DA041229

CA200417

DA009158

DA021696

**Title:** Cannabinoid CB2 receptors on epidermal keratinocytes and langerhans cells as potential targets for blocking paclitaxel-induced neuropathic pain

**Authors: X. LIN**[1], L. DENG[1], K. MACKIE[1], J. ROMERO[3], C. J. HILLARD[4], P. J. ALBRECHT[5], F. L. RICE[5], *A. G. HOHMANN[2]
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**Abstract:** Cannabinoid CB2 receptors are a promising therapeutic target because their activation suppresses pathological pain without unwanted psychotropic effects caused by activating CB1 receptors in the CNS. However, the cell types that contain CB2 receptors and mediate these analgesic effects are poorly understood due to questionable specificity of existing CB2 antibodies. Previously, we showed that the cannabilactone CB2 agonist AM1710 suppressed development and maintenance of paclitaxel-induced mechanical and cold allodynia without producing tolerance or physical dependence (Deng et al. (2015) Biological Psychiatry 77: 475-487). Moreover, anti-allodynic effects of AM1710 were absent in CB2 knockout (KO) mice but fully preserved in CB1 KO mice, confirming mediation by CB2. Here we show that the anti-allodynic efficacy of systemic AM1710 in the paclitaxel model is specifically blocked by SR144528, a CB2 receptor antagonist with limited CNS penetration. These observations suggest that CB2 receptors outside the CNS underlie the CB2-mediated suppression of chemotherapy-induced neuropathic pain induced by the CB2 agonist. We, therefore, developed mice with a CB2-promoter driven expression of green fluorescent protein (GFP) to identify CB2-expressing cell types in glabrous paw skin. Mice received repeated injections of paclitaxel (4 mg/kg i.p. on day 0, 2, 4 and 6) or its cremophor-based vehicle and were perfused during the maintenance stage of neuropathic pain when allodynia was maximal (i.e. day 15 following initiation of paclitaxel dosing). A separate group of mice were killed 1 h following an acute challenge with paclitaxel (4 mg/kg i.p.) and tissue was processed concurrently. GFP was differentially expressed on epidermal keratinocytes in stratified patterns that shifted and increased in intensity, especially after repeated paclitaxel injections. GFP was intensely expressed on dendritic cells, with the Langerhans subtype, invading the epidermis already after the acute and increasing after the repeated injections. As reported previously (Ibrahim et al. (2005) PNAS 102: 3093-3098) our studies confirm the expression of CB2 receptors on keratinocytes and revealed a novel expression on dendritic and Langerhans cells as potential targets for treating paclitaxel-induced neuropathic pain.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.04/AA8

**Topic:** D.03. Somatosensation: Pain

**Support:** NSFC81271241

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NSFC81320108012

**Title:** Activation of EphB receptor facilitates calcium influx via modulation of NMDA receptor in dorsal root ganglion neurons

**Authors: *P.-C. MA**[1,2], Z.-L. ZHOU[2], F. RAO[1,2], X.-J. SONG[1,2]
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**Abstract:** EphB receptor tyrosine kinases were first found to play important roles during development, but recently have been implicated in synaptic plasticity in mature nervous system and pain processing. We have previously reported that ephrinB-EphB receptor signaling contributes to neuropathic pain by regulating neural excitability and synaptic plasticity in the dorsal horn neruons. How EphB receptors influence the excitability of dorsal root ganglion (DRG) neurons, however, remains elusive. We investigated roles of activity of ephrinB-EphB receptor signaling in neural excitability and its underlying mechanisms in acutely dissociated-, primary cultured-, or excised intact-DRG neurons from Sprague-Dawley rats. The results showed that EphB1 and EphB2 receptors are expressed in the large- and medium-sized as well as the small DRG neurons, but not in the satellite glial cells. Bath application of EphB receptor activator ephrinB2-Fc caused NMDA receptor-independent $Ca^{2+}$ influx and amplified NMDA induced-$Ca^{2+}$ influx in primary cultured DRG neurons. Blocking Src kinase by Src kinase inhibitor PP2 reversed ephrinB2-Fc-induced increase of NMDA-dependent $Ca^{2+}$ influx and phosphorylation of NR2B subunit. The mRNA and protein expressions of EphB1 and EphB2 receptors are greatly upregulated in DRG neurons following peripheral nerve injury. Intracellular concentration of $Ca^{2+}$ was significantly increased in the nerve injured-DRG neurons. In addition, the nerve injured-DRG neurons exhibited increased $Ca^{2+}$ response to NMDA that was reversed by EphB receptor blocker EphB2-Fc. Blocking EphB receptor activation also reversed nerve injury-induced upregulation of phosphorylation of NR2B receptor and other neurochemical markers. These findings demonstrate that EphB receptors can facilitate calcium influx of DRG neurons directly and via modulation of NMDA receptor and thus provide underlying mechanisms for its contribution to neuropathic pain.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.05/AA9

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH grant NS094664

NIH grant NS094224

**Title:** Overexpressing Tet1 in primary sensory neurons attenuated nerve injury-induced nociceptive hypersensitivity

**Authors:** ***Z. PAN**[1], Q. WU[2], G. WEI[3], S. WU[3], L. LIANG[3], A. BEKKER[3], Y.-X. TAO[3]
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**Abstract:** DNMT3a-mediated DNA methylation contributes to the nerve injury-induced neuropathic pain genesis through the epigenetic silencing of mu opioid receptor (*Oprm1*) and *Kcna2* genes in the primary sensory neurons of dorsal root ganglion (DRG). Tet (ten eleven translocation) family of oxygenases catalyse the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), resulting in DNA demethylation and promotion of the gene transcription in biological processes. The present study investigated whether overexpressing Tet1 in the DRG affected nerve injury-induced pain hypersensitivity. As expected, DRG microinjection of herpes simplex viruses (HSV) expressing full-length Tet1 (HSV-Tet1) into the ipsilateral L5 DRG significantly increased the level of TET1 in the injected DRG. This increase attenuated the SNL-induced elevation of 5mC level and correspondingly rescued the SNL-induced decrease of 5hmc in promoter regions of *Oprm1* and *Kcna2 genes*, resulting in the reverse in the SNL-induced downregulation of MOR and KV 1.2 in the ipsilateral DRG. Behavioral observations revealed that microinjection of HSV-Tet1 into the ipsilateral L5 DRG alleviated mechanical allodynia, thermal hyperalgesia and cold allodynia in the development and maintenance of neuropathic pain without affecting locomotor function and basal responses to mechanical, thermal and cold stimuli. Moreover, microinjection of HSV-Tet1 into the ipsilateral L5 DRG improved morphine analgesia and prevented the morphine analgesic tolerance under neuropathic pain conditions. These results suggest that TE1 overexpression in DRG has the potential function in treatment of neuropathic pain through blocking nerve injury-induced DNA methylation in *Oprm1* and *Kcna2* genes in the DRG.

**Disclosures:** **Z. Pan:** None. **Q. Wu:** None. **G. Wei:** None. **S. Wu:** None. **L. Liang:** None. **A. Bekker:** None. **Y. Tao:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

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**Program#/Poster#:** 581.06/AA10

**Topic:** D.03. Somatosensation: Pain

**Support:** NS094664

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HL117684

Research Fellowship grant

**Title:** Contribution of DNMT3a to paclitaxel-induced neuropathic pain via silencing K2p1.1 expression in primary sensory neurons

**Authors: *J. XIAO**, Q. MAO, S. WU, L. LIANG, K. MO., A. BEKKER, Y.-X. TAO
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**Abstract:** Chemotherapy-induced neuropathic pain (CINP) is one of the major dose-limiting toxicities of cytostatic pharmacotherapy. It can affect up to 80% of patients and lead to reduction or discontinuation of the cancer treatment. Epigenetic modulation by DNA methylation, which in mammals occurs at cytosine residues catalyzed by DNA methyltransferase (DNMTs), plays an important role in the dynamic regulation of gene expression. DNA methylation triggered by chemotherapy has largely unexplored therapeutic consequence. Our study found that after administration of paclitaxel, K2p1.1 (KCNK1), one of the two-pore potassium channels, was decreased significantly in mouse DRG neurons, whereas DNMT3a (DNA methyltransferase 3a), but not DNMT1 and DNMT3b, was increased significantly in mouse DRG. DRG K2p1.1 decrease led to the elevations in rest membrane potential and neuronal hyperexcitability of DRG neurons. Blocking the increased DNMT3a in the DRG rescued the decrease of K2p1.1 in DRG and alleviated mechanical allodynia and thermal hyperalgesia in paclitaxel-treated mice. DNMT3a was co-localized with K2p1.1 in DRG neuron. Our results suggest that DNMT3a-triggered K2p1.1 down-regulation in DRG may be required for the development of CINP.

**Disclosures:** **J. Xiao:** None. **Q. Mao:** None. **S. Wu:** None. **L. Liang:** None. **K. Mo.:** None. **A. Bekker:** None. **Y. Tao:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** D.03. Somatosensation: Pain

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**Title:** MicroRNA 143 contributes to neuropathic pain through DNMT3a-triggered epigenetic silencing of MOR in DRG

**Authors:** ***S. WU**, B. XU, K. MO, L. LIANG, A. BEKKER, Y.-X. TAO
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**Abstract:** Dysregulation of microRNAs (miRNAs) in dorsal root ganglion (DRG) and spinal cord has been found following peripheral nerve injury. However, how miRNAs contribute to neuropathic pain remains elusive. The present study showed that the level of miR-143 was significantly decreased in the ipsilateral L5 DRG after spinal nerve ligation (SNL) at day 3 and 7 post-SNL in the rats. Blocking this decrease through pre-microinjection of miR-143 mimics into the ipsilateral L5 DRG prevented the SNL-induced pain hypersensitivities. In contrast, mimicking this decrease through microinjection of miR-143 inhibitors into L4/5DRG led to neuropathic pain-like symtoms in naive rats. Mechanismtically, miR-143 suppressed the expression of DNA methyltransferase 3A (Dnmt3a) post-transcriptionally in the DRG. Downregulating miR-143 significantly increased the expression of Dnmt3a, resulting in the suppression of the expression of mu opioid receptor (MOR), a downstream target of Dnmt3a, in the DRG. DRG overexpression of miR-143 blocked the SNL-induced increase in Dnmt3a and rescued the SNL-induced decrease in MOR in the ipsilateral DRG. These findings suggest that miR-143 reduction in the DRG contributes to neuropathic pain through Dnmt3a-triggered epigenetic silencing of MOR in the ipsilateral DRG.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

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**Program#/Poster#:** 581.08/AA12

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant NS094664

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**Title:** The transcription factor CEBPβ in the dorsal root ganglion contributes to peripheral nerve trauma-induced nociceptive hypersensitivity

**Authors: *Y. TAO**[1], Y. MAO[2], L. LIANG[3], S. WU[2], W. CAI[2], A. BEKKER[2]
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**Abstract:** The alterations in gene transcription in the dorsal root ganglion (DRG) following nerve trauma contribute to the genesis of neuropathic pain. Transcription factors gate gene expression. We report here that peripheral nerve trauma caused by chronic constriction injury (CCI) increased the abundance of the transcription factor C/EBPβ (CCAAT/enhancer-binding protein-β) in the DRG. Blocking this increase mitigated the development and maintenance of CCI-induced mechanical, thermal, and cold pain hypersensitivities, without affecting basal responses to acute pain and locomotor activity. Mimicking this increase produced mechanical, thermal, and cold pain hypersensitivities. Mechanistically, C/EBPβ participated in CCI-induced decreases in the abundance of mRNAs and proteins of the voltage-gated potassium channel subunit Kv1.2 and mu opioid receptor through C/EBPβ-triggered activation of euchromatic histone-lysine N-methyltransferase 2 gene in the ipsilateral DRG. C/EBPβ is likely an endogenous initiator of neuropathic pain and may serve as a potential target for the prevention and treatment of this disorder.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

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**Program#/Poster#:** 581.09/AA13

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant R01NS094664

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NIH Grant R01DA033390

**Title:** The involvement of Nrcam variants in nerve injury-induced neuropathic pain in mice

**Authors: *L. LIANG**[1], S. WU[2], Z. PAN[3], Y.-J. CHANG[4], B. LUTZ[5], A. BEKKER[5], Y.-X. TAO[5]
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**Abstract:** Neuropathic pain is a serious clinical disease. The dorsal root ganglion (DRG) is a major target to explore the peripheral mechanisms of neuropathic pain. Alternative splicing contributes to multiple physiological and pathological events including nociceptive processing. NrCAM, a neuronal cell adhesion molecule of the L1 family of immunoglobulins, has been indicated in neural development and disorders such as drug addiction and autism. However, it is unknown if Nrcam alternative splicing is involved in neuropathic pain. Using a mouse model of the fourth spinal nerve ligation (SNL)-induced neuropathic pain, we compared the junction reads of RNA-sequencing data obtained from both SNL and sham DRG by MISO (Mixture of Isoforms probablistic model for RNA-Seq). This bioinformatics approaches identified that SNL led to decreased exon skipping of Nrcam gene exon 10 in DRG. The expression of Nrcam RNA with exon 10, but not the total mRNA of Nrcam, was significantly increased in the ipsilateral DRG by reverse transcription PCR validation. Suppression of exon 10 inclusion via the specific antisense oligonucleotides targeting the splicing sites markedly reduced the SNL-induced pain hypersensitivity. Our findings suggests that Nrcam pre-mRNA alternative splicing may be a potential analgesic target for neuropathic pain.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.10/AA14

**Topic:** D.03. Somatosensation: Pain

**Title:** Characterization of a humanized $Na_v1.7$ rat model for the study of neuropathic pain

**Authors: *B. GRUBINSKA**[1], C. YANG[1], N. RAMPAL[2], K. TABORN[1], D. MATSON[1], M. ZHANG[2], T. KORNECOOK[2], S. LEHTO[2], B. MOYER[2], J. GINGRAS[1]
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**Abstract:** Clinical genetic studies have shown that loss-of-function of the $Na_v1.7$ voltage-gated sodium ion channel (*SCN9A*) leads to complete inability to perceive pain (**C**ongenital **I**ndifference to **P**ain - **CIP**), whereas $Na_v1.7$ gain-of-function alleles cause or contribute to chronic spontaneous pain. Mechanistically, $Na_v1.7$ governs the excitability of peripheral nociceptive sensory neurons and the transmission of noxious nociceptive signals into the spinal cord, possibly by facilitating action potential propagation across fine neuronal branch points. Mounting evidence indicates that $Na_v1.7$ plays a major role in transducing pain in acquired clinical neuropathic pain syndromes. To further our knowledge on the role of this channel in pain processing, we developed the first viable global $Na_v1.7$ knockout mouse (Gingras et al, 2014). These mice were anatomically normal, reached adulthood, and had phenotype wholly analogous to human congenital indifference to pain (**CIP**). Although the model had proven useful in various aspects of $Na_v1.7$ antagonist research, but the difficulties around breeding large number of animals due to the hand feeding requirements left a few unrequited questions, particularly regarding the role of $Na_v1.7$ in chronic pain. We report the generation of a humanized chimeric rat model designed to overcome identified species differences that would allow for unique chemical matter testing. Surprisingly, upon thorough characterization, it was observed that the animals had a protein translation deficit, which resulted in a surrogate $Na_v1.7$ KO-like phenotype. Here we show the full characterization of the animals at the molecular, protein and behavioral level in direct comparison to the report mouse global KO phenotype and highlight its novel experimental applications.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.11/AA15

**Topic:** D.03. Somatosensation: Pain

**Title:** Paclitaxel alters enzymes involved in the biosynthesis of nicotinamide adenine dinucleotide ($NAD^+$) in the dorsal root ganglia and dorsal horn of the rat: reversal by nicotinamide riboside

**Authors: *D. L. HAMMOND**[1], R. Y. WALDER[2], G. OURTIES[3], S. R. WHITE[4], M. V. HAMITY[4]
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**Abstract:** Nicotinamide riboside (NR) is a form of vitamin B3 and a precursor of nicotinamide adenine dinucleotide ($NAD^{+)}$. Daily gavage with NR prevents the cold and tactile hypersensitivity and suppresses place escape avoidance behaviors in rats treated with paclitaxel. This study assessed the effects of paclitaxel on enzymes involved in *de novo* and salvage pathways for synthesis of $NAD^+$, and further tested whether NR could prevent these changes. Female Sprague Dawley rats received a cumulative dose of 19.8 mg/kg paclitaxel or Kolliphor:ethanol:saline (KES) vehicle i.v. and were killed six or 14 days later. Another cohort of rats was gavaged daily with 200 mg/kg NR or vehicle daily for one week before, during and continuing for 14 days after i.v. administration of paclitaxel or KES vehicle at which time the rats were killed. This dose of NR increases blood levels of $NAD^+$ by 50%. Levels of transcript for nicotinamide riboside kinases (*Nmrk*) 1 and 2, sirtuins (*Sirt*) 1 and 3, sterile alpha and TIR motif containing 1 (*Sarm-1*), nicotinamide mononucleotide adenylyl transferases (*Nmnat*) 1-3, and nicotinamide adenine phosphoribosyl transferase (*Nampt*) were determined in the L4 and L5 dorsal root ganglia (DRG) and spinal cord dorsal horn. All transcripts were determined in the same sample by RT-qPCR and normalized to hypoxanthine phosphoribosyltransferase 1 (*Hprt-1*) as a reference gene. In the dorsal horn, *Sirt-1* levels were decreased by 50% both six and 14 days after paclitaxel. No other transcripts were altered by paclitaxel. Treatment with NR prevented the decrease in *Sirt-1*. The reduction in *Sirt*-1, an $NAD^+$ consumer, in the dorsal horn may reflect a reduction in $NAD^+$ levels. In the DRG, *Nmnat*-1 and *Nmnat*-2 levels increased two-fold within 14 days of paclitaxel. Pretreatment with NR prevented these increases. Levels of transcript for *Nmrk*-1 and *Nampt* trended higher after paclitaxel. Of note, NR by itself increased transcripts for *Sarm-1*, *Nampt*, *Nmnat-2* and *Nmrk-1* by two-fold in the DRG. Levels of *Nmrk-2* in the DRG and dorsal horn were too low to quantitate. In comparison, sciatic nerve transection in male rats decreased *Sirt-3* and *Nmrk-1*, and did not alter *Sarm-1* or *Nampt* in the L4 and L5 DRG. Whether

corresponding changes in protein expression occur in the DRG and dorsal horn after paclitaxel and NR treatment remains to be determined. However, these results suggest that sensory neurons or glia in the DRG may be able to utilize NR to maintain levels of $NAD^+$ as a possible mechanism for relief of neuropathy induced by paclitaxel, but not nerve-injury.

**Disclosures:** **D.L. Hammond:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ChromaDex. **R.Y. Walder:** None. **G. Ourties:** None. **S.R. White:** None. **M.V. Hamity:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ChromaDex.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.12/AA16

**Topic:** D.03. Somatosensation: Pain

**Support:** Oberely Seed Grant Program (Holden Comprehensive Cancer Center at the University of Iowa and its National Cancer Institute Award P30CA086862)

College of Medicine

**Title:** The nicotinamide adenine dinucleotide ($NAD^+$) precursor nicotinamide riboside (NR) relieves paclitaxel-induced peripheral neuropathy in tumor bearing rats and does not facilitate tumor growth or interfere with the effects of paclitaxel

**Authors: *M. V. HAMITY**[1], S. WHITE[1], K. GIBSON-CORLEY[2], D. HAMMOND[1]
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**Abstract:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common disabling and dose-limiting adverse effect of many chemotherapeutic drugs, for which an effective treatment remains elusive. We recently reported NR, a naturally occurring vitamin B3 precursor of $NAD^+$, can prevent and relieve established CIPN induced by paclitaxel in tumor-naïve rats. However, studies in tumor-naïve rats do not fully model the clinical situation in that factors secreted by tumors not only alter the microenvironment, but can also cause systemic inflammation. It is also critical that drugs that alleviate CIPN do so without interfering with the chemotherapy. In the case of NR, there are additional concerns that an increase in $NAD^+$ levels could promote tumorigenesis. This study re-examined the effects of NR in tumor-bearing rats and determined whether NR treatment facilitates tumorigenesis or interferes with the chemotherapeutic effect of paclitaxel. Mammary gland tumors were induced in female rats by injection of 50 mg/kg i.p. NMU (N-methyl-N-nitrosourea) at 3 weeks of age. Rats were examined twice a week, and volume and number of tumors were recorded for up to 12 weeks after NMU injection. Upon the

appearance of a palpable tumor ~ 6 weeks later, NMU- treated rats were injected with a cumulative dose of 19.8 mg/kg i.v. paclitaxel over five days. NR (200 mg/kg) or vehicle was administered daily by gavage concomitant with the paclitaxel treatment and continuing for four weeks. Mechanical and cold sensitivity were assessed 14 and 21 days after paclitaxel to establish if NR treatment ameliorates CIPN in tumor-bearing rats. To evaluate if NR treatment promotes tumorigenesis, another set of rats was dosed daily with NR or vehicle starting 6 weeks after NMU treatment. To evaluate if NR interferes with chemotherapy effects, measurements of tumor number and size were also made in the rats treated with paclitaxel for a period of four weeks. The results indicate that NR diminished paclitaxel-induced mechanical hypersensitivity and cold sensitivity in tumor-bearing rats as it did in tumor-naïve rats. Importantly, daily gavage with NR did not facilitate tumor growth (size or number) compared to vehicle-treated rats. Paclitaxel reduced tumor size to an equivalent extent in NR and vehicle-treated rats, and rebound tumor growth after paclitaxel effects waned also did not differ between NR- and vehicle-treated rats. This study is one of the first to test the effects of a novel therapeutic in a tumor-bearing rodent model of CIPN induced by a clinically relevant dose of paclitaxel. These findings provide additional support for a clinical trial of NR for the relief of taxane-induced peripheral neuropathy.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

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**Topic:** D.03. Somatosensation: Pain

**Support:** NIH grant RO1 NS031680

**Title:** Role of Adenosine monophosphate-activated kinase in mechanical allodynia mediated by reactive oxygen species

**Authors: *K. M. HANKERD**, J. WANG, J.-H. LA, J. M. CHUNG
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**Abstract:** Production of excess reactive oxygen species (ROS) has been shown to mediate pathologic mechanical pain in several pain models, including the intradermal capsaicin injection model. However, it has not yet been elucidated which signaling molecules downstream of excessive ROS mediate and perpetuate pain. Adenosine monophosphate-activated kinase (AMPK) is a redox-sensitive protein kinase whose pharmacological activators were shown to reduce neuropathic mechanical pain. We thus hypothesized that AMPK would play a role in

ROS-mediated pathologic mechanical pain. C57BL/6N mice developed secondary mechanical allodynia and hyperalgesia after intradermal capsaicin injection. The ROS scavenger phenyl-N-tert-butylnitrone (PBN) selectively alleviated mechanical allodynia but not hyperalgesia. Similarly, activators of AMPK, metformin and A769662, inhibited only mechanical allodynia. Directly increasing ROS levels in the cerebrospinal fluid by intrathecal ROS donor injection produced mechanical allodynia and hyperalgesia. Metformin and A769662 also selectively attenuated mechanical allodynia in this intrathecal ROS donor injection model. AMPK activator treatment prior to intradermal capsaicin or intrathecal ROS donor injection significantly hampered the development of mechanical allodynia. These results suggest that decreased AMPK activity due to excess ROS contributes to ROS-mediated mechanical allodynia.

**Disclosures: K.M. Hankerd:** None. **J. Wang:** None. **J. La:** None. **J.M. Chung:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.14/AA18

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant RO1 NS031680

**Title:** Peripheral nerve activity maintains neuropathic mechanical allodynia but not hyperalgesia

**Authors: *J.-H. LA**, J. WANG, H. SHIM, J. CHUNG
Dept. of Neurosci. and Cell Biol., Univ. of Texas Med. Br., Galveston, TX

**Abstract:** Peripheral neuropathic injury often causes chronic mechanical allodynia (pain from an innocuous touch) and hyperalgesia (increased pain from a normally painful mechanical stimulus). Sensitization of nociceptive system has been suggested to be accountable for the chronic neuropathic pain symptoms. However, it remains unclear how the nociceptive system sensitization is persistently maintained. We hypothesized that it is maintained by ongoing peripheral nerve activities originating at the symptomatic area. To test this hypothesis, we injured L5 spinal nerve by tight ligation (L5 SNL) in the mouse and examined effects of local anesthesia on mechanical allodynia and hyperalgesia at two symptomatic areas in the hind paw plantar skin. Mechanical allodynia and hyperalgesia were measured as % occurrence of paw withdrawals from low- and high-intensity von Frey filament stimulations. L5 SNL-induced mechanical allodynia was robust at the base of 3$^{rd}$ and 4$^{th}$ toes (Site 1) but less pronounced at the center of the paw (Site 2). When a local anesthetic (bupivacaine 0.75%, 3 μl) was injected at Site 1, mechanical allodynia, but not hyperalgesia, at Site 2 was alleviated. Selective silencing of either TRPV1/A1-expressing nociceptors or TLR5-expressing Aβ-fibers at Site 1 did not affect mechanical allodynia and hyperalgesia at Site 2. These results suggest that nociceptive system

sensitization for chronic neuropathic mechanical allodynia is dynamically maintained by ongoing peripheral activities arising from the symptomatic areas, whereas that for chronic neuropathic mechanical hyperalgesia persists without being dependent on such peripheral inputs. Peripheral activities of TRPV1/A1-expressing nociceptors and TLR5-expressing Aβ-fibers do not appear to contribute to the dynamic maintenance of sensitization for chronic neuropathic mechanical allodynia.

**Disclosures:** **J. La:** None. **J. Wang:** None. **H. Shim:** None. **J. Chung:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.15/AA19

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant NS031680

NIH Grant NS079166

NIH Grant DA036165

**Title:** Decrease in excitatory synaptic response of spinal dorsal horn GABAergic neurons during burst inputs

**Authors: *C. BAE**, J.-H. LA, H. SHIM, S.-J. TANG, J. M. CHUNG
Dept. of Neurosci. and Cell Biol., Univ. of Texas Med. Br. At Galveston, Galveston, TX

**Abstract:** Decreased function of inhibitory interneurons (disinhibition) in the spinal cord has been suggested as an important mechanism for mechanical allodynia. To maintain mechanical allodynia, abnormal ongoing afferent input was shown to be necessary. Thus, we hypothesize that disinhibition is dynamically dependent on abnormal ongoing afferent input. To test this hypothesis, we examined the excitatory postsynaptic potential (EPSP) in spinal GABAergic neurons (GABAn) in the mouse spinal cord slices before, during, and after burst synaptic input simulating abnormal ongoing afferent inputs. Stimulation parameters for the burst input were adopted from studies inducing long-term potentiation of excitatory synaptic transmission in the spinal dorsal horn *in vivo*. GABAn were identified by green fluorescent protein (GFP) expression driven by GAD67 promoter. Synaptic inputs evoking excitatory postsynaptic currents with consistent onset latency and no failure at the burst stimulation frequency were selected for EPSP recordings. As results, lamina II GABAn (GFP-positive) showed a gradual decrease in EPSP amplitude during the burst input. After cessation of the burst input, GABAn EPSP amplitude returned to the baseline level. Unlike GABAn, unidentified (GFP-negative) lamina II neurons showed an increase in EPSP amplitude during the burst input, and this increase was maintained

long-term even after cessation of the burst input. Demonstrating that GABAn excitability to synaptic inputs decreases only while burst input is ongoing, these results suggest that disinhibition is dynamically dependent on abnormal ongoing afferent input.

**Disclosures: C. Bae:** None. **J. La:** None. **H. Shim:** None. **S. Tang:** None. **J.M. Chung:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.16/AA20

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH Grant R01DE022750

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NIH Training Grant T32 OD011089

Neurosurgery Pain Research Institute at Johns Hopkins

**Title:** Nerve injury induces distinct changes in central terminals among nociceptor and mechanoreceptor subpopulations

**Authors: *L. K. CRAWFORD**[1], S. JEON[2], D. CHANG[3], D. D. GINTY[5], M. J. CATERINA[4]
[1]Dept of Mol. and Comparative Pathobiology, [2]Biol. Chem., [3]Biochem., Cellular, and Mol. Biol., [4]Neurosurg., Johns Hopkins Sch. of Med., Baltimore, MD; [5]Dept Neurosci, Harvard Med. Sch., Boston, MA

**Abstract:** Neuropathic pain affects a wide variety of patients, yet, due to our incomplete understanding of its underlying pathophysiology, current therapies for this condition are inadequate. In addition to pain neurons, or nociceptors, there is growing evidence that touch neurons known as low-threshold mechanoreceptors (LTMRs) also play an important role in painful pathologic conditions. Mechanical allodynia occurs when light touch, which would typically activate LTMRs in healthy skin, instead elicits a painful response. However, the role of LTMRs in mechanical allodynia is poorly understood. With the advent of genetic tools that enable targeted labeling of specific LTMR subtypes we can now examine how LTMRs might change in the pathologic setting and more closely evaluate differential roles for LTMR subtypes in allodynia. Inducible Cre mouse lines were crossed to reporter mouse lines to generate offspring with genetically labeled Adelta LTMRs and rapidly adapting Abeta LTMRs. The SNI model of neuropathic pain was used to generate mechanical allodynia in these mice, and the hypersensitive phenotype was quantified in behavioral tests. Spinal cord tissue from the level of nerve injury was examined to characterize central terminals of nociceptor and LTMR

subpopulations of sensory neurons. Preliminary results suggest that there may be differential loss of labeled axon terminals across sensory neuron subpopulations. Following nerve injury we often observed a regional decrease in IB4, a marker of non-peptidergic nociceptors, presumably due to a loss of axon terminals as a result of nerve injury. However, this response was not uniform across sensory neuron populations. By comparing patterns of axon terminals identified by markers of nociceptors or by lineage-specific genetic labeling, we found distinct changes across nociceptor and LTMR subtypes, suggesting distinct regulation of sensory neuron subtypes in a model of neuropathic pain. These data demonstrate that nociceptors and LTMRs do not show uniform responses to nerve injury and underscore the mechanistic clarity that more precise evaluation of neuronal subtypes can provide.

**Disclosures: L.K. Crawford:** None. **S. Jeon:** None. **D. Chang:** None. **D.D. Ginty:** None. **M.J. Caterina:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.17/AA21

**Topic:** D.03. Somatosensation: Pain

**Support:** VA Rehabilitation Research and Development Grant (I01RX001940)

**Title:** GFAP promoter determines gene transfer to satellite glial cells following intraganglionic delivery in adult rats

**Authors: *H. YU**[1], H. XIANG[2], H. XU[2], Q. H. HOGAN[3]
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**Abstract:** Satellite glial cells (SGCs) are the main type of glial cells in sensory ganglia and are a potential gene-therapy target for chronic pain. Recombinant adeno-associated viral (AAV)-mediated therapeutic gene transfer is an effective and safe tool for treating chronic pain. However, AAV with various ubiquitous cell-potent promoters leads to gene transfer predominantly to neurons while glial cells are refractory to AAV transduction in the peripheral nervous system (PNS). The present study evaluated whether *in vivo* SGC transduction in the dorsal root ganglia (DRG) could be enhanced by SGC-specific GFAP promoter and by using AAVshH10 and AAVshH19 that are engineered capsid variants with glia-prone transduction. Titer-matched AAV6 (as control), AAVshH10, and AAVshH19 variants, all encoding the enhanced green fluorescent protein (EGFP) reporter driven by the ubiquitous cell-potent CMV promoter, as well as AAV6-EGFP driven by a GFAP promoter (AAV6-GFAP-EGFP) were injected into DRGs of adult rats. Neurotropism of gene transfer was determined and compared

by immunohistochemistry. Results showed that injection of AAV6-GFAP-EGFP induces efficient EGFP expression selectively in SGCs, whereas injection of either AAVshH10-CMV-EGFP or AAVshH19-CMV-EGFP into DRGs resulted in a similar *in vivo* transduction profile to AAV6-CMV-EGFP, all showing efficient transduction of sensory neurons without significant transduction of glial cell populations. Co-injection of AAV6-CMV-mCherry and AAV6-GFAP-EGFP induces transgene expression in neurons and SGCs separately. This report, together with our prior studies, demonstrate that the GFAP promoter rather than capsid tropism determines selective gene expression in SGCs following intraganglionic AAV delivery in adult rats. A dual AAV system, one with GFAP promoter and the other with CMV promoter, can efficiently express transgenes selectively in neurons vs. SGCs.

**Disclosures:** **H. Yu:** None. **H. Xiang:** None. **H. Xu:** None. **Q.H. Hogan:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.18/AA22

**Topic:** D.03. Somatosensation: Pain

**Title:** Effects of peripheral nerve injury on the kinetics of electrically evoked GABA receptor-mediated currents in GABAergic and non-GABAergic neurons of the spinal dorsal horn

**Authors: T. AJIMA**, E. KATO, S. TANAKA, Y. HORI, *T. FUKUSHIMA
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**Abstract:** In the spinal dorsal horn (SDH), several different types of neurons, including GABAergic inhibitory neurons and glutamatergic excitatory neurons, play important roles in modulating the synaptic transmission of sensory information, such as pain sensations. GABA is an inhibitory neurotransmitter that is crucially involved in the modulation of nociceptive transmission in the SDH. In this study, we characterized the GABA receptor-mediated currents recorded from the SDH neurons and investigated the effects of peripheral nerve injury on these currents. Experiments were performed on glutamate decarboxylase 67-green fluorescent protein (GFP) knock-in mice at the age of six to eight weeks. Spinal cord slices from the lumbar enlargement were prepared, and tight-seal whole-cell recordings were made from GFP-positive and GFP-negative neurons located in the SDH. Postsynaptic currents were evoked by internuncial electrical stimulation. GABA receptor-mediated inhibitory postsynaptic currents (GABA IPSCs) were pharmacologically isolated in the presence of the glycine receptor blocker strychnine, the NMDA receptor blocker CNQX, and the NMDA receptor blocker APV. The recordings were made at the holding potential of 0 mV. Partial sciatic nerve ligation (PNL) was performed according to the methods described by Seltzer et al. (1990). In sham-operated control mice, the peak amplitude of the GABA IPSCs recorded from the GFP-negative neurons was

larger than that from the GFP-positive neurons. However, the time decay of the GABA IPSCs was slower in the GFP-positive neurons than in the GFP-negative neurons. The PNL did not affect the amplitude of the GABA IPSCs but increased the rate of their decay over time in the GFP-negative neurons. In contrast, in the GFP-positive neurons, the PNL decreased the amplitude and slowed the time decay of the GABA IPSCs. The amount of charge transferred during the GABA IPSCs was decreased by the PNL in the GFP-negative neurons. It is presumed in the literature that the GFP-negative neurons in the SDH are predominantly glutamatergic excitatory neurons. If this assumption is true, our results indicate that the PNL decreases GABAergic inhibitory synaptic inputs to excitatory neurons in the SDH. The PNL-induced disinhibition of excitatory neurons may be involved in the mechanisms for peripheral nerve injury-induced mechanical allodynia. In order to elucidate the molecular adjustment that underlies our observed changes in the characteristics of GABA IPSCs, we are currently pursuing real-time quantative RT-PCR analysis to clarify how the PNL affects $GABA_A$ receptor subunit composition.

**Disclosures:** **T. Ajima:** None. **E. Kato:** None. **S. Tanaka:** None. **Y. Hori:** None. **T. Fukushima:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.19/AA23

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH NS045594

**Title:** Localized sympathectomy reduces regeneration and associated mechanical pain in the spinal nerve ligation model

**Authors: *W. XIE**, J. A. STRONG, J.-M. ZHANG
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**Abstract:** It is known that the sympathetic nervous system plays an important role in regulating pain and inflammatory responses. Our previous studies have demonstrated that mechanical hypersensitivity evoked by either spinal nerve ligation (SNL), localized inflammation of the DRG (LID; a low back pain model) or injecting the hindpaw with Complete Freund's Adjuvant (CFA; an inflammatory pain model), was greatly reduced by localized sympathectomy achieved by cutting the grey rami to the L4 and L5 sensory ganglia. Also, we recently found that in the SNL model the injured spinal nerve underwent functional regeneration and target reinnervation. The actively regenerating nerve was closely associated with spontaneous afferent activity and pain, which were both reduced by inhibiting nerve regeneration. In this study, we examined

whether inhibiting nerve regeneration is one of the mechanisms underlying pain relief by localized sympathectomy in the SNL model. The L5 spinal nerve was ligated and cut with or without concurrently cutting the grey rami to the L5 and L4 sensory ganglia on the same side. The regenerated nerve emerging from the nerve stump proximal to the ligature was observed in vivo, and was found to be thinner and paler in the rats with localized sympathectomy at all examined time points (POD 14, 28 and 42). *In vivo* fiber recording (from filaments teased from the L5 dorsal root and disconnected centrally) showed localized sympathectomy greatly reduced spontaneous activity, which was previously shown to originate in newly regenerated nerve. Functional regeneration and distal target reinnervation were also inhibited by localized sympathectomy: by day 42, the majority of receptive fields responses conducting through regenerated nerve could be detected only in the thigh and upper leg, unlike in the SNL model without sympathectomy, in which receptive fields in the knee, lower leg, and paw could be progressively detected starting from day 28. Previous studies suggest that macrophage infiltration around the axotomized sensory neuronal cell bodies plays a critical role in promoting nerve regeneration. We found that localized sympathectomy significantly reduced the macrophage accumulation in L5 dorsal root ganglion induced by axotomy. This results indicate that sympathetic nerve activity is important for peripheral nerve regeneration and reinnervation. Sympathetic nerves may be involved in promoting recruitment of macrophage to axotomized DRG neuron to stimulate nerve regeneration. Sympathetic blockade may relieve chronic pain partially via inhibiting nerve regeneration.

**Disclosures:** **W. Xie:** None. **J.A. Strong:** None. **J. Zhang:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.20/AA24

**Topic:** D.03. Somatosensation: Pain

**Support:** Evangelisches Studienwerk Villigst

graduate program in Pharmacology and Experimental Therapeutics at the University of Cologne in collaboration with and funded by Bayer

**Title:** Collagens modulate sensitization signaling and CGRP expression of nociceptive neurons

**Authors: *K. MÖLLER**[1], C. LOOS[2], J. ISENSEE[1], J. HASENAUER[2], T. HUCHO[1]
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**Abstract:** Painful tissue alterations such as wounds, tumors or arthritis are accompanied by changes in the extracellular matrix (ECM). Even though ECM has been extensively studied for its impact on neurite outgrowth and regeneration, knowledge about its influence on pain sensitization signaling in nociceptive neurons is sparse. Thus, we aimed to identify ECM components which alter sensitization signaling in nociceptive neurons, and to analyze their subgroup specificity, cellular mechanism and downstream targets.
We analyzed about 1 million cultured primary sensory neurons from male rat dorsal root ganglia for an influence of 17 ECM proteins on basal and NGF-, 5-HT-, and OncostatinM-induced phosphorylation of endogenous PKA subunits and/or Erk1/2 at 7 time points (510 conditions), using immunocytochemistry and a novel High-Content-Screening (HCS) microscopy approach. Based on single-cell data of candidates, a mechanistic computational model was established and analyzed for mechanistic changes using ordinary differential equation constrained mixture modelling (ODE-MM). Further subgroup and mechanistic analysis was pursued via HCS microscopy.
We identified the class of collagens to modulate NGF-induced pErk1/2 but not pRII-PKA kinetics. In contrast, none of the other ECM proteins altered basal and induced Erk1/2 and RII-PKA phosphorylation. Neuronal subgroup comparability was validated by HCS based subgroup analysis and ODE-MM. Collagens did not modulate GDNF-induced pErk1/2 kinetics and thus growth factor signaling of another neuronal subgroup. Mechanistic analysis excluded changes in basal TrkA and Erk expression, ligand affinity and dephosphorylation, and identified NGF-mediated effects on the TrkA receptor level to be the reason for differential pErk1/2 signaling. Furthermore, we could show that collagens induce long-lasting elevations of pErk1/2 levels and downstream CGRP expression.
Collagen content and composition is strongly modified in painful tissue alterations such as wounds, tumors or arthritis. Our results suggest that collagens augment nociceptor sensitivity long-lastingly in such pathologies. The observed elevated CGRP expression proposes a further increased pain signaling by para- and autocrine CGRP activity.

**Disclosures: K. Möller:** None. **C. Loos:** None. **J. Isensee:** None. **J. Hasenauer:** None. **T. Hucho:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.21/AA25

**Topic:** D.03. Somatosensation: Pain

**Support:** Else-Kröner-Fresenius Foundation

Funds from the University of Wuerzburg

**Title:** GSK-3 and microRNA-29 regulating blood nerve barrier tightness in neuropathy

**Authors: *H. L. RITTNER**[1], J. T. CHEN[1], S. YANG[2], S. SAUER[1], L. HU[2], A. BRACK[1]
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**Abstract:** Background: In peripheral nerves, the blood-nerve barrier (BNB) consisting of the perineurium surrounding nerve fascicles and the endothelium of endoneurial blood vessels protects neurons against nerve damage. Perineurial cells or endoneurial cells are connected by adherens junctions and sealed by tight junction proteins (TJP) like claudins or occludin. BNB tightness after controlled opening for drug delivery e.g. by claudin-1 specific peptides is governed by glycogen kinase synthase 3 (GSK-3) reulating the beta-catenin pathway. In pathological states like neuropathy, the BNB is opened leading to diffusion of possible toxic metabolites and immune cell invasion. Here, we explored mechanisms controlling BNB tightness in neuropathy.
Methods: Animal experiments were approved by local authorities. Neuropathy was induced in male Wistar rats via chronic constriction injury of the sciatic nerve. Thermal and mechanical nociceptive thresholds as well as tight junction protein expression and selected micro-RNAs were analyzed in CCI and after treatment with glycogen-3 synthase kinase inhibitors.
Results: Chronic constriction injury resulted in a downregulation of claudin-1 and ZO-1 mRNA and protein in the sciatic nerve compared to sham animals. Also, microRNA-29 was downregulated starting after 1 d and persistent for up to 14 d in the peripheral nerve. In silico, microRNA-29 bound to the 3'UTR of GSK-3beta. In the early phase of inflammation phosphorylation of GSK-3beta was enhanced. Short-term treatment with a GSK-3 inhibitor prevented TJP downregulation and the development of allodynia. However, no significant long-term reversal of allodynia and thermal hypersensitivity or prevention of TJP downregulation was observed.
Conclusion: GSK-3beta is only involved in early BNB regulation possibly via to downregulation of microRNA-29.

**Disclosures: H.L. Rittner:** None. **J.T. Chen:** None. **S. Yang:** None. **S. Sauer:** None. **L. Hu:** None. **A. Brack:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.22/AA26

**Topic:** D.03. Somatosensation: Pain

**Title:** Nanotechnology-based non-viral approaches for gene delivery to peripheral nerves

**Authors: *N. HIGUITA-CASTRO**[1], C. G. WIER[2], J. MOORE[3], A. SUNYECZ[3], C. K. SEN[1], S. J. KOLB[2], D. GALLEGO-PEREZ[1]
[1]Dept. of Surgery, [2]Dept. of Neurol., [3]The Ohio State Univ., Columbus, OH

**Abstract:** Nerve injury and neuropathies result in pain, loss of sensation, weakness, and systemic complications. Direct gene delivery to peripheral nerve has the potential to enable a host of novel therapies for these conditions. However, current gene delivery methods face numerous practical and translational hurdles, including heavy reliance on viral infection, stochasticity, and cellular damage. Additionally, delivery of targeted therapies to nerves is especially challenging because of the unique morphology of the neurons contained within, where cell bodies on the motor and sensory neurons reside within the central nervous system (CNS) and yet their axons can extend for over 1 meter into the body to either innervate muscle or sensory end organs. We developed a novel, facile, and powerful nanochannel-based technique for non-viral cargo delivery into nerves in a highly deterministic and benign manner. Cleanroom-based methods were used to fabricate millimeter-sized chip platforms for nanochannel-mediated gene delivery into the sciatic nerve (SN) of adult mice. Nanochannels are used to controllably nanoporate the cell membranes on the nerve tissue and electrophoretically deliver a wide variety of genes. We specifically delivered reprogramming angiogenic genes as potential strategy for the treatment of peripheral neuropathies. Delivery efficiency and retrograde transport from the SN to the CNS was assessed via immunofluorescence microscopy and qRT-PCR at the SN, dorsal root ganglion (DRG) and spinal cord (SC) levels. Tissue sections collected shortly after transfection revealed successful cargo delivery following the implementation of a short-lived (<100 ms) pulsed electric field across the nanostructured platform. Immunofluorescence analysis and qRT-PCR confirmed tissue transfection and strong pro-endothelial gene activity at the SN, DRG and SC levels. No appreciable behavioral changes (e.g., paw clenching, gait perturbations) were noted in mice that underwent the procedure. We were able to efficiently transfect peripheral nerve tissue using our nanostructured platform via non-viral approaches. This study supports the feasibility of gene delivery to targeted individual peripheral nerves to enhance regeneration following a focal injury to that nerve. Moreover, the retrograde delivery to the CNS expands the potential applicability of our method to the treatment of focal and regional CNS insults. Ongoing studies are currently focused on monitoring induced tissue plasticity following delivery of reprogramming factors, as well as developing pro-angiogenic reprogramming-based cell therapies for injury-induced neuropathies.

**Disclosures:** **N. Higuita-Castro:** None. **C.G. Wier:** None. **J. Moore:** None. **A. Sunyecz:** None. **C.K. Sen:** None. **S.J. Kolb:** None. **D. Gallego-Perez:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.23/AA27

**Topic:** D.03. Somatosensation: Pain

**Support:** NS87988

DE17794

NS91779

**Title:** Optogenetic manipulation of pain: Characterization of VGluT1:ChR2 dorsal root ganglion neurons and its implication in mechanical pain

**Authors: *M. YOUNG**[1], A. CHAMESSIAN[2], T. VAN DE VEN[2], R.-R. JI[2]
[1]Neurobio., [2]Anesthesiol., Duke Univ., Durham, NC

**Abstract:** Understanding the peripheral mechanisms underlying mechanical allodynia in neuropathic pain has proven challenging. The development of novel genetic lines in order to manipulate particular neurons within peripheral pain and somatosensitive circuits has facilitated the study of the underlying mechanisms in neuropathic pain. Previous work has shown that VGluT1 is primarily expressed in Aβ mechanoreceptors and proprioceptors offering a tool for the study of this population of neurons in mechanical pain, especially mechanical allodynia. In order to further study the role of Aβ mechanoreceptors in pain, we are currently using electrophysiology and microscopy to characterize a population of primary afferents expressing channelrhodopsin under the vesicular glutamate transporter type 1 Cre-recombinase driver (VGluT1:ChR2). In cultured dorsal root ganglion (DRG) neurons we find that VGluT1:ChR2 cells are enriched in large-diameter neurons. VGluT1:ChR2 neurons show narrow spike waveforms and lower spike thresholds consistent with Aβ mechanoreceptors. Importantly, activation of these neurons by optical stimuli lead to responses consistent with those elicited by direct current injection and the majority of neurons responded faithfully to optical stimuli up to 10 Hz. Immunohistochemistry performed on hairy and glabrous skin preparations revealed that VGluT1:ChR2 afferent endings terminated onto structures consistent with a mixed population of slowly and rapidly adapting mechanoreceptors including merkel cell-neurite complexes and lanceolate endings. The majority of VGlut1:ChR2 afferents terminated along the dermal-epidermal border and did not overlap with peptidergic nociceptor endings. Interestingly, a subset of terminals did however penetrate into the epidermis more consistent with free nerve endings. Taken together, this data suggests that the VGluT1:ChR2 subpopulation of DRG is enriched in low-threshold mechanoreceptors and validates the use of this model in studying the role of these cell-types in the expression of mechanical allodynia.

**Disclosures:** **M. Young:** None. **A. Chamessian:** None. **T. Van de Ven:** None. **R. Ji:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.24/AA28

**Topic:** D.03. Somatosensation: Pain

**Support:** Ministry of Science and Technology, Taiwan (103-2320-B-016 -016 -MY3)

**Title:** Sex difference of oxytocin-induced antiallodynia

**Authors: *J.-H. KAO**[1,2], L.-H. CHOW[3], Y.-H. CHEN[4], Y.-J. CHEN[2], E.-K. HUANG[2]
[2]Dept. of Pharmacol., [1]Natl. Def. Med. Ctr., Taipei, Taiwan; [3]Dept. of Anesthesiol., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [4]Dept. of Neurolog. Surgery, Tri-Service Gen. Hospital, Natl. Def. Med. Ctr., Taipei, Taiwan

**Abstract:** Background: We demonstrated previously that angiotensin IV (Ang IV) and LVV-hemorphin 7 (LVV-H7) act through the blockade of insulin-regulated aminopeptidase (IRAP) to decrease oxytocin degradation, thereby causing antihyperalgesia at the spinal level in rats. We determined that intrathecal oxytocin can induce significant antihyperalgesia in male rats with inflammation but not in female rats. Thus, we speculate that Ang IV, LVV-H7, and oxytocin can induce antiallodynia, which could be of great therapeutic potential. Because the anti-hyperalgesia by these peptides was with sex difference, their possible antiallodynia was examined in male and female mice for comparison. We investigated whether Ang IV, LVV-H7, and oxytocin produce antiallodynia at the spinal level in mice and whether this antiallodynia differs between the sexes.Methods: Partial sciatic nerve ligation (PSNL) surgery was performed on adult male and female C57BL/6 mice from the same litter (25-30 g). The effects of intrathecal injections of Ang IV (25.8 nmole), LVV-H7 (27.2 nmole), and oxytocin (0.125 nmole or 1.25 nmole) were assessed through the von Frey test 3 days after PSNL.Results: Intrathecal injection of Ang IV, LVV-H7, and oxytocin all produced a potent antiallodynia in male mice. However, these antiallodynia effects were either extremely weak or absent in female mice at the same dose.Conclusions: Intrathecal Ang IV, LVV-H7, and oxytocin can all cause significant antiallodynia in male mice. The Ang IV-, LVV-H7-, and oxytocin-induced antiallodynia effects differed between the sexes at the spinal level in mice.

**Disclosures: J. Kao:** None. **L. Chow:** None. **Y. Chen:** None. **Y. Chen:** None. **E. Huang:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.25/AA29

**Topic:** D.03. Somatosensation: Pain

**Support:** T32DA07234

T32DA007097

R21AT007098

R01DA03531

**Title:** Percutaneous electrical nerve stimulation elevates agmatine in spinal cords of nerve-injured rats

**Authors: *S. J. ERB**[1], C. PETERSON[2], R. H. SPELTZ[3], K. F. KITTO[3], K. A. SLUKA[6], G. L. WILCOX[4], C. A. FAIRBANKS[5]
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**Abstract: Background:** Decarboxylated L-arginine (agmatine) prevents or reduces the development of opioid tolerance and self-administration, as well as manifestations of neuropathic pain in rodents. Since agmatine is endogenously expressed, we hypothesized that its release in response to percutaneous nerve stimulation (electroacupuncture, EA) may contribute to alleviation of neuropathic pain. We report that endogenous agmatine levels in two rat strains is correlated with the degree of nerve injury-induced hyperalgesia, that agmatine levels in spinal dialysate are elevated during EA, and that spinal agmatine levels are diminished following nerve injury. These observations are consistent with the hypothesis that agmatine is an anti-hyperalgesic modulator released into spinal microdialysate during and following EA stimulation.
**Methods:** Male Sprague Dawley and Long Evans rats were subjected to nerve injury. Development of tactile hypersensitivity was monitored by von Frey monofilament stimulation. Spinal cord extracts were derivatized for agmatine analysis via fluorescence HPLC. Nerve-injured Sprague Dawley rats were also implanted with transverse spinal cord microdialysis fibers (AN69 Hospal), where dialysate was perfused at 5 µL/min and collected in 15-min bins. Baseline samples were collected under isoflurane for 1 hr before and during a 30-min electro-acupuncture stimulation (4 Hz, Zusanli acupoint (ST36)). Control subjects were anesthetized, but not stimulated. Samples were collected for 3 hrs following stimulation and anesthesia. Again, mechanical allodynia was assessed using von Frey monofilaments and spinal cord extracts

derivatized for agmatine analysis via fluorescence HPLC and LC-MS/MS. **Results:** The magnitude of nerve injury-induced tactile hypersensitivity was significantly lower in Long Evans compared to Sprague Dawley rats. The tissue concentration of spinal agmatine was substantially higher in Long Evans compared to Sprague Dawley rats. In nerve-injured Sprague Dawley rats subjected to percutaneous EA, we observed an elevation in agmatine concentration in spinal microdialysate, relative to unstimulated controls. **Conclusion**: These observations support a role for agmatine in alleviation of nerve injury-induced neuropathic pain.

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**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.26/AA30

**Topic:** E.05. Brain-Machine Interface

**Support:** NSF-CRCNS grant IIS-130764

NINDS grant R01-NS100016

NIGMS grant K08-GM1026911

NIGMS grant R01-GM115384

**Title:** A real-time rodent neural interface for deciphering acute pain signals

**Authors: *S. HU**[1], Q. ZHANG[2], J. WANG[3], Z. CHEN[4]
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**Abstract:** In recent years, brain machine interfaces (BMI) have attracted accumulating interest with goals to map, assist, augment or repair the cognitive or sensory-motor functions of human and animal brains. Pain is a common experience in our daily lives. However, the neural mechanisms of pain are still poorly understood. To improve our understanding of neural mechanisms of nociceptive pain, we developed a novel rodent neural interface aimed at detecting acute pain signals based on simultaneously recorded ensemble spike activity from multiple brain areas. Specifically, a custom tetrode microdrive was used to simultaneously record neuronal ensemble activity from the rat primary somatosensory cortex (S1) and anterior cingulate cortex (ACC). Three sequential change-point detection algorithms were used to detect pain onset based on online-sorted neuronal ensemble spikes. One is a model-free method called CUSUM

algorithm, and the other two are model-based methods, including PLDS (Poisson Linear Dynamical System) and TLDS (Transformed Linear Dynamical System). The model parameters of PLDS and TLDS were identified by the expectation-maximization (EM) methods, and the latent state was inferred using recursive filtering methods to identify a change in common sensory input that drives the population spike activity. The system has two parallel workflows based on multithread: one for online system identification, and the other for real-time filtering based on the pre-identified system parameters. We implemented two models independently for S1 and ACC recordings. The ultimate change-point detection was determined by integrating information from two parallel decision systems. The detection accuracy was measured by the sensitivity and specificity. Our preliminary results showed that all three methods had low false negative rates (FNR <5%). PLDS and TLDS yielded a similar performance in false positive rate (FPR), whereas the model-free method had very poor FPR. In terms of detection timing, PLDS achieved earlier detection than TLDS in the majority of trials (82%), and they had similar timing in 12% of trials. In summary, we have developed the first rodent neural interface that is capable of detecting acute pain onset signals in real time.

**Disclosures:** **S. Hu:** None. **Q. Zhang:** None. **J. Wang:** None. **Z. Chen:** None.

**Poster**

**581. Mechanisms of Peripheral Neuropathic Pain I**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 581.27/AA31

**Topic:** D.03. Somatosensation: Pain

**Support:** 1F31NS095421

R01NS088518

**Title:** Interaction of VGF-derived peptides in microglial signaling

**Authors: *J. L. COOK**[1], K. KITTO[1], M. S. RIEDL[3], C. N. HONDA[2], C. A. FAIRBANKS[4], L. VULCHANOVA[5]
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**Abstract:** VGF is a neuropeptide precursor whose expression is rapidly and robustly increased in dorsal root ganglion and dorsal horn neurons following peripheral nerve injury. We have demonstrated that the VGF-derived peptide TLQP-21 contributes to both the development and maintenance of hypersensitivity after peripheral nerve injury and inflammation, and that intrathecal administration of TLQP-21 results in thermal hyperalgesia. TLQP-21 signals through

the C3aR1 receptor, and we have demonstrated that TLQP-21 elicits $Ca^{2+}$ transients in microglia in spinal cord slices and primary glial cultures. The c-terminus of VGF includes a number of peptides, and TLQP-21 is cleaved from a larger peptide with another bioactive peptide AQEE-30. It is likely that the two peptides are exerting their effects in parallel, and therefore we used behavioral and calcium imaging assays to determine if they interact functionally. When AQEE-30 and TLQP-21 were co-administered intrathecally, maximal thermal hyperalgesia was achieved at lower doses than administration of each peptide alone. In primary microglial cultures, TLQP-21 evoked calcium transients in a concentration-dependent manner, whereas AQEE-30 failed to elicit responses. However, TLQP-21 evoked transients were potentiated in the presence of equimolar concentrations of AQEE-30. To explore the potential downstream effectors of TLQP-21-evoked microglial activation, a semi-quantitative cytokine array was performed on supernatant collected from cells stimulated with control media or TLQP-21. A number of pro-inflammatory and chemotactic immune factors that are implicated in neuropathic pain were significantly increased in TLQP-21-treated media and further work will examine whether a combination of TLQP-21 and AQEE-30 changes the profile of downstream microglial signaling. In the context of increased VGF expression after nerve injury and the microglial effects of TLQP-21, the interaction of TLQP-21 and AQEE-30 could be important in neuroimmune signaling under conditions of neuropathic pain.

**Disclosures:** **J.L. Cook:** None. **K. Kitto:** None. **M.S. Riedl:** None. **C.N. Honda:** None. **C.A. Fairbanks:** None. **L. Vulchanova:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.01/AA32

**Topic:** D.03. Somatosensation: Pain

**Support:** R21 AA023051

R01 DA018156

T32-AA014127

P50 AA022534

**Title:** Enduring pathological effects of prenatal alcohol exposure on touch hypersensitivity following peripheral nerve damage via neuroimmune mechanisms in adult rat offspring

**Authors:** ***J. SANCHEZ**[1], J. E. SANCHEZ[1], M. V. NYSUS[2], J. P. NORENBURG[2], D. D. SAVAGE[1], E. D. MILLIGAN[1]

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**Abstract:** Allodynia (hypersensitivity to light mechanical touch) is a clinical neuropathic pain condition shown to be mediated by activated spinal glia (astrocytes and microglia) and is associated with sciatic nerve chronic constriction injury (CCI) induced by loose ligation of 4 chromic gut sutures. We recently demonstrated that young prenatal alcohol exposed (PAE) rats develop potentiated allodynia following CCI with elevated spinal glial activation (Noor et al., 2017). The goal of the current study was to determine whether following PAE if (1) the pain effects are enduring, (2) a minor CCI injury (1-suture model) produces allodynia, and (3) blocking the activation of lymphocyte function-associated antigen-1 (LFA-1), an adhesion molecule expressed on trafficking immune cells and microglia, could suppress allodynia.
PAE and Saccharin control rat offspring (as described by Savage et al., 2010) were used for all experiments. For goals 1 and 2, middle-aged male (1 yr) or young male (4 mo) and female (4 mo) rats were assessed for hindpaw responses to light mechanical touch (von Frey fiber test) prior to and after either a standard (4-suture) or a minor (1-suture) CCI or sham surgery. For goal 3, young male & female rat hindpaw responses were assessed prior to & after CCI or sham surgery and after spinal (intrathecal) BIRT-377 (an LFA-1 antagonist) or vehicle injection until peak drug efficacy. Tissue from middle-aged and young males were prepared for immunohistochemical (IHC) detection of GFAP for astrocyte activation, Iba1 for microglia & peripheral macrophages, and the microglial specific marker, transmembrane protein 119 (TMEM119). A separate group of young females was given intravenous BIRT-377 or vehicle and reassessed for 4 days.
Results show standard CCI potentiated allodynia in middle-aged PAE rats while minor CCI generated unilateral allodynia similarly to standard CCI injury in non-PAE rats. Potentiated allodynia coincided with exaggerated expression of all glial activation markers whereas minor injury induced increased astrocyte GFAP expression. No changes in expression of microglial activation was observed despite ongoing allodynia. Young males given spinal BIRT-377 reversed from allodynia while females did not. Spinal cords from these behaviorally verified rats revealed astrocyte reactivity was greatest in young PAE allodynic males while microglial reactivity was elevated under all CCI conditions. PAE females revealed full allodynic reversal following intravenous BIRT-377. These results indicate PAE creates enduring pathological susceptibility to neuropathy with potentially distinct spinal neuroimmune mechanisms underlying sex differences.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.02/AA33

**Topic:** D.03. Somatosensation: Pain

**Support:** Natural Science Foundation of China, NO. 81671100

Natural Science Foundation of China, NO. 81200867

**Title:** Neuroglia activated by TREZ compression injury on trigeminal neuralgia animal model

**Authors: *D. LUO**, Y. YU
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**Abstract:** Objects: Trigeminal root entry zone (TREZ) is a segment of trigeminal nerve adjacent to the pons, and it is a transitional zone between central nervous system (CNS) and peripheral nervous system (PNS). TREZ compressed by microvascular is the major cause of the primary trigeminal neuralgia (TN) in patients, but the pathogenesis of TN is still unclear. To study the pathomorphological changes of neuroglia cells under TN in the TREZ, a novel animal model of TN by chronic compression of the trigeminal nerve root (CCT) from inferior orbital fissure in the male SD rats was established.
Methods: Paraformaldehyde-fixed TREZ were processed into 30-um thick serial longitudinal sections on post-operation day 7, 14, 21 and 28 in CCT animal model group and sham operation group, respectively. GFAP, IBA-1, P75NTR and MBP as different glial markers were detected in the TREZ after operation by use of immunofluorescent staining.
Results: Immunofluorescent staining showed, GFAP immunoreactivity was very strong in the TREZ and formed the dome boundary of CNS-PNS glial barrier. A great number of the glia cells were activated after CCT operation. The interface of dome boundary stained by GFAP was highly irregular after trigeminal nerve root compression, and it extended distally from CNS to PNS compartment. Both IBA-1 immunoreactive microglia and P75NTR immunoreactivities in CCT group increased on day 7, and gradually decreased from day 14 after operation, which were higher than those in sham operation group ( $p < 0.05$). MBP immunoreactivities were also activated in CCT group after operation, and the arrangement of oligodendroglia in CNS compartment of TREZ was disordered.
Conclusion: Trigeminal nerve root compression activated most of the neuroglia cells in the TREZ of TN animal model, and the CNS-PNS boundary of myelin sheath formed by myelinating glia was also changed after mechanical injury. TREZ was fragile to chronic compression, and the neuroglia cells activation may participated in the pathogenesis of TN.

**Disclosures:** **D. Luo:** A. Employment/Salary (full or part-time):; Department of Anatomy, Histology and Embryology, Basic Medical College, Fujian Medical University/Full time. 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.; Natural Science Foundation of China, NO. 81671100, 81200867. **Y. Yu:** A. Employment/Salary (full or part-time):; Department of Anatomy, Histology and Embryology, Basic Medical College, Fujian Medical University/part-time.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.03/AA34

**Topic:** D.03. Somatosensation: Pain

**Support:** R21 AA023051

T32-AA014127

P50 AA022534

**Title:** Characterizing immune cell phenotype and function in middle-aged male prenatal alcohol-exposed rats

**Authors:** ***S. NOOR**[1], J. J. SANCHEZ[1], A. G. VANDERWALL[1,2], S. DAVIES[1], D. D. SAVAGE[1], E. D. MILLIGAN[1]
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**Abstract:** A growing body of evidence indicates that prenatal alcohol exposure (PAE) may predispose individuals to secondary medical disabilities later in life. Animal models of PAE reveal neuroimmune sequelae such as elevated brain glial (astrocyte and microglial) activation with corresponding increases in proinflammatory cytokines. Our recent work demonstrates that young adult PAE rats display enhanced pathological sensitivity to light mechanical touch (allodynia) following adult-onset sciatic nerve injury (Noor *et al.*, 2016*),* which is mediated by increased spinal cord glial and cytokine actions that hyper-excite pain neurons. Corresponding elevations in spinal leukocyte activation and sciatic nerve proinflammatory cytokines also occur, suggesting that PAE-augmented immune responses extend beyond spinal glial cells to include alterations in peripheral immune cells. Therefore, we examined whether PAE-induced peripheral immune cell activation persists later in adulthood. Here, we have generated an overall peripheral immune cell profile in middle aged (~30 yrs human equivalent) male PAE rats using *ex vivo* flow

cytometry from the immune organs of the spleen, thymus, and medial iliac lymph node using the same moderate PAE paradigm reported previously (Savage *et al.,* 2010). Additionally, immune cell composition was characterized from peritoneal exudate cells (PECs) and peripheral blood. Functional responses of splenocytes and PECs to immune stimulation were further examined. While no significant change in the overall T and B cell numbers compared to non-PAE controls was observed, data suggest basal increases in natural killer cells numbers is present in the lymph node. Moreover, the proportions of CD11b/c+ leukocytes increase both in the spleen and lymph node, indicating basal activation in the absence of immune challenge. Interestingly, peripheral leukocytes (splenocytes and PECs) from PAE rats reveal exaggerated expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) and interleukin (IL)-1β following *in vitro* immune stimulation. These data indicate that peripheral immune cells are primed in PAE rats to produce aberrant immune responses and may underlie a susceptibility to developing autoimmune disease and inflammatory conditions following immune challenge in adulthood. Together, these results provide novel insight into the vulnerability that PAE produces in predisposing individuals to lifelong neuropathological conditions. Ongoing studies are investigating possible sex differences in PAE neuroimmune responses in adult PAE rats following peripheral nerve injury.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.04/AA35

**Topic:** D.03. Somatosensation: Pain

**Support:** 5T32DA035165-02

**Title:** The hippocampal extracellular matrix regulates pain and memory dysfunction after peripheral injury

**Authors: *M. TAJERIAN**[1], V. HUNG[3], H. NGUYEN[1], T.-T. HUANG[1], D. CLARK[2]
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**Abstract: Background:** Chronic pain is a heavy individual and societal burden, presenting with co-morbid psychiatric disorders (mood alterations and cognitive impairment) that parallel signs of brain neuroplasticity. Hippocampal involvement has been studied due to its role in cognition/memory and in modulating the overall pain experience. The functional, anatomical, and biochemical changes in patients and preclinical models suggest a pivotal role for hippocampal plasticity in the maintenance of chronic pain and implies a level of flexibility in the

extracellular environment in which cells function; a line of investigation not pursued to date. **Hypothesis**: Changes in the hippocampal extracellular matrix (ECM) support maladaptive cellular and behavioral plasticity after peripheral injury. **Methods**: We used a C57bl/6 male mouse model of chronic pain due to tibia fracture. Seven weeks post-injury, animals were tested for mechanical sensitivity and memory function. Slice electrophysiology was carried out in the hippocampal pyramidal layer. Adeno-associated virus-labeled hippocampal neurons were analyzed for dendritic complexity. Hippocampi were decellularized and used for scanning electron microscopy and atomic force microscopy. ECM components were measured using western blotting and ELISA. Immunohistochemistry was used to measure the abundance of stable inhibitory interneurons in the hippocampus. **Results:** We report deficits in working and location memory that are associated with increased hippocampal LTP, decreased dendritic complexity, altered ECM microarchitecture and decreased ECM rigidity. Furthermore, we observed biochemical changes in the ECM, including a decrease in aggrecan, an increase in matrix metalloprotease 8, and an increase in tissue inhibitor of matrix metalloprotease 2. We also report a reduction in specialized ECM nets around inhibitory interneurons, potentially accounting for the increased LTP. The reported anatomical, physiological, and biochemical changes in the hippocampal ECM reflect a local imbalance between plasticity-inhibiting and plasticity-promoting cues to help the organism adapt to a new environment that includes the multitude of co-morbidities that accompany injury and chronic pain. However, this prolonged imbalance can reach a pathological state thereby participating in the chronification of the overall pain experience and its resistance to traditional therapies. **Conclusions**: Targeting ECM plasticity in the chronic pain brain goes beyond examining nociceptive pathways themselves, and addresses structural factors supporting both pain and its related consequences.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

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**Program#/Poster#:** 582.05/AA36

**Topic:** D.03. Somatosensation: Pain

**Support:** NINDSR01NS08644401

**Title:** A key role of RGS4 protein in the maintenance of chronic pain

**Authors: *K. AVRAMPOU**, F. CARR, S. GASPARI, E. LOH, V. MITSI, A. OBRADOVIC, L. SHEN, V. ZACHARIOU
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**Abstract:** Regulator of G protein signaling 4 (RGS4) is a G-Protein Coupled Receptor (GPCR) modulator expressed in several CNS regions associated with pain transmission and perception. RGS4 controls the duration and direction of GPCR signaling by associating with activated Galpha ($Galpha_i$ and $Galpha_q$) subunits. Our previous studies used a neuropathic pain model to demonstrate that RGS4 is a positive modulator of the antiallodynic actions of monoamine-targeting antidepressants. Here, using the murine Complete Freud's adjuvant (CFA) and Spared Nerve Injury (SNI) models of chronic pain, we demonstrate a key role of RGS4 in the maintenance of sensitized behaviors. RGS4 Wild-type (RGS4WT) mice show prolonged mechanical and cold allodynia in response to peripheral inflammation or nerve injury, whereas mice lacking the Rgs4 gene (RGS4 Knock-out, RGS4KO) recover from mechanical and cold allodynia. This phenotype is observed both in female and male mice. RGS4 acts in a modality specific-manner as prevention of RGS4 affects mechanical and cold allodynia whereas it does not affect thermal hyperalgesia. Consistent with the behavioral studies, qPCR analysis highlights that RGS4 is uniquely regulated by long term peripheral inflammation or nerve injury in the spinal cord, the periaqueductal gray and the thalamus. Using viral mediated gene transfer for conditional knockdown of RGS4 in specific brain regions of adult floxed RGS4 mice, we also show that prevention of RGS4 action in the ventral posteromedial thalamic nucleus (VPm) results in the same phenotype to that observed in constitutive RGS4KO mice. Prevention of RGS4 action in a subset of nociceptive neurons expressing Vallinoid type-1 receptors (TRPV1) has a small but significant effect on recovery from CFA-induced allodynia. Finally, we applied RNA sequencing methodology in order to further understand the impact of RGS4 in gene regulation induced by peripheral inflammation in the thalamus. This analysis reveals different intracellular adaptations in the RGS4WT thalamus compared to RGS4KO groups in response to long term peripheral inflammation. Our data provide new information on the mechanisms underlying the maintenance of long-term pain states and point to RGS4 as a target for pain management.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

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**Program#/Poster#:** 582.06/BB1

**Topic:** D.03. Somatosensation: Pain

**Support:** Rita Allen Foundation

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American Pain Society

**Title:** Neural circuits for mechanical allodynia in the spinal dorsal horn

**Authors: *C. PEIRS**[1], X. ZHAO[1,2], S.-P. G. WILLIAMS[1], J. GEDEON[1], R. P. SEAL[1]
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**Abstract:** Pain continues to be a significant clinical problem due to its high prevalence, marked negative impact on quality of life and limited treatment options. The most common treatments such as opioid analgesics and non-steroidal anti-inflammatory drugs are frequently ineffective or have serious side effects. Development of more effective, well-tolerated pain treatments will come from a more complete understanding of the neural networks that mediate pain. This will then allow for the identification of the many key synaptic and molecular mechanisms that underlie this form of pain. Here we use a combination of chemogenetics, immunohistochemistry, electrophysiology and behavior to identify the neural network that mediates mechanical allodynia, a prevalent condition in which light touch or movement becomes painful with injury or disease. Our studies identify several critical neuronal populations that participate in the dorsal horn network for mechanical allodynia. Interestingly, the data also reveal the existence of distinct pathways depending on the type of injury. This latter finding has important implications not only for understanding at a basic level how the nervous system transmits mechanical allodynia, but it also reinforces that notion that etiology must be considered when implementing and developing treatment strategies.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

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**Title:** Separate spinal substrates transmitting dynamic versus static mechanical pain

**Authors: *L. CHENG**[1,2], B. DUAN[3,2], T. HUANG[2], Y. ZHANG[2], X.-J. SONG[4], O. BRITZ[5], L. GARCIA-CAMPMANY[6], X. REN[8], Y. CHEN[1], Q. ZHANG[1], X. XIE[1], R. ZHANG[1], J. WEI[1], Q. SUI[1], M. D. GOULDING[7], Y. WANG[1], Q. MA[2]
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**Abstract:** Pain evoked by innocuous mechanical stimuli or mechanical allodynia is a debilitating symptom suffered by millions of chronic pain patients. It exists in two forms, dynamic and static, which 1) are evoked by skin touch and skin pressure, respectively, 2) produce distinct perceptions, and 3) exhibit differential morphine sensitivity. However, neural circuits differentially transmitting these forms of allodynia remain unknown. Here we report that spinal neurons marked by developmental VGLUT3 expression form a morphine-resistant circuit required selectively to transmit dynamic mechanical pain. In contrast, static allodynia is likely mediated via both morphine-sensitive and morphine-resistant pathways that are preserved in VGLUT3 lineage neuron-ablated mice, but eliminated in somatostatin lineage neuron-ablated mice. The studies reveal for the first time separate spinal substrates transmitting dynamic versus static mechanical pain. Furthermore, acute silencing of VGLUT3 lineage neurons can attenuate pre-exisitng dynamic allodynia. The studies reveal for the first time separate spinal substrates transmitting dynamic versus static allodynia, and identify new cellular targets for treating these forms of neuropathic pain.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

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**Topic:** D.03. Somatosensation: Pain

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Chinese Academy of Sciences Hundreds of Talents Program

Youth Thousand Plan

Strategic Priority Research Program of the Chinese Academy of Sciences No. XDB02010000

**Title:** Functional roles of the spinoparabrachial pathway in nociception

**Authors: *J. DENG**[1,2], D. MU[1,2], Y. SUN[1]
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**Abstract:** Somatosensory signals information isare conveyed to the brain through by projection neurons in the dorsal spinal cord. The parabrachial nucleus (PBN), one of the major targets of the spinal cordprojection neurons, has been reported to be involved in pain sensation. However, the functional role of spinoparabrachial pathway in pain sensation is still unknown. Here, we investigated the anatomical and functional property of the spinoparabrachial pathway with a combination of viral tracing, slice electrophysiological and optogenetic techniques. We found that neurons in the spinal cord send projections to dorsal lateral PBN bilaterally. Use ofing the retrograde FuGB-Cre virus, we demonstrated that left PBN-projecting neurons in the spinal cord also send projections to right PBN and other brain areas, such as thalamus and periaqueductal grey. Moreover, neurons in PBN receive AMPA receptors-mediated excitatory input from the spinal dorsal horn. Optogenetic activation of the ipsilateral rather than contralateral spinoparabrachial projection induces pain related licking and flinching behaviors. While the emotion related behaviors, vocalization and conditional place avoidance (CPA), were only observed when the contralateral spinoparabrachial pathway was activated. Our study demonstrates the different roles of ipsi- and contralateral spinoparabrachial pathway in pain processing.
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**Disclosures:** **J. Deng:** None. **D. Mu:** None. **Y. Sun:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.09/BB4

**Topic:** D.03. Somatosensation: Pain

**Title:** Neuropathic pain creates an enduring deficit in medial prefrontal cortex-dependent behavioral performance that is resistant to gabapentin treatment but reversed by metformin

**Authors: *S. SHIERS**, G. PRADHAN, G. MEJIA, S. KROENER, T. PRICE
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**Abstract:** Chronic pain patients suffer from cognitive impairments reflected by deficits in working memory, emotion based decision-making, and cognitive flexibility even while taking commonly prescribed analgesics. These deficits are linked to problems in the medial prefrontal cortex (mPFC) in patients leading to the hypothesis that pain-related maladaptive plasticity occurs in this region disrupting healthy cortical activity. We sought to model this mPFC dysfunction in mice to examine molecular mechanisms of maladaptive plasticity and to assess the effect of therapeutics. Male mice with spared nerve injury (SNI) or sham surgery were given a single dose of intrathecal clonidine or 7-day treatment with systemic gabapentin or metformin. We assessed possible impairment in cognitive flexibility using an attentional set shifting task. SNI resulted in a profound impairment in cognitive flexibility which was not reversed by short-term pain relief using clonidine (10ug). Long-term treatment with gabapentin (100 mg/kg twice daily for 7 days) completely reversed mechanical hypersensitivity but had no positive effect on attentional set shifting. In stark contrast, metformin (200 mg/kg once daily for 7 days) completely reversed SNI-induced deficits in attentional set shifting. To examine molecular mechanisms of mPFC dysfunction we used immunohistochemistry to investigate changes in parvalbumin (an inhibitory interneuron marker), glial fibrillary acidic protein (GFAP; an astrocyte marker) and axon initial segment length (Ankyrin-G) in several mPFC subregions. While no changes in astrocytes or axon initial segment length have yet to be observed, there was significant loss of parvalbumin in the infralimbic cortex, a mPFC subregion involved in cognitive flexibility. Future analysis will examine whether gabapentin and metformin affect parvalbumin immunoreactivity. Our results highlight persistent changes in mPFC function in neuropathic pain and suggest that disease-modifying treatments are needed to positively influence this pathology.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.10/BB5

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH NS080889

**Title:** Long-term changes in sensory input to dorsal horn interneurons after neonatal tissue injury

**Authors: *J. LI**[1], M. L. BACCEI[2]
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**Abstract:** Recent work indicates that tissue damage during early life disrupts the balance between primary afferent-evoked excitation and inhibition of lamina I projection neurons in the adult spinal cord and facilitates spike timing-dependent LTP (t-LTP) at sensory synapses. However, whether this shift reflects cell type-specific alterations at synapses onto ascending projection neurons or are indicative of global changes in synaptic signaling across the mature superficial dorsal horn (SDH) remains unknown. To address this issue, the present study investigated the effects of neonatal surgical incision on primary afferent synaptic input to GABAergic and presumed glutamatergic interneurons in the adult mouse SDH using in vitro patch clamp recordings from spinal cord slices. Hindpaw incision at postnatal day (P) 3 strengthened primary afferent drive to mature Gad67-GFP neurons compared to naïve littermate controls, which included an increased prevalence of low-threshold sensory input. Afferent-evoked polysynaptic inhibition of these same neurons was reduced, resulting in a significant elevation in the excitation:inhibition (E:I) ratio. Paired presynaptic and postsynaptic stimulation at intervals that exclusively produced t-LTP in projection neurons evoked LTD in GABAergic neurons. There was a trend towards increased t-LTP in these neurons after P3 incision, although this did not reach statistical significance. Similar changes in the E:I ratio were observed in adjacent non-GFP neurons after neonatal incision, suggesting that early injury persistently shapes sensory input to excitatory interneurons in the SDH. Meanwhile, P21 incision failed to alter the E:I ratio in either population of interneurons during adulthood. Collectively, the results suggest that tissue damage during a critical period of early life evokes widespread changes in the processing of sensory information by interneurons within the spinal nociceptive circuit.

**Disclosures: J. Li:** None. **M.L. Baccei:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.11/BB6

**Topic:** D.03. Somatosensation: Pain

**Title:** Locus coeruleus (LC)-noradrenergic system plays a different role at short- and long-term of a neuropathic pain model

**Authors: *M. LLORCA-TORRALBA**[1,2,3], I. SUAREZ-PEREIRA[1,3], C. CAMARENA-DELGADO[1,3], L. BRAVO[1,2,3], J. A. GARCIA-PARTIDA[1,3], H. WEI[4], A. PERTOVAARA[5], J. A. MICO[1,2,3], E. BERROCOSO[1,2,3]
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**Abstract:** Patients suffering chronic pain often development long-lasting affective disorders. Accordingly, many preclinical studies have reported that prolonged experimental pain leads to depressive-like, anxiety-like behavior and cognitive deficit in rodents. Locus coeruleus (LC) is the major source of noradrenaline in the CNS and although its role in the descending inhibition of acute pain is well established, it has been reported a contribution to the facilitation of chronic pain. In addition, basolateral amygdala (BLA) has been focused as the locus of pain aversive memory formation and affective anxiety-like states via noradrenergic LC in long-term neuropathic pain. In the present study, we evaluated the time-dependent changes in the descending and ascending pathways of LC during the development of neuropathic pain model. Also, we evaluated the role of LC-BLA pathway on sensory, cognitive and anxiety-like aspect in long-term neuropathic pain. Sprague-Dawley and Long Evans-Tg(TH-CRE)3.1.Desis male rats were used. CCI was used as neuropathic pain model: 2 (short-term), 7 (middle-term) and 30 (long-term) days after surgery. A combination of behavioral, pharmacology and chemogenetics techniques were used for testing pain threshold, cognitive and anxio-depressive-like behaviors. In short-term neuropathy the LC is involved in descending pain pathway contributing to endogenous analgesia. In long-term neuropathy there is a LC functional sensitization that may involve the LC-BLA ascending pathway, implicated in pain-related anxiety-like, depression-like and cognitive disorders. Instituto de Salud Carlos III, FEDER, CIBERSAM G18, PI12/00915, CTS-510, CTS-7748, Ministerio de Economía y Competitividad (SAF2015-68647-R), 2015 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation, Cátedra Externa del Dolor-Grünenthal-UCA, Fundación Española del Dolor (PI2015-FED-007).

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.12/BB7

**Topic:** D.03. Somatosensation: Pain

**Support:** MD Anderson Cancer Center Internal Funds

**Title:** Microglial activation mapping in sensory and affective brain regions following peripheral nerve injury

**Authors: *M. J. LACAGNINA**, T. J. FABISIAK, P. M. GRACE
Dept. of Critical Care & Resp. Care Res., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

**Abstract:** Neuropathic pain is a debilitating condition in which an injury or dysfunction of the nervous system results in persistent pain, and its manifestation includes both sensory and affective dimensions. Microglia are the resident immune cells of the central nervous system, and their activation as a consequence of peripheral nerve fiber injury can amplify nociceptive signaling through a variety of mechanisms (including the release of cytokines, chemokines, or other neuroimmune mediators), thereby contributing to the development and maintenance of chronic pain. Investigations regarding the contribution of microglia to persistent pain have largely been restricted to the spinal cord. However, the landscape of microglial activation in supraspinal brain regions has not yet been systemically mapped, and existing reports are limited by the number of brain regions analyzed or the number of timepoints assessed post-injury. To address this issue, we used a rodent model of peripheral nerve injury and quantified microglial activation at several timepoints and across multiple brain regions that encode sensory and affective components of pain. Adult male Sprague Dawley rats received unilateral chronic constriction injury (CCI) of the sciatic nerve, and the spinal cord and brain tissues were collected either prior to surgery or at 7 and 28 days following injury. Microglial activation was assessed by performing densitometry analysis of fixed tissue stained for CD11b. We observed transient upregulation of CD11b expression in structures primarily associated with encoding the sensory aspects of pain. In contrast, CD11b expression was persistently elevated in nuclei thought to mediate affective pain components. These results provide evidence in male rats that an injury in peripheral tissues can produce central microglial activation in distributed brain regions associated with pain processing, and this glial reactivity is both time-sensitive and brain region-specific. Moreover, the pattern and time course of CD11b expression suggests that microglia

may be persistently dysregulated in networks encoding negative affect, which warrants further investigation.

**Disclosures:** **M.J. Lacagnina:** None. **T.J. Fabisiak:** None. **P.M. Grace:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.13/BB8

**Topic:** D.03. Somatosensation: Pain

**Support:** NSFC Grant 81371243

**Title:** Involvement of $GABAB_B$ receptor within the CSF-contacting nucleus in neuropathic pain of rats

**Authors: *L.-C. ZHANG**[1], S.-S. CHEN[2], S.-Y. SONG[2]
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**Abstract:** A special nucleus in the brain parenchyma, the cerebrospinal fluid contacting nucleus (CSF contacting nucleus), is just recognized in the central nervous system. The outstanding feature of this nucleus is that the neural somas locate in the parenchyma, and their processes stretch into CSF , which has the unique structural features participating in both neuro- neural and neuro- humoral crosstalk. It has been proved that CSF contacting nucleus play an role in the modulation of pain, but the molecular mechanism and exact pathway of that remains obscure. The aim of this study is to observe the expression of $GABA_B$ receptors in CSF-contacting nucleus and further to investigate its role in neuropathic pain. The study provide evidence of morphology and substance for revealing that the CSF-contacting nucleus is involved in the pain regulation. 1.$GABA_B$ receptors were observed in the CSF-contacting nucleus of normal and CCI neuropathic pain rats. Results :there was a basic expression of $GABA_B$ receptors in the CSF-contacting nucleus of normal rats. Compared to normal group, The proportion of $GABA_BR$ in CSF-contacting nucleus was significantly increased in neuropathic pain rats.There was no difference between left and right side of the CSF-contacting nucleus in neuropathic pain rats. 2.To observed the correlation between expression of $GABA_B$ receptors in the CSF-contacting nucleus of neuropathic pain and pain behavior at different time. The results indicated that the expression levels of $GABA_B$ receptors notably upregulated on day 3, and the elevation was maintained till day 14, with the peak on day 7. Further analysis of the correlation between the expression of $GABA_B$ receptors with pain behavior, there was a significant negative correlation between them. 3.Effects of i.c.v injection of baclofen ($GABA_B$ receptors agonist )on CCI-induced pain behavior in rats without CSF-contacting nuclues.The results are that after the

injection of baclfen into the LV on day 7 after the CCI, CCI-associated neuropathic pain was attenuated. However, this antinociception was of baclofen was inhibited by CGP55845, such that the TWL of CCI rats treated with CGP55845 and baclofen was lower than that of CCI rats treated with baclofen alone. No significant difference was detected in TWL between the CCI and CCI+saline groups. It suggested that $GABA_B$ receptors in CSF-contacting nucleus was significantly increased in neuropathic pain rats and the expression of GABAB receptors had a significant negative correlation with neuropathic pain behavior. GABAB receptors within CSF-contacting nucleus might play a functional role in modulating neuropathic pain.

**Disclosures: L. Zhang:** None. **S. Chen:** None. **S. Song:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

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**Program#/Poster#:** 582.14/BB9

**Topic:** D.03. Somatosensation: Pain

**Support:** NIH grant R01 NS088518

Training grant T32GM008471

**Title:** Time- and sex-dependent TrkB activation after nerve injury

**Authors: *R. GORE**, A. G. SKORPUT, M. S. RIEDL, J. L. COOK, C. HUFFMAN, L. VULCHANOVA
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**Abstract:** Brain derived neurotrophic factor (BDNF) signals via its cognate receptor Tropomyosin receptor kinase B (TrkB), which undergoes autophosphorylation upon binding of BDNF. This BDNF/TrkB signaling contributes to neuroplastic mechanisms of central sensitization under conditions of chronic pain. While BDNF is required for the development and maintenance of nerve injury-induced hypersensitivity in male rodents, it is dispensable for the maintenance of neuropathic pain in female mice, and the mechanism of this difference remains unknown. We hypothesized that comparison of TrkB activation in male and female mice will provide insight into this disparity. We utilized immunofluorescence directed against phosphorylation at the Tyr816 site of TrkB to examine the distribution of phosphorylated/activated TrkB (pTrkB) following nerve injury (spared nerve injury model, SNI) in male and female ICR mice. The observed pattern of labeling under normal conditions was consistent with previously reported expression of TrkB on dorsal horn primary afferent terminals, spinal neurons, and microglia. Selectivity of labeling was confirmed by the observation that it was substantially reduced following incubation of the tissue with Lambda

protein phosphatase. Preliminary immunofluorescent analysis following nerve injury shows that pTrkB is upregulated in dorsal horn of male and female mice 3 days post-SNI, and is at control levels by day 28 post-SNI. These initial observations suggest that BDNF signaling is increased in both sexes during the period of development of hypersensitivity. By comprehensive quantification of the time course and cellular identify of pTrkB immunofluorescence in control and SNI male verse female mice we hope to generate mechanistic insight into BDNF induced spinal neuroplasticity and sex differences in neuropathic pain.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.15/BB10

**Topic:** D.03. Somatosensation: Pain

**Support:** DOD CDMRP award MR130295

**Title:** The chemokine receptor cxcr2 supports nociceptive sensitization after traumatic brain injury

**Authors: *D.-Y. LIANG**, X. SHI, P. LIU, Y. SUN, P. SAHBAIE, W.-W. LI, D. C. YEOMANS, D. J. CLARK
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**Abstract:** Chronic pain after traumatic brain injury (TBI) is very common, but the mechanisms linking TBI to pain and the pain-related interactions of TBI with peripheral injuries are poorly understood. In the current work we pursue the hypothesis that epigenetic changes induced by TBI support chronic pain sensitization. We used the rat lateral fluid percussion model to induce mild and moderate TBI. Some animals received hindpaw incisions as well. Mechanical allodynia was measured for up to eight weeks post-injury. Inhibitors of histone acetyltransferase (HAT) and histone deacetylase (HDAC) were used to probe epigenetic mechanisms. We followed serum markers including glial fibrillary acidic protein (GFAP), neuron-specific enolase 2 (ENO2) myelin basic protein (MBP) and S100β to gauge TBI injury severity. Our results showed that both mild and moderate TBI caused mechanical allodynia in the hindpaws of the rats lasting 3 or more weeks. Hindpaws contralateral to TBI showed more rapid and profound changes. The inhibition of HAT using curcumin 50 mg/kg s.c reduced mechanical sensitization while the HDAC inhibitor suberoylanilide hydroxamic acid 50mg/kg i.p. prolonged sensitization in the mild injury model rats. These agents did not systematically change serum markers in ways suggesting regulation of the degree of brain injury constituted a complete explanation for the

observations. Immunohistochemical analyses of spinal cord tissue localized changes in the level of acetylation of the H3K9 histone mark to dorsal horn neurons. Taken together, these findings demonstrate that TBI induces persistent pain sensitization, and changes in spinal neuronal histone proteins may play an important role.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.16/BB11

**Topic:** D.03. Somatosensation: Pain

**Title:** Involvement of IFN-gamma of trigeminal spinal sub nucleus caudal neurons in neuropathic pain in ruts with infraorbital nerve injury

**Authors: *S. YUKA**[1,2], A. OKADA-OGAWA[2], M. SHINODA[3], Y. IMAMURA[2], K. IWATA[3]
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**Abstract:** It is well known that the trigeminal nerve injury such as tooth extraction or orthodontic surgery causes severe pain (neuropathic pain) in the orofacial regions. The orofacial neuropathic pain caused by trigeminal nerve injury is difficult to diagnose and treat because of the complexity of its neuronal mechanism. It is very important to evaluate the neuronal mechanisms underlying orofacial neuropathic pain, in order to develop the appropriate approaches to treat neuropathic pain patients. For this purpose, we developed the neuropathic pain model with infraorbital nerve injury in rats. Recently, it has been reported that the peripheral nerve injury causes an upregulation of cytokine Interferon-gamma (IFN-gamma). Intrathecal administration of the IFN-gamma induced mechanical allodynia, contributing to the development of neuropathic pain. We hypothesized that IFN-gamma play a crucial role of a glia-neuronal interaction in the trigeminal spinal subnucleus caudalis (Vc), involving in the development of orofacial neuropathic pain in the whisker pad skin. We introduced a neuropathic pain model with infraorbital nerve injury following tight ligation of half of the infraorbital nerve (IONI). Mechanical nocifensive behavior, phosphorylated extracellular signal-regulated kinase (pERK), phosphorylated GluR1 (pGluR1), Iba1 (a marker of microglia) and IFN-gammaR immunohistochemistry were precisely analyzed in the IONI rats and the sham rats. Head-withdrawal threshold to mechanical stimulation of the whisker pad skin innervated by infraorbital nerve decreased at day 4 after nerve injury in IONI rat but not in sham rat. The pERK, pGluR1 and Iba1 expression significantly increased in Vc at day 4 after IONI. The decrements of head withdrawal threshold to mechanical stimulation were reversed during

intrathecal (i.t.) administration of MAPK/ERK kinase 1/2 inhibitor, PD98059 and inhibitor of microglia, minocycline. On the otherhands, i.t. administration of IFN-gamma induced decrements of head withdrawal threshold to mechanical stimulation in sham rats. The present findings have demonstrated that mechanical allodynia and hyperalgesia occur in the whisker pad area following the infraorbital nerve injury. It also suggest that GluR1 phosphorylation of Vc neurons and activation of microglia in Vc area are involved in the neuropathic pain, and IFN-gamma might play a important role of the glia-neuronal interaction.

**Disclosures:** **S. Yuka:** None. **A. Okada-Ogawa:** None. **M. Shinoda:** None. **Y. Imamura:** None. **K. Iwata:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.17/BB12

**Topic:** D.03. Somatosensation: Pain

**Support:** Grants-in-Aid and by special coordination funds from Grants-in-Aid for Scientific Research (C) (16K10988) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

**Title:** Involvement of the HMGB1-TLR4 signaling in the development of central post-stroke pain

**Authors: *S. TOKUYAMA**, W. MATSUURA, S. HARADA
Kobe Gakuin Univ., Kobe, Japan

**Abstract:** Central post-stroke pain (CPSP) is known as a sequelae occurring after cerebral stroke. The treatment of CPSP remains incomplete due to its resistance to both pharmacological and non-pharmacological therapies in approximately half of CPSP patients. Since cure of central post-stroke pain has been difficult, establishment of a novel treatment method is desired. High-mobility group box 1 (HMGB1) and the receptor for advanced glycation end products (RAGE, one of the receptors of HMGB1) have recently been shown to be critical in the modulation of nociceptive transduction following peripheral neuropathy. We have been suggested that spinal HMGB1 might be involved in the induction of CPSP. The aim of this study is to determine that HMGB1-toll like receptor 4 (TLR4), one of receptor of HMGB1, interaction in spinal glial cell is directly involved in induction of CPSP.
Male ddY mice were subjected to 30 min of bilateral carotid artery occlusion (BCAO). The development of hind paw mechanical hyperalgesia was measured using the von Frey test.
The expression of HMGB1 protein in the spinal cord was significantly increased on day 3 after BCAO. In addition, spinal microglia and astrocyte were clearly activated by BCAO. HMGB1

was colocalized with neurons, activated microglia and astrocyte after BCAO. On day 3 after BCAO, although TLR4 expression was no change compared with sham mice, intrathecal injection of LPS-RS (5 or 10 μg/mice, TLR4 antagonist) significantly blocked the mechanical allodynia on day 3 after BCAO using the von Frey test. We also confirmed that TLR4 was colocalized with HMGB1 and microglia in the spinal cord. Furthermore, BCAO-induced spinal microglia activation was suppressed by intravenous anti-HMGB1 antibody and intrathecal LPS-RS administration.
We concluded that ischemic-stress evokes HMGB1 release from spinal neurons and glia cells, and subsequent activation of spinal microglia by HMGB1 maintains the ischemic stress-induced pain. Additionally, microglial HMGB1-TLR4 interaction is directly involved in the pathogenesis of CPSP. Our findings are helpful for better understanding of the pathological mechanism in CPSP.

**Disclosures: S. Tokuyama:** None. **W. Matsuura:** None. **S. Harada:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.18/BB13

**Topic:** D.03. Somatosensation: Pain

**Title:** Possible involvement of spinal nicotinic receptor subtypes in a rat model of trigeminal neuropathic pain

**Authors: *K. NAKAI**[1], A. NAKAE[2], T. KUBO[3], Y. MINEGISHI[4], K. HOSOKAWA[3]
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**Abstract:** (Background) Nicotinic acetylcholine receptors (nAChRs) are widely expressed in the spinal cord and modulate nociceptive transmission. Activation of cholinergic pathways by the nicotinic agonists has been shown to elicit antinociceptive effects. The contribution of spinal nAChRs in inhibiting the trigeminal neuropathic pain remained unclear. Chronic constriction injury to the infraorbital nerve (ION-CCI) has proven a useful model for trigeminal neuropathic pain. The present study evaluated the possible role of spinal nAChRs in ION-CCI rat model. (Material and Methods) Male Sprague Dawley rats underwent unilateral CCI to the right ION. Two nylon (5-0) ligatures were tied around the ION. Series of von Frey filaments were used to determine pain hypersensitivity to mechanical stimulation on day 14 after surgery. A polyethylene (PE-10) catheter was implanted for upper cervical spinal injection of drugs. The rats were allowed to recover for 7 days. The potential anti-allodynic effects from intrathecal

administration of a nAChR agonist nicotine, an alpha4beta2 nAChR agonist RJR2403, and an alpha7 nAChR agonist PNU 282987 were examined. In addition, the role of nAChR, alpha4beta2 nAChR, and alpha7 nAChR in these effects was assessed by determine whether the anti-allodynic effects were prevented by intrathecal administration of a nAChR antagonist mecamylamine, an alpha4beta2 nAChR antagonist dihydro-beta-erythroidine, or an alpha7 nAChR antagonist methyllycaconitine. The time course data for the dose-response effects were analyzed by two-way analysis of variance and Tukey-Kramer multiple-comparison test. (Results) Intrathecal administration of nicotine, RJR2403, and PNU 282987 increased mechanical thresholds ($P$ <0.05). Mecamylamine significantly reduced the anti-allodynic effect of nicotine. Dihydro-beta-erythroidine significantly reduced the anti-allodynic effect of RJR2403. Methyllycaconitine significantly reduced the anti-allodynic effect of PNU 282987. (Conclusions) The results indicated that spinal nAChRs, alpha4beta2 nAChRs, and alpha7 nAChRs may play an important role in a rat model of trigeminal neuropathic pain.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.19/BB14

**Topic:** D.03. Somatosensation: Pain

**Support:** Grant-in-Aid for Scientific Research (B) Grant No 16H05460

**Title:** Activated spinal astrocytes sensitize the nociceptive transmission through the release of L-lactate

**Authors: K. MIYAMOTO**, K.-I. ISHIKURA, K. KUME, *M. OHSAWA
Nagoya City Univ., Aichi, Japan

**Abstract:** Neuropathic pain induces the functional changes of the central nervous systems. Among them, the morphological changes of the astrocytes by activation are important and play roles in the expression and maintenance of neuropathic pain. We previously revealed that intrathecal (i.t.) administration of L-lactate induced mechanical hyperalgesia, which was attenuated by i.t. treatment with α-cyano-4-hydroxycinnamate (4-CIN), a monocarboxylic acid transporter that transports L-lactate into astrocytes and neurons. In this study, we investigated the mechanisms of this L-lactate-induced mechanical hyperalgesia. L-lactate-induced lowering of mechanical withdrawal threshold was attenuated by i.t. treatment with isosafrole that inhibits lactate dehydrogenase 1 (LDH1) and LDH5 which convert L-lactate to pyruvate. I.t. administration of L-lactate increased the expressions of c-Fos (a marker for neuronal activity) in

the spinal cord dorsal horn, which was attenuated by i.t. treatment with 4-CIN. Lowered mechanical withdrawal threshold on seven days after the partial sciatic nerve ligation was attenuated by i.t. treatment with 4-CIN and isosafrole. Then, we microinjected an adeno-associated virus (AAV) vector expressing hM3Dq designer receptor exclusively activated by a designer drug (DREADD) under the control of the glial fibrillary acidic protein (GFAP) promotor into the L4 dorsal horn of spinal cord. Intraperitoneal (i.p.) administration of clozapine N-oxide (CNO), which is the designered ligand of hM3Dq, induced hyperalgesia in ipsilateral, but not contralateral side of AAV microinjection. This CNO-induced mechanical hyperalgesia was continued until 8 hours after administration, which was attenuated by i.t. treatment with 4-CIN. These results suggest that L-lactate released from reactive astrocytes in neuropathic pain is one of the key mediators to induce hyperalgesia through the direct sensitization of nociceptive transmission in the spinal cord.

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**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.20/BB15

**Topic:** F.10. Food Intake and Energy Balance

**Title:** Synergistic antinociceptive interaction between Syzygium aromaticum and Rosmarinus officinalis

**Authors: *K. BELTRÁN-VILLALOBOS**[1], M. DÉCIGA- CAMPOS[1], H. AGUILAR-MARISCAL[2], J. LÓPEZ- MUÑOZ[3]
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**Abstract:** Herb-Herb pharmacological interactions are present when the active compounds of the herbs alter their pharmacodynamic or pharmacokinetic process. The interactions could be synergistic, additive, or antagonistic, and the modified effect could be therapeutic or toxic. Thus, the analysis of the results of herb-herb combinations is important for demonstrating the possible therapeutic utility of the combination treatment and to identify their potential use. The aim of the present study was to determine whether *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Mirtaceae), is able to increase the antinociceptive effects of *Rosmarinus officinalis* L. (Lamiaceae). The peripheral antinociceptive effect was assayed in the formalin test in the rat. Rats received local pretreatment with saline, *Syzygium aromaticum* oil or *Rosmarinus officinalis* ethanolic extract followed by 50 µl of either 1% formalin. *Syzygium aromaticum* ($ED_{50}$=31.12±1.6 mg/kg) and *Rosmarinus officinalis* ($ED_{50}$=11.09±0.5 mg/kg) each showed a dose-dependent anti-nociceptive effect. The pharmacological interaction was investigated by an

isobologhaphic study using the $ED_{50}$ of each component in a fixed 1:1 ratio. The isobolographic analysis showed a synergistic interaction between *Syzygium aromaticum* and *Rosmarinus officinalis*, the experimental value Zexp= 5.6± 0.5 was lower than the theoretical value Zadd=21.1±1.1. These results provide evidence that this medicinal combination herb-herb are effective in this model of pain.

**Disclosures: K. Beltrán-Villalobos:** None. **M. Déciga- Campos:** None. **H. Aguilar- Mariscal:** None. **J. López- Muñoz:** None.

**Poster**

**582. Mechanisms of Central Neuropathic Pain**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 582.21/BB16

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NS34696 (DJS)

**Title:** Circuit-specific adaptations in mesoaccumbal circuitry modulate distinct features of chronic pain

**Authors: *W. REN**[1], M. V. CENTENO[1], X. WEI[1], I. WICKERSHAM[2], M. MARTINA[1], A. V. APKARIAN[1], D. J. SURMEIER[1]
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**Abstract:** The mesolimbic circuitry - including the ventral tegmental area (VTA) and nucleus accumbens (NAc) - shape chronic pain. We found that nociceptive signaling suppresses the activity of VTA dopamine neurons projecting to the medial shell of the NAc (msNAc), triggering cell-specific adaptations there and augmented chronic pain (tactile allodynia). However, recent work by other groups has shown that neurons in the neighboring core of the NAc (cNAc) manifest a very different set of adaptations in chronic pain models, suggesting that the dopaminergic innervation of this region by the VTA was responding differently to chronic nociceptive signaling. In support of this conclusion, anatomical studies have shown that msNAc and cNAc are innervated by distinct populations of VTA neuron.
To test the hypothesis that the circuitry of the msNAc and cNAc respond differently to chronic nociceptive signaling, cNAc spiny projection neurons (SPNs) and VTA dopaminergic neurons were studied in ex vivo brain slices from the spared nerve injury (SNI) rodent model of persistent neuropathic pain. Indeed, the intrinsic excitability of indirect pathway SPNs (iSPNs) in the cNAc

was lower and the strength of synaptic connections with prefrontal cortex was augmented in tissue from SNI mice - in sharp contrast with the adaptations in the msNAc. Using a non-toxic rabies virus carrying a Cre-recombinase expression construct, it was found that the VTA dopaminergic neurons innervating cNAc and msNAc were largely distinct, in agreement with previous studies. Moreover, the intrinsic excitability of these two VTA populations change in opposing ways following SNI - falling in cells innervating msNAc and rising in cells innervating cNAc - mirroring the changes in cNAc and msNAc. To test the hypothesis that these changes were causally linked to the pain state, chemogenetic approaches were used. Normalizing the excitability of the VTA-cNAc circuit diminished SNI-induced social recognition deficits, without affecting allodynia, contrasting it with our previous work showing chemogenetic correction of msNAc excitability alleviated allodynia. Thus, there are parallel VTA-NAc circuits that are causally linked to distinct features of the chronic pain state. The differential modulation of these circuits by SNI appears to stem from the circuitry controlling the VTA. Studies are underway to dissect this circuitry.

**Disclosures:** **W. Ren:** None. **M.V. Centeno:** None. **X. Wei:** None. **I. Wickersham:** None. **M. Martina:** None. **A.V. Apkarian:** None. **D.J. Surmeier:** None.

**Poster**

**583. Somatosensation: Thalamocortical Processing**

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**Topic:** D.04. Somatosensation: Touch

**Support:** NIH Grant 1R01GM116916-01

**Title:** Effect of isoflurane on selectively activated afferent pathways in neocortex

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**Abstract:** The mechanisms underlying loss of consciousness (LOC) under anesthesia remain unknown; current studies suggest that anesthetic-induced LOC is mediated by the corticothalamic network. While some studies have proposed that anesthetics gate ascending information via thalamocortical (TC) projections, recent evidence suggests that feedback corticocortical (CC) projections may be preferentially affected by anesthetics. For example, current sinks triggered by stimulation of layer 1 afferents to primary auditory cortex are suppressed by isoflurane to a greater extent than layer 4 TC afferents. However, layer 1 contains axons originating from both TC and CC projections. We compared the effect of isoflurane on layer 1 versus layer 4 TC pathways to determine whether isoflurane-sensitive afferents in layer 1 might originate in the thalamus. We separately measured the effect of isoflurane on layer 1

currents following specific activation of CC feedback connections. Posterior parietal cortex (PPC) and secondary visual cortex (V2) both integrate information from higher order thalamus along with primary sensory and higher order cortical areas. Because activity in these areas is specifically linked to awareness, we investigated the effect of isoflurane on the properties of monosynaptic current sinks following stimulation of TC and CC afferents to PPC and V2, respectively. Channelrhodopsin was expressed in either posterior thalamus (Po) or cingulate cortex (Cg). Axon terminals in layers 1 and 4 of PPC or layer 1 of V2 were activated. Isoflurane was delivered to slices via artificial cerebral spinal fluid, and current source densities (CSD) were recorded using 16channel electrode arrays oriented orthogonally to the cortical laminae. Monosynaptic current sinks in respective layers were compared between control and drug conditions for each pathway. Consistent with previous findings, isoflurane only modestly suppressed the magnitude of monosynaptic current sinks following stimulus of TC afferents in layer 4. Interestingly, layer 1 TC current sinks were blocked to the same extent as layer 4 sinks, suggesting the most isoflurane-sensitive layer 1 fibers are not of thalamic origin. We also observed a concentration-dependent block by isoflurane of layer 1 CC feedback pathways to V2 following optogenetic stimulation.

**Disclosures:** **C. Murphy:** None. **B. Krause:** None. **S. Grady:** None. **M.I. Banks:** None.

**Poster**

**583. Somatosensation: Thalamocortical Processing**

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**Title:** Quantifying sensory encoding from thalamus to cortex in topographically aligned neuron pairs

**Authors: *Y. LIEW**[1], C. J. WHITMIRE[1], A. PALA[1], W. A. STOY[1], P. Y. BORDEN[1], A. D. ORTIZ[1], B. YANG[2], C. R. FOREST[1,2], G. B. STANLEY[1]
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**Abstract:** The processing of external stimuli requires the transmission of sensory information across brain regions. However, the nature and efficiency of synaptic transmission across neuronal circuits in the intact brain, in the context of information transformation, remain unclear.

Here, we take advantage of the well-known anatomical and functional organization of the rodent whisker thalamocortical pathway to perform simultaneous in vivo recording in the topographically aligned neurons from the ventroposteromedial nucleus of the thalamus (VPm) and layer 4 of primary somatosensory cortex (S1) to understand how thalamic neurons drive their downstream cortical targets. Using weak stimulation of the rodent whisker, we first inferred the functional connectivity between pairs of simultaneously recorded thalamocortical (TC) neurons using a cross-correlation technique. We then quantified synaptic efficacy, defined as the probability of eliciting a cortical spike in a short time window following a thalamic spike, for putatively connected pairs of neurons to investigate the effect of thalamic firing mode (e.g. burst vs tonic) on the synaptic efficacy of TC network. Preliminary analysis suggests a complicated relationship between thalamic firing mode and synaptic efficacy at the TC synapse. Additionally, it is well established that a single neuron in cortex receives inputs from multiple thalamic neurons and that synchronous activation of this population of thalamic inputs could maximally drive cortical neurons. Here, we are evaluating the effect of sensory stimulation on thalamic synchrony within and across barreloids as well as the implications of thalamic population activity for downstream cortical targets using a 32-channel probe in VPm combined with single unit recordings from S1. Preliminary analysis suggests that punctate whisker stimuli evoked synchronized response within and across thalamic barreloids, and this could potentially affect cortical integration of information downstream. Finally, applying white noise stimuli to a single whisker, we assessed stimulus feature selectivity in pairs of putatively connected and non-connected neurons, using traditional reverse correlation analysis. Preliminary results show that the feature selectivity between putatively connected neurons is not identical, instead suggesting that the cortical neuron is pooling its selectivity from the distinct selectivity of its thalamic inputs. Taken together, this work utilizes the simultaneous multi-site recording framework to provide a step towards establishing the relationship between sensory percepts and the neural circuits that underlie them.

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**Poster**

**583. Somatosensation: Thalamocortical Processing**

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SNSF Early Postdoc Mobility Fellowship

**Title:** Thalamic state modulation of somatosensory encoding in the thalamocortical circuit

**Authors: *C. J. WHITMIRE**, Y. LIEW, A. PALA, G. B. STANLEY
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**Abstract:** While traditionally labelled as a relay nucleus, it is now widely accepted that the thalamus plays an important role in transforming sensory information traveling from the sensory periphery to primary sensory cortex. Furthermore, the distinct burst/tonic firing modes of the thalamus may represent different information processing states such that the regulation of thalamic firing modes could function as one mechanism for controlling how information is conveyed to downstream cortical targets. Yet how this gating mechanism impacts precise feature selectivity in the thalamocortical circuit remains unknown. Using in-vivo single unit recordings in the rat vibrissa thalamus (VPm), feature selectivity was assessed using white noise single-whisker stimulation while simultaneously manipulating the firing mode of the thalamic neurons using optogenetic hyperpolarization. In response to a white-noise stimulus delivered to the whisker, individual thalamic neurons displayed reliable responses containing a similar number of spikes irrespective of their level of hyperpolarization. However, the sensory evoked firing of the thalamic neurons shifted from tonic to bursting when optogenetically hyperpolarized. Furthermore, this shift in firing mode led to a loss of the feature selectivity as assessed using traditional reverse correlation techniques. Because the output of the thalamic neurons, or the spiking activity, is fundamentally different as a function of firing mode, the state-dependent thalamic input to cortex may induce significant shifts in cortical feature selectivity and encoding. To measure the impact of this state-dependent change in thalamic feature selectivity on neocortical feature selectivity and encoding, single unit cortical activity was recorded along a cortical column in primary somatosensory cortex (S1) using a 32-channel linear probe while optogenetically manipulating thalamic state. Consistent with the thalamic findings, cortical units with recoverable linear filters showed a reduction in the amplitude of their kernels with thalamic hyperpolarization. Furthermore, fast spiking cortical units were significantly more likely to exhibit sensory feature selectivity than regular spiking units suggesting differential processing of the incoming sensory information by different cell types. These results could have broad implications for a more comprehensive coding strategy whereby ongoing sensory stimulation as well as a host of endogenous and exogenous influences dynamically alters the state of the thalamus to fundamentally shape the functional encoding of the thalamocortical pathway.

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**Poster**

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**Title:** Thalamic control of sensory evoked spatiotemporal cortical responses

**Authors: *P. Y. BORDEN**[1], I. KOLB[1], A. D. ORTIZ[1], A. J. SEDERBERG[1], C. WAIBLINGER[1], W. STOY[1], C. FOREST[2,3], A. MORRISSETTE[4], D. JAEGER[4], G. B. STANLEY[1]
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Georgia Tech., Atlanta, GA; [3]Mechanical Engin., Georgia Inst. Of Technol., Atlanta, GA; [4]Biol., Emory Univ., Atlanta, GA

**Abstract:** The thalamus is a central junction that processes both sensory and motor signals. Although many neurological disorders are linked to thalamic dysfunction, basic information about ongoing thalamic processing is still unknown. Specifically, it is unclear how ongoing changes in membrane polarization, firing modes, and overall firing rate alter the transmission of information to and from cortical regions. Thalamic neurons have complex firing modes (i.e., tonic and burst) and receive neuromodulatory inputs that shape ongoing activity. Although ongoing thalamic activity has been shown to dynamically alter ongoing cortical activity, it is unclear how distinct thalamic firing modes (i.e., state) change sensory percepts. Here, we utilized a genetically encoded voltage indicator, ArcLight, to measure large scale cortical sensory evoked response while measuring different thalamic states using thalamic single unit extracellular recordings. Specifically, we modulated the thalamic state through optogenetic hyperpolarization of the primary whisker thalamic nucleus (VPM) with viral delivery of halorhodopsin (eNph3.0). We found that by briefly shifting the thalamus to a more hyperpolarized state, we increased both the thalamic and cortical sensory evoked response. Furthermore, we found that by titrating the levels of thalamic hyperpolarization, we could control the spatial and temporal primary somatosensory cortical responses. Through *in vivo* extracellular single cell and *in vitro* intracellular whole cell recordings, we predict that thalamic hyperpolarization enables synchronized sensory evoked calcium bursting events. These busting events produce a larger

downstream cortical spatial response that theoretically should increase the detectability of sensory stimuli, at a cost of discriminability. Based on these results, we demonstrate the importance of thalamic state on controlling the flow of sensory information across cortex as a possible mechanism to control the perception of sensory information.

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**Poster**

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**Title:** Closed loop optogenetic control of thalamocortical activity

**Authors:** ***M. F. BOLUS**[1], A. A. WILLATS[1], C. J. WHITMIRE[1], C. J. ROZELL[2], G. B. STANLEY[1]
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**Abstract:** When investigating tightly interconnected neural circuits such as the thalamocortical loop, isolating the function of individual components in the network is a powerful approach to understanding complex function. In contrast to irreversible lesioning techniques or slow pharmacological manipulations, optogenetics offers cell-type specific actuation at fast timescales. While widely used for such circuit dissection, optogenetic stimulation is nearly always designed before an experiment and presented in “open-loop”. The effectiveness of this approach is limited by changes in ongoing neural activity, variability in opsin expression, *etc*. A more robust approach is to “close the loop” around the activity of interest and update stimulation in real-time to compensate for such variability. We have developed a closed-loop control strategy where optical stimulation is updated in real-time to elicit a desired pattern of neural firing. We applied this technique to the stimulation of neurons in the somatosensory thalamus of fentanyl-anesthetized rats expressing channelrhodopsin (ChR2). Single-unit spiking isolated from extracellular recordings was smoothed into an online estimate of firing rate. A proportional-

integral controller operated on the error between the instantaneous firing rate and a desired pattern of firing to modulate the intensity of optical stimulation: blue light delivered to thalamus via an optic fiber. Simultaneous recordings in S1 provided insight into the downstream effects of thalamic control. Using a principled design strategy, we have found that this simple-to-implement control scheme is effective not only at holding firing rate of thalamic neurons steady (e.g., Newman *et al.* 2015) but also at eliciting time-varying trajectories in firing rate. We demonstrate the ability to track sinusoidal firing rates as well as to elicit patterns of rate modulation observed in the thalamus of the awake rat. Importantly, we find that the control strategy is more robust across neurons and results in less variability in response to optical stimulation than the *de facto* standard open-loop stimulation. Finally, we show that the thalamic responses to pulsatile inputs of the type used most often with ChR2 produce large, widespread responses in LFP recorded in cortex which may be due to highly synchronized thalamic inputs. In contrast, more continuously-modulated light inputs have subtle downstream effects in cortex, possibly indicating a less synchronous engagement of thalamus using our methodology. Taken together, our results represent an exciting maturation of closed-loop optogenetic control for circuit dissection.

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**Poster**

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R01 NS069679

**Title:** Encoding of whisker motion versus arousal by secondary somatosensory thalamus

**Authors: *A. K. KINNISCHTZKE**[1], Y. HONG[2], R. M. BRUNO[3]
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**Abstract:** Sensory information about the external world reaches the cerebral cortex through the thalamus. Thalamic nuclei relay visual, auditory, somatosensory, and cognitive information to relevant cortical areas for further processing. Primary thalamic nuclei transmit information from the periphery to primary sensory cortices, whereas ‘higher-order’ thalamic nuclei are bi-directionally connected to multiple cortical areas, including primary sensory cortex, and are suggested to be involved in attention, perception, and cognition. Here, our overall goal is to

obtain a better understanding of how higher-order thalamic nuclei contribute to sensory processing. The higher-order somatosensory thalamus in mice, the posteromedial (POm) thalamic nucleus, integrates signals from and provides input to primary somatosensory cortex (S1), secondary somatosensory cortex (S2), motor cortex (M1), and other associational cortices. Previous research has suggested POm contains less information regarding sensory stimuli applied to the whiskers than primary whisker thalamus (VPM) and may instead predominately relay signals related to whisker motion. In electrophysiological recordings from awake, freely whisking mice, we observe that the activity of most POm neurons is modulated based the whisking state of the animal, suggesting a possible role for POm in encoding whisker motion. We additionally find, however, that whisking does not typically happen in isolation but usually co-occurs with other behavioral states, including changes in arousal and locomotion. Currently, we are examining whether whisking-related POm activity actually represents whisking or in fact reflects arousal.

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**Poster**

**583. Somatosensation: Thalamocortical Processing**

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**Title:** A sensorimotor pathway via higher order thalamus

**Authors: *C. MO**[1], S. SHERMAN[2]
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**Abstract:** According to traditional wiring of a sensory pathway, once information reaches cortex, further processing occurs only within cortico-cortical connections. However, we now find that projections from primary to higher order sensory cortex have a parallel route through thalamus: cortico-thalamo-cortical pathways. These transthalamic pathways may play a significant role in ongoing cortical processing because they are glutamatergic driver projections, which are well-suited to the fast, efficient transfer of information. Transthalamic pathways have been found in visual, somatosensory and auditory pathways, but it is unknown if they exist beyond purely sensory systems. Given evidence of a direct, driver pathway from primary somatosensory cortex (S1) to primary motor cortex (M1) in the mouse, we hypothesized a parallel transthalamic pathway from S1 to M1 via the posterior medial nucleus (POm) in

thalamus.
Since transthalamic projections originate from layer 5 of cortex, we injected AAV-EF1a-DIO-ChR2-eYFP into S1 of Rbp4-Cre mice to express ChR2-eYFP in S1 layer 5 cells. Three weeks later, we injected the retrograde tracer Fluororuby into M1 of the same mouse, and after an additional 5 days, we could histologically verify M1-projecting cell bodies amidst S1 layer 5 terminals in POm. In another group of animals with the same injections, we performed whole cell patch recordings of Fluororuby-labeled cell bodies (which project to M1) and confirmed connectivity from S1 layer 5 by focal laser stimulation of ChR2-expressing terminals. Ten of 36 (28%) labeled cells and 17 of 27 (63%) unlabeled cells responded to activation within the zone of fluorescent overlap. Responses corresponded with driver properties: ionotropic receptor-dependent, large, monosynaptic EPSCs and paired-pulse depression to a 10Hz train. These were seen both when activated by laser at pre-synaptic terminals or their axons and with electrical stimulation of the axons. Preliminary data for experiments of the POm to M1 projection using ChR2 activation also shows driver properties. These data generalize the concept of transthalamic sensory cortices to a sensory-motor example.

**Disclosures: C. Mo:** None. **S. Sherman:** None.

**Poster**

**583. Somatosensation: Thalamocortical Processing**

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International Foundation for Research on Paraplegia (chair Alain Rossier to AH)

**Title:** Diverse coding of higher-order thalamocortical projections during sensory perception

**Authors: R. CHEREAU**, S. PAGÈS, T. BAWA, *A. HOLTMAAT
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**Abstract:** The cortex plays a critical role in the integration of multisensory information in order to produce a relevant behavioral response. Emerging models suggest that the underlying mechanisms include not only corticocortical feedback but also thalamocortical feedback that shape the activity of a large array of cortical areas. The thalamus is a major relay for information streams between deep brain structures and the cortex. It is composed of nuclei that primarily transmit incoming sensory inputs to the cortex (first-order nuclei) and others that primarily receive input from the cortex and send information back to a larger array of cortical areas

(higher-order nuclei). How the output of higher-order nuclei affects cortical activity during sensory perception and learning remains poorly understood. Previous work from our lab showed that the posteromedial (POm) area of the thalamus, which is the higher-order nucleus for the somatosensory system, mediates sensory-evoked synaptic long-term potentiation of pyramidal neurons in the barrel cortex.
Here, we investigated the role of the POm in a reward-based sensory discrimination task. We used calcium imaging to track the activity of individual POm axonal boutons in layer 1 (L1) of the somatosensory cortex over days during the acquisition of a whisker-mediated texture discrimination task. We correlated the activity of the boutons with the performance of the mouse during this task. We observed diverse responses, correlating with the various sensory stimuli, as well as with the various behavioral outcomes of the task. We found the activity of a sub-population of POm boutons to be predictive of the discrimination of the different textures after the mice had become experts in the task. This study indicates that POm conveys sensory discriminatory information to the barrel cortex, which thereby may play a role in sensory perception and learning.

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**Title:** The inhibitory influences in the somatosensory thalamus

**Authors:** ***J. YI**[1], E. IAVARONE[2], C. O'REILLY[2], Y. SHI[2], R. PERIN[1], H. MARKRAM[2]
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**Abstract:** The ventral posterolateral (VPL) nucleus of the thalamus serves as a relay center for the somatosensory system, receiving various forms of tactile and proprioception information from the periphery. The incoming sensory information is then shaped by neighboring GABAergic neurons of the reticular nucleus which have been observed to form both reciprocal (closed-loop) connections with VPL relay cells as well as disynaptic (open-loop) connections, an organization presumably designed for the propagation of oscillatory activity and lateral

inhibition of neighboring relay cells. In addition, recent immunogold work suggests that local interneurons represent about 3.7% of the neuronal population in the VPL of rats and mice. Although such a sparse population might seem negligible, interneuronal processes can contain richly branched arborizations with hundreds of terminals. Thus, it remains a possibility that local interneurons provide a significant source of inhibition to VPL network activity. The synaptic dynamics and physiological significance of these reticular neurons and local interneurons within the VPL remain to be elucidated. Here, we systematically investigated the electrophysiological and morphological characteristics of the VPL inhibitory influences in wistar han rats and GAD67-eGFP mice of age postnatal 14-18 days. We obtained whole cell single- and multi-patch clamp recordings of reticular neurons and local interneurons in acute slices and stained and morphologically reconstructed the neurons. We also investigated the properties of short term plasticity between connected pairs of VPL relay cells and inhibitory neurons. The data gathered from inhibitory interactions within the VPL is being integrated into an *in silico* model of the rat somatosensory cortex (Markram et al. 2015) to reconstruct and simulate this thalamocortical pathway. Thus, we explored how inhibition from the reticular nucleus and local interneurons within the VPL contribute to the excitatory tone of salient stimuli and the consequent impact on sensory perception.

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**Title:** Impact of the superior colliculus on cortical processing of somatosensory (whisker) input

**Authors: *S. GHARAEI**, E. ARABZADEH, G. J. STUART
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**Abstract:** Superior colliculus (SC) is an evolutionary ancient midbrain structure, which is highly conserved across species. Functionally, SC is thought to be important for detecting salient or novel sensory stimuli and eliciting an appropriate behavioral response. It is well established that SC receives direct input from the primary sensory cortices. What is not known is whether (and if so how) the SC modulates information processing in primary sensory cortex. To address this question, we combined optogenetic activation of SC with recordings in primary vibrissal

somatosensory cortex (vS1) of adult C57BL6/J mice. Adeno-associated virus was injected into SC to induce ChR2 expression (n=28). Two to four weeks after injection, we performed whole-cell and extracellular recordings under urethane anesthesia from vS1 while activating SC via an optic fiber. Using multi-electrode arrays in SC, we identified whisker responsive neurons located mainly in the intermediate layers of SC and confirmed their reliable activation with blue light. Consistent with the idea that SC can have an impact on cortical processing, photo-activation of ChR2 in SC led to light-evoked responses in vS1 neurons. These responses were manifested in the local field potential, as well as depolarization of the membrane potential and increased spiking activity. Furthermore, we found that photo-activation of SC led to a leftward shift in the input-output relationship of cortical neurons resulting in an enhanced capacity to detect low intensity stimuli. Given the absence of a direct anatomical projection from SC to vS1, we investigated two potential pathways for this functional modulation: (i) a projection from SC to facial nucleus, which is responsible for whisker movements though the facial nerve and (ii) an indirect thalamic pathway from SC to vS1 through the rostral sector of the posterior nucleus of the thalamus (Pom). Monitoring the whiskers under high-speed camera revealed whisker protractions after photo-activation of SC (latency = 20 ms). These movements were abolished after cutting the facial nerve, however, this procedure had no impact on vS1 responses following photo-activation of SC. Consistent with the idea that SC modulates vS1 via Pom, multi-electrode array recording indicated that photo-activation of SC led to increased firing of Pom neurons. Taken together, our results suggest that the SC, which plays a key role in attentional network, modulates sensory processing in primary somatosensory cortex via an indirect pathway through the thalamus.

**Disclosures:** **S. Gharaei:** None. **E. Arabzadeh:** None. **G.J. Stuart:** None.

**Poster**

**583. Somatosensation: Thalamocortical Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 583.11/BB27

**Topic:** D.04. Somatosensation: Touch

**Support:** NIH/NINDS grant 1R21NS09014801A1

**Title:** Functional benefits of spatially inhomogeneous cortical state

**Authors: T. NUR**[1], S. GAUTAM[1], L. PINTO[2], M. GOARD[3], J. A. STENKEN[4], *W. L. SHEW[5]
[1]Univ. of Arkansas, Fayetteville, AR; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Univ. of California, Santa Barbara, Santa Barbara, CA; [4]Chem, [5]Univ. of Arkansas Fayetteville, Fayetteville, AR

**Abstract:** The dynamical regime of cortical neuronal networks is not static. Rather, it shifts between different states with different collective population dynamics, often driven by changes in cholinergic neuromodulation. Such changes in cortical state are associated with changes in how neurons respond to sensory input, i.e. coding is state-dependent. These facts raise some basic questions. Which cortical states are optimal for which types of coding? In general, does state-dependent coding imply tradeoffs, improving one type of coding at the cost of degrading another type of coding? In principle, such tradeoffs could be avoided if cortical state is spatially inhomogeneous. Little is known about such spatial variability of cortical state compared to well-studied temporal changes in state. Here we hypothesize that if one part of the network is in one state, another part of the network could be simultaneously in a different state. In this way, each part could be well-suited to different types of coding, thus avoiding compromised function at the level of the whole network.We tested this hypothesis in the context of somatosensory detection in anesthetized rats and visual detection in awake mice. Cholinergic changes in cortical state were imposed by microdialysis in the rats and by optogenetic stimulation of nucleus basalis in the mice. In the rats, dual microdialysis probes were used to infuse different spatial patterns of acetylcholine or neostigmine. In the transgenic mice (expressing ChR2-EYFP under the choline acetyltransferase promoter), an optic fiber was inserted into basal forebrain. First, using microelectrode array recordings we demonstrated that changes in cortical state could be quite spatially inhomogeneous. Changes in state at one location were often quite different than changes in state at a nearby location (~200 microns away). Second, we demonstrated that changes in state are associated with dramatic changes in the efficacy of sensory detection for single neurons or small groups of neurons (multi-unit activity at a single electrode). However, when considering larger, spatially-distributed populations of neurons, sensory detection can be maintained. Our findings suggest that spatial inhomogeneity of cortical state can be important and beneficial for sensory coding.

**Disclosures: T. Nur:** None. **S. Gautam:** None. **L. Pinto:** None. **M. Goard:** None. **J.A. Stenken:** None. **W.L. Shew:** None.

**Poster**

**583. Somatosensation: Thalamocortical Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 583.12/CC1

**Topic:** D.04. Somatosensation: Touch

**Title:** Basal forebrain activation changes the vibrotactile responses of neurons in the hindpaw representation of rat SI cortex

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**Abstract:** Current understanding of attentional mechanisms in the cortex lacks detailed information within the tactile modality. We had previously measured the differential effects of cholinergic inputs, which are assumed to be involved in attentional modulation, on the vibrotactile responses of cortical neurons in the rat SI cortex via microinjection of acetylcholine and atropine. It was found that responses were mostly modulated by muscarinic receptors in deeper layers. In the current study, we electrically stimulated the basal forebrain (BF), which is the main source of cholinergic projections to the cortex. We recorded single-unit spike activity in the hindpaw representation of rat SI cortex from 16 neurons of 5 anesthetized Wistar albino rats. The vibrotactile responses were measured with and without BF stimulation (0.5-ms bipolar pulses at 100 Hz; duration: 0.5 s; amplitude: 50 µA). If present, BF stimulation just preceded the vibrotactile stimuli applied on the glabrous skin of the hindpaw (bursts of 5-, 40-, and 250-Hz sinusoidal displacements; duration: 0.5 s; zero-to-peak amplitude: 50 µm upon 0.5-mm static indentation). Each condition was repeated for 10 trials. Average firing rates were calculated for different time periods with respect to the vibrotactile stimulus ($R_b$: background; $R_o$: during the initial 100-ms of stimulus duration, $R_d$*: during the last 400-ms of stimulus duration). To quantify the changes in entrainment, vector strengths (VS) were also calculated for the entire vibrotactile stimulus duration. The short and long-term (after 30 min.) effects of BF activation were analyzed for different vibrotactile frequencies and cortical layers by using repeated measures ANOVA. Preliminary data show that BF activation produces both short and long-term increases in $R_b$ ($p=0.049$ and $p=0.036$, respectively), while there was only a long-term effect on $R_d$* ($p=0.004$). On the other hand, no significant effects of BF activation were found on $R_o$ ($p=0.838$ for short-term, $p=0.732$ for long-term) and VS ($p=0.622$ for short-term, $p=0.856$ for long-term). These results suggest that BF activation causes hyperactivity in the SI cortex, but the relative effects of vibrotactile inputs seem to be reduced in the pilot data. This may be due to stronger cholinergic connections onto inhibitory neurons and warrants more studies. In contrast to the microinjection of cholinergic agents, BF activation had long-term effects, probably reflecting the fact that BF projections are wide and diffuse, and they can alter the global activity pattern in the brain. We are currently collecting more data to model the local network dynamics in the hindpaw representation.

**Disclosures: B. Vardar:** None. **B. Güçlü:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** D.04. Somatosensation: Touch

**Support:** Klingenstein-Simons Fellowship

Johns Hopkins Science of Learning Grant

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NINDS (NS050274)

**Title:** Transcriptional variation across cortical neurons reflects axonal projection bias, laminar position and neuronal activity state

**Authors: *M. CHEVEE**[1], J. D. ROBERTSON[2], G. H. CANNON[2], S. P. BROWN[3], L. A. GOFF[2]
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**Abstract:** Single cell RNA sequencing technologies have generated the first catalogs of transcriptionally defined neuronal subtypes of the brain. However, the biologically informative cellular processes that contribute to neuronal subtype specification and heterogeneity remain unclear. By comparing the gene expression profiles of individual layer 6 corticothalamic neurons (L6CThN) in somatosensory cortex, we demonstrate that the variance in gene expression across individual neurons is associated with long-range axonal target, sublaminar position within the cortex and neuronal activity state. These three basis vectors of identity contributed to both subtype identity and heterogeneity within each subtype, suggesting that changes in one of these basic defining features would influence not only gene expression but also the relative transcriptional definitions of each subtype. We tested this hypothesis by analyzing the transcriptomes of 1023 single L6CThNs collected at baseline and one and seven days following manipulation of the sensory inputs to barrel cortex. Analysis of the L6CThN population revealed that altering the activity of L6CThNs increased the heterogeneity within each transcriptional subtype and enhanced the distinction between the transcriptional subtypes of L6CThN. Pseudotemporal analysis of the molecular responses to altered sensory inputs showed that subtype specific biases in the choice of transcriptional state following manipulation of neuronal activity contributed to the increase in subtype differences and revealed distinct cellular mechanisms preferentially engaged along each response. Our results demonstrate that, in contrast to bulk analysis, single cell RNA sequencing approaches enable the assignment of canonical functional features to transcriptionally defined subtypes, generating a more comprehensive definition of neuronal cell types. Together, they reveal that neuronal activity state, axonal projection pattern and sublaminar position within the cortex are significant sources of transcriptional variation contributing to the identity of individual neurons.

**Disclosures: M. Chevee:** None. **J.D. Robertson:** None. **G.H. Cannon:** None. **S.P. Brown:** None. **L.A. Goff:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.02/CC3

**Topic:** D.04. Somatosensation: Touch

**Support:** NIGMS GM122657-01

SCROG 80232-25 17

**Title:** A quantitative morphological analysis of supragranular neurons in the mouse barrel cortex

**Authors: *F. PALAGUACHI**[1], D. KANDOVA[2], A. EDMUND[2], A. TSIMOUNIS[3], J. C. BRUMBERG[4]
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**Abstract:** Layers 2 and 3 of the mouse somatosensory neocortex are critical in distributing incoming whisker sensations to neighboring cortical areas. Initially processed by the barrels of layer 4, these sensations are unilaterally transmitted to layers 2 and 3. Here, the signals are further processed and then relayed by supragranular projecting neurons to the contralateral primary somatosensory cortex (S1), ipsilateral primary motor cortex (M1) and ipsilateral secondary somatosensory cortex (S2). Although specific morphologies have been associated with the supragranular neuron's circuit networks, the number of functional classes of neurons remains unidentified. Specifically, it remains unknown whether neurons that project to the same cortical areas exhibit similar or different intrinsic properties. A morphological approach was adopted to analyze supragranular projecting neurons associated with M1, S1, and S2 circuits. The principal objective sought to determine if specific morphological groups are associated with each of the three projection sites (S1, M1, S2). Individual neurons in brain sections from CD-1 mice were labeled using Diolistics in conjunction with prior in vivo retrograde bead labeling. This allowed for the identification of the projecting neurons, which were then reconstructed from confocal images. Morphological measurements were made in Neurolucida and then analyzed using principal component analysis followed by a cluster analysis, revealing distinct groups of neurons. Our results suggest that layers 2 and 3 neurons projecting to different cortical locations exhibit distinct morphological characteristics.

**Disclosures: F. Palaguachi:** None. **D. Kandova:** None. **A. Edmund:** None. **A. Tsimounis:** None. **J.C. Brumberg:** None.

**Poster**

**584. Barrel Cortex**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** D.04. Somatosensation: Touch

**Support:** DFG Grant Sta 431/8-1, 10-1

**Title:** Diversity of VIP neurons: Insights into electrophysiological types and the influence of neuromodulation

**Authors: *A. PRÖNNEKE**, M. MÖCK, M. WITTE, J. F. STAIGER
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**Abstract:** As an essential component of cortical circuitry VIP neurons are highly diverse in their electrophysiological profile. Whole-cell patch clamp recordings of individual VIP neurons across all layers of the mouse barrel cortex revealed at least 5 different electrophysiological types. We identified these by a novel unsupervised classification method based on firing pattern adaptation and frequency spectrum. One of these types, bursting VIP neurons, was exclusively located in upper layer II/III. Further experiments on bursting VIP neurons showed that their typical burst firing depended on membrane potential because in a more depolarized state their firing mode changed to tonic. We identified noradrenalin, acetylcholine (ACh), and serotonin (5HT) as neuromodulators depolarizing all layer II/III VIP neurons. Whereas cholinergic depolarization was mediated by non-α7 nicotinic receptors, serotonergic modulation was more complex. Our experiments revealed that only 50% of all VIP neurons displayed $5HT_{3a}$ receptor functionality despite their classification as $5HT_{3a}$ receptor expressing interneurons. Interestingly, all VIP neurons in layer II/III were depolarized by metabotropic $5HT_2$ receptors. In this manner, brain states caused by neuromodulators can be differentially integrated by the population of VIP neurons. Whereas bursting neurons change their firing behavior during neuromodulation, all other VIP neurons become more excitable without changes to their firing pattern. Additionally, ACh and 5HT can be differentiated because only 50% of all VIP neurons react with a strong and transient depolarization to 5HT, whereas all VIP neurons in layer II/III of the mouse barrel cortex are recruited by cholinergic input.

**Disclosures:** **A. Prönneke:** None. **M. Möck:** None. **M. Witte:** None. **J.F. Staiger:** None.

**Poster**

**584. Barrel Cortex**

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**Topic:** D.04. Somatosensation: Touch

**Support:** DFG-SFB 1089

**Title:** Coupling of tactile LFP signals between mouse cortex and olfactory bulb

**Authors: *A. PARABUCKI**, I. LAMPL
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**Abstract:** Broad-band field potentials are important and powerful tools when investigating and interpreting brain function. The reasons to believe so are ranging from the evidence of coupling local neuronal activity with FPs to ability to overcome physical impairments through brain machine interfaces. Thus, it is not a surprise that in the last decade the recording of local field potentials (LFP) has gained increasing popularity in basic and applied electrophysiology. However, there is an uncertainty over LFP regarding its locality and its possible contamination by volume conductance coming from the distant generators.
Using standard LFP recording techniques, we revealed prominent whisker evoked LFP signal in the olfactory bulb (OB) of anesthetized mice, highly correlated with the activity in the barrel cortex (BC). To test whether the LFP response observed in the OB upon whisker stimulation depends on the BC, we employed optogenetic and pharmacological tools. First, light activation of BC in Thy1-ChR2 mouse line induced a strong LFP response in the OB. Next, light-silencing of BC using ChR2-GAD-Cre mice attenuated whisker evoked LFP responses in both BC and OB. However, the lack of unit responses urged to investigate the origins of the whisker-evoked LFP in OB. Therefore, we have recorded from different brain structures and found that whisker LFP responses in the ventro-lateral orbitofrontal cortex (vlOrb) exhibited near zero latency when compared to the OB. Next, we performed multichannel recording of the LFPs in the OB followed by inverse current source density (iCSD) analysis. iCSD analysis could not identify sinks and sources of the current in the OB, strongly suggesting that the observed signal does not emerge by synaptic inputs within the OB. Finally, we tested this hypothesis by recording whisker-evoked LFP response following complete separation of the OB from the rest of the brain. As expected for the case of volume conductance, cutting all the connections between OB and the rest of the brain did not attenuate the LFP response to whisker stimulation.
In conclusion, LFP signal in the OB, dependent on barrel cortex activation and highly correlated with its local activity, represented a pure volume conductance signal that was sourced back to the activity in the vlOrb, located a few millimeters away. Therefore, our findings, demonstrating a

pure case of volume conductance, add on to the rising concern regarding interpretation of LFP data and consequently urge for careful experimental approach when dealing with LFPs.

**Disclosures: A. Parabucki:** None. **I. Lampl:** None.

**Poster**

**584. Barrel Cortex**

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**Topic:** D.04. Somatosensation: Touch

**Support:** NIH R37 NS092367

**Title:** Organization of the whisker receptive field map in L2 of somatosensory cortex in awake behaving mice

**Authors: *H. WANG**, A. M. LEMESSURIER, D. E. FELDMAN
Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA

**Abstract:** Layer (L) 2/3 of primary sensory cortex provides the major output to higher cortical areas; therefore sensory coding in L2/3 is critical to understanding cortical information processing and perception. In mouse primary somatosensory cortex (S1), whisker receptive fields and maps have been well characterized under anesthesia, but not in awake mice, where active whisking precludes calibrated deflections of multiple whiskers. We developed a head-fixed whisker discrimination task suitable for mapping receptive fields in awake, whisker-attentive mice. Mice expressing GCaMP6s in L2/3 neurons had all whiskers intact, with 9 whiskers inserted into a 3 x 3 piezo array, and mice were trained to lick to occasional all-whisker stimuli (S+) but not to single-whisker stimuli (S-). Using 2-photon $Ca^{2+}$ imaging, we measured whisker-evoked $Ca^{2+}$ transients in L2 neurons during S- trials (12 imaging fields in 5 animals, total 1352 cells). 19% of neurons showed significant whisker-evoked responses, consistent with known sparse coding in L2/3 (Peron et al., 2015). Of responsive neurons, ~70% were narrowly tuned with a single best whisker evoking statistically greatest responses; other showed broader tuning with statistically equivalent responses driven by 2-5 whiskers. Cells were localized to specific anatomical barrel columns by post hoc cytochrome oxidase staining. Only 45% of responsive neurons within a barrel column were tuned to the columnar whisker; the remainders were tuned to one of the neighboring whiskers. Differently tuned neurons were spatially intermixed in a salt-and-pepper organization, but with average subcolumnar somatotopy aligned to the neighboring columns. As a result, the ensemble of neurons co-tuned for a single whisker was scattered over multiple anatomical columns in L2. Similar results were observed with pyramidal cell-specific expression of GCaMP6s. Thus, L2 neurons show substantial local tuning

heterogeneity in awake mice, superimposed on an orderly cross-columnar and subcolumnar whisker map. We are currently studying the effect of attention on whisker representation in L2.

**Disclosures: H. Wang:** None. **A.M. LeMessurier:** None. **D.E. Feldman:** None.

**Poster**

**584. Barrel Cortex**

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**Topic:** D.04. Somatosensation: Touch

**Support:** NIH R37 NS092367

**Title:** Tactile enrichment refines whisker map subcolumnar structure and population coding in L2/3 of S1

**Authors: *A. M. LEMESSURIER**[1], D. E. FELDMAN[2]
[1]Helen Wills Neurosci. Inst., UC Berkeley, Oakland, CA; [2]Molec & Cell Biology; Helen Wills Neurosci Inst., UC Berkeley, Berkeley, CA

**Abstract:** Sensory experience can powerfully alter whisker map topography in developing and adult somatosensory cortex (S1). To understand how natural tactile experience influences map development, we studied the effects of tactile enrichment on micro-scale organization of the whisker map in L2/3 and L4 of S1. Mice were raised with tactile enrichment (EN) or normal housing (NH) beginning at weaning (P21). GCaMP6s was expressed virally in L2/3 or L4 excitatory cells, using Drd3-Cre (L2/3) or Scnn1a-Cre (L4) mice. At P50 ± 10 days, we measured whisker responses and 9-whisker receptive fields from S1 neurons using 2-photon imaging through a chronic cranial window. After imaging was complete, cells were localized relative to anatomical (CO-stained) column boundaries. Within a single anatomical column, L2/3 cells were heterogeneously tuned to different whiskers in a spatially intermixed, salt-and-pepper fashion. Enrichment increased somatotopic precision in L2/3: 84% of cells within 100 um of a column center were tuned to the columnar whisker (CW) in EN, while only 57% were CW-tuned in NH. In both groups, this dropped to 20% at a radius of 350 um from the column center. CW-evoked response magnitude (mean $\Delta F/F$) was also elevated in column centers in EN mice. In L4 in both groups, the spread of neurons tuned to one whisker was narrower than in L2/3, suggesting a more focused whisker representation. EN mice showed substantially reduced spontaneous activity in both L2/3 and L4, and stronger CW-evoked responses in L2/3. To study the effects of enrichment on the spatial and temporal aspects of population coding, we compared pairwise noise correlations and signal correlations of whisker responses for neurons within each column. While signal correlations - which reflect overall tuning similarity - were increased for within-column pairs in EN compared to NH, noise correlations - which reflect trial-to-trial

response fluctuations - were decreased by 10% in EN relative to NH. In both groups, noise correlations decreased with distance between neurons, but this fall-off was spatially sharper in EN mice. These findings demonstrate a strong impact of juvenile sensory experience on sensory responsiveness and subcolumnar map topography, and suggest that enrichment may sharpen local excitatory subnetworks in L2/3 to promote more homogeneous whisker tuning and reduced firing correlations within single columns.

**Disclosures: A.M. Lemessurier:** None. **D.E. Feldman:** None.

**Poster**

**584. Barrel Cortex**

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NSF Graduate Fellow

**Title:** Selectivity for multi-whisker stimuli by single neurons in mouse somatosensory cortex

**Authors: *K. J. LABOY-JUAREZ**[1], S. AHN[2], D. E. FELDMAN[3]
[1]Univ. of California Berkeley, Berkeley, CA; [2]Mol. Cell Biol., Univ. of California, Berkeley, Berkeley, CA; [3]Molec & Cell Biology; Helen Wills Neurosci Inst., UC Berkeley, Berkeley, CA

**Abstract:** Active whisker sensation generates complex multi-whisker patterns of deflection, but coding of multi-whisker stimuli remains poorly understood in whisker somatosensory cortex (S1). We characterized multi-whisker tuning and integration in mouse S1 by extensively mapping neural responses to 2-whisker combinations and sequences, an important subset of multi-whisker stimuli. Recordings were made in the D1 column with silicon probes in anesthetized mice. 52% of S1 single units were significantly tuned for specific 2-whisker sequences at specific inter-whisker-deflection-intervals (Δt, range: ±50 ms). This spatiotemporal tuning was strongest in L2/3 and L5, suggesting that multi-whisker tuning is enhanced by intracortical processing. Multi-whisker responses were generally sublinear, with prominent sublinear suppression of non-preferred stimuli, and more linear responses to preferred stimuli. As a result, multi-whisker tuning was significantly sharper than predicted by linear summation. The strongest spatiotemporal tuning was observed in neurons whose single best whisker was a non-columnar (non-D1) whisker. 2-whisker sequence responses were strongly affected by Δt. The mean Δt tuning curve showed a significant preference for positive Δt's (columnar whisker leading surround whisker) and strong inhibition at nearly simultaneous deflections. 80% of the variance in Δt tuning could be described by three principal components, representing order

tuning (whether surround or columnar whisker was deflected first) and suppression during simultaneous whisker deflections (Δt=0). Order tuning strongly sharpened spatial selectivity for specific 2-whisker sequences. These findings show that multi-whisker patterns are represented in single S1 columns due to a combination of linear and non-linear computations in single neurons.

**Disclosures: K.J. Laboy-Juarez:** None. **S. Ahn:** None. **D.E. Feldman:** None.

**Poster**

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**Support:** Deutsche Forschungsgemeinschaft (SFB 870)

European Research Council Advanced Grants to A.K.

**Title:** A subset of 'super-responding' neurons dominates output signaling in layer 5A of mouse barrel cortex

**Authors: *A. BIRKNER**, Z. VARGA, V. D. J. BONFARDIN, M. TOHMI, B. SAKMANN, A. KONNERTH
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**Abstract:** Electrical recordings from different cortical regions of the mammalian brain indicate that the level of activity in layer 5 (L5) is often stronger than that in other cortical layers. However, it is difficult to verify this assumption directly because methods of functional imaging with single-cell resolution, like two-photon calcium imaging, were mostly restricted to the upper cortical layers. Here we implemented a recently developed method of deep-two photon imaging involving the use of the red-shifted calcium indicator Cal-590 (Tischbirek et al., PNAS 2015, Birkner et al., Cell Calcium 2016) for the analysis of L5 in the mouse barrel cortex. When recording from the upper sheet of L5, at a depth of 770-820 µm, we observed that the majority of neurons responded only weakly to whisker stimulation (1 s, 10 Hz). Instead, whisker stimulation evoked in a small subset of neurons (< 5%) large amplitude somatic calcium transients. These ‘super-responding’ neurons were activated extremely reliably by repeated stimulation of the whisker corresponding to the investigated barrel region (= principal whisker), mostly without any failure. Instead, surround whiskers evoked only rarely small calcium transients in these neurons. By using two-photon imaging-guided cell-attached recordings, we determined that each of the large calcium transients of the ‘super-responders’ reflects the activity of 10-20 action potentials. Next we wondered about the dendritic activity underlying ‘super-responsiveness’. For this analysis, we performed a two-step imaging experiment in which we first identified in L5

‘super-responsive’ neurons by using the red indicator Cal-590 AM for population staining, and then electroporated such neurons with the green calcium indicator OGB-1. The morphological inspection of the apical dendrites demonstrated that all tested ‘super-responders’ (10/10) belonged to the class of ‘thin’-tufted L5 pyramidal neurons. Two-photon calcium dendrite imaging revealed whisker stimulation-evoked presumed calcium spikes that extended over all branches of the tuft. The synaptic origin of these dendritic spikes was demonstrated by locally blocking in L1 synaptic excitation by a mix of CNQX/APV. Tuft dendritic spikes were reliably evoked by stimulation of the principle whisker and less effectively when stimulating one of the surround whiskers. They were only rarely encountered during spontaneous activity. In conclusion, our results reveal an unexpected sparseness of the activity in the upper L5, the presence of a small subset of thin-tufted ‘super-responding’ neurons and a key role of L1 tuft dendritic spikes for their output activity.

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**Poster**

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**Topic:** D.04. Somatosensation: Touch

**Support:** R01-NS045130

Fulbright Graduate Study Award

Brown Institute for Brain Sciences Graduate Research Award

**Title:** Fast-spiking interneurons of barrel cortex show increased activity on detected trials in a vibrissae deflection detection task

**Authors: *H. SHIN**, J. BOTROS, S. R. JONES, C. I. MOORE
Brown University, Neurosci., Providence, RI

**Abstract:** Fast-spiking interneurons of sensory neocortex are widely regarded as contributing to perceptual success through controlling local excitatory output. Their precise role (e.g. suppression, enhancement or synchronization) is, however, a topic of intense debate. To address this question, we recorded extracellularly from 4 mice trained to perform a vibrissae deflection detection task, using the 16-tetrode flexDrive. We analyzed 84 sessions from well-trained mice showing lawful psychometric behavior. In 191 single units with fast-spiking waveform (FS), we observed at the population level enhanced sensory evoked activity on detected trials compared to non-detected trials (matched in the magnitude of the sensory drive). We also observed enhanced

evoked activity in 195 regular-spiking units (RS), in agreement with prior studies. We hypothesize that this concurrent increase of excitatory and inhibitory activity is possible due to the synchronization of FS firing, a signature of which is FS-gamma (i.e. FS synchrony repeating in ~25ms intervals). Therefore, we assessed when FS synchronization occurred with regard to the stimulus onset. Optogenetic trains of FS stimulation initiating before sensory input and continuing after it (peristimulus) have been shown to enhance tactile detection under some conditions. Prior hypotheses have posited a role for prestimulus FS-gamma, that can align a pre-established "window of opportunity" to coincide with initial excitatory sensory input arriving from lemniscal thalamus. In apparent conflict with this prediction, we found decreased FS activity prestimulus on detected trials, and lower power in the gamma band (30~80 Hz) in the local field potentials (LFP) in this time period. We hypothesize that the beneficial effect of FS-gamma is largely realized by sensory evoked feedforward FS activity, as opposed to optimal alignment of prestimulus gamma. Ongoing optogenetic experiments have replicated prior peristimulus findings, and are now being applied to dissect the causal role of prestimulus versus poststimulus FS-gamma.

**Disclosures:** **H. Shin:** None. **J. Botros:** None. **S.R. Jones:** None. **C.I. Moore:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.10/CC11

**Topic:** D.04. Somatosensation: Touch

**Support:** Career Award at the Scientific Interface from the Burroughs Wellcome Fund

**Title:** Phase dependent differences in excitatory and inhibitory modulation of somatosensory cortex during active touch

**Authors: *S. SUNIL**, J. B. SCHROEDER, J. T. RITT
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**Abstract:** Active sensing incorporates a closed-loop behavioral selection of information during sensory acquisition. To understand the role of primary somatosensory cortex (SI) in coordinating active sensing motions, we developed a real time feedback system that delivers optogenetic stimulation to SI in actively exploring mice, time locked to whisker motions. Water-restricted mice, trained to alternately traverse a linear track for water rewards, were implanted with custom hyperdrives over left SI for neural recording and stimulation, and with bilateral facial electromyography (EMG) electrodes in the whisker pad, to estimate whisker motions in real time. Online processing identified protractions and retractions during active whisker motions as animals traversed the track. Stimulation of excitatory neurons (thy1-ChR2 mice) lead to an

increase in regularity of whisking, whether locked to protractions or retractions. In addition, stimulation timed to protractions sped up the following whisk, with weak effects for retractions. Simultaneous neural recordings from SI showed pulse-induced increases in neural activity when stimulation was timed to protractions, but not when timed to retractions. Stimulation of inhibitory interneurons (PV-ChR2 mice) showed a similar dependence on motion of SI responsiveness, where stimulation locked to protractions resulted in a decrease in neural activity, while stimulation locked to retractions had a muted effect. These results suggest a cyclic modulation of excitation and inhibition in SI with each whisk, that may reflect that incoming sensory information is naturally acquired when whiskers are moving forward. However, the interwhisk interval following inhibitory stimulation appeared to depend more strongly than excitation on the stimulated phase of protraction. Inhibitory stimulation earlier in protractions appeared to speed up the following whisk, while inhibitory stimulation later in protractions appeared to slow down the following whisk. Overall, downstream areas may show preferential tuning to the timing of SI activity relative to self-motion, with differential timing precision across cell classes and/or layers. We relate these findings to "window of opportunity" models for active sensory processing. Moreover, these findings suggest that SI is likely to play a role in guiding sensory motions, in addition to encoding incoming sensory information.

**Disclosures:** **S. Sunil:** None. **J.B. Schroeder:** None. **J.T. Ritt:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.11/CC12

**Topic:** D.04. Somatosensation: Touch

**Support:** HHMI

**Title:** Precisely-timed feedback inhibition in the barrel cortex

**Authors:** ***J. YU**[1], A. AGMON[2], K. SVOBODA[3]
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**Abstract:** Somatostatin (SST)-expressing interneurons are a major subgroup of GABAergic interneurons in the barrel cortex. In thalamic recipient layers, SST interneurons receive only weak thalamic input but connect strongly to the local excitatory neurons. SST interneurons might thus be a source of feedback inhibition. The dynamics of this feedback inhibition is unknown and the evidence that SST interneurons are driven by the somatosensory input is lacking. In fact, prior studies suggest that SST interneurons might be primarily modulated by cholinergic input and other types of interneurons as a result of behavioral arousal. We thus measured the dynamics

of SST interneurons in mice performing an active, whisker-mediated object detection task. Whisker movements were tracked with millisecond (ms)-timescale resolution. SST interneurons were recorded in vivo using channelrhodopsin-guided juxtacellular and whole-cell recordings, in barrel columns corresponding to the whiskers with which the mouse solved the behavioral task.In the absence of whisker movement and touch, the baseline spike rate of SST interneurons was 3.3 ± 4.6 spikes / s (mean ± s.d.). After the onset of exploratory whisker movement, we observed either a modest increase (62% of neurons) or a decrease (38%) in spike rate (average during whisker movement, 4.8 ± 6.9 spikes / s). The reduction of spike rate in some SST neurons could be caused by fast-spiking (FS) interneurons, which are strongly driven by whisker movement. Indeed, reducing the spike rate of FS interneurons increased the spike rate of some SST interneurons. Touch between whisker and object elicited phasic spikes (0.7 ± 0.7 spikes/touch) in SST interneurons across all cortical layers (2-6). The latency of touch-evoked spikes in the SST interneurons is 12.8 ± 4.6 ms (median, 12.0 ms), longer than for excitatory neurons (8.9 ± 4.4 ms, median, 7.8 ms), fast-spiking interneurons (5.2 ± 2.7 ms, median, 4.2 ms), or the latency for touch-evoked thalamic input (4 ms). Touch induced a delayed (8-10 ms) membrane potential depolarization in the SST interneurons, which matches the spike latency of local excitatory neurons. This suggests that the SST neurons are excited by local excitatory neurons. We conclude that SST interneurons are a major source of millisecond-timescale feedback inhibition in the cortical circuits.

**Disclosures:** **J. Yu:** None. **A. Agmon:** None. **K. Svoboda:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.12/CC13

**Topic:** D.04. Somatosensation: Touch

**Support:** CNRS

iCode

Universite Paris Saclay

HFSP

EU Marie Curie

**Title:** Spatio-temporal coding in the rat primary and secondary somatosensory cortices

**Authors: *M. A. GOLDIN**, E. R. HARRELL, L. ESTEBANEZ, D. E. SHULZ
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**Abstract:** The rodent vibrissal system plays a key role in the identification of objects in the environment. Such tactile recognition is likely to depend on the spatiotemporal dynamics of multi-whisker contacts with an object. However, the coding of these multiple contacts in the whisker somatosensory cortices is not well understood. To characterize the multi-whisker representations in the two largest somatosensory cortical regions, we applied with a 24 whisker piezoelectric stimulator three different types of stimuli to the caudal 24 macrovibrissae of anesthetized (Isofluorane) rats while recording the activity of multiple single neurons in either primary or secondary whisker somatosensory cortex (wSI or wSII). First, we applied identical Gaussian white noise deflections to all whiskers and analyzed the neuronal responses with reverse correlation techniques. We found that both cortices have a predominant low dimensional filter space, with wSII neurons filtering the stimulus in extended temporal windows compared with wSI. Second, in a stimulation context where each whisker receives its own, uncorrelated Gaussian stimulus, we found that the reverse correlation analysis was not suitable for wSII responses. However, using the region-specific temporal filters for whisker movements across time found in the correlated context, it became feasible to study both wSI and wSII responses in the uncorrelated condition. Our new method reduces the dimensionality of the spike triggered ensemble 50 times by transforming it to the meaningful stimulus coordinates of the respective sensory areas . This powerful approach allowed identifying for the first time, spatio-temporal receptive fields at a whisker-pad scale during continuous, simultaneous multi-whisker stimulation for an extended time window of 200ms. An analysis of the dynamics of the responses in wSI and wSII in the two dense stimulation contexts revealed that these regions contain specialized whisker representations. In wSI, the population encodes fine features of whisker movements on precise spatial and temporal scales. In wSII, many whiskers contribute equally to the firing of a single neuron, and these contributions occur over longer time scales than what is found in wSI. The predicted outcome of these coding principles were then tested and validated in a sparse noise stimulation context where each whisker was deflected independently but not simultaneously. In conclusion, the whisker system encodes both fine scale tactile features and broader tactile scene statistics at different timescales and these complementary representations are organized into distinct and specialized cortical regions.

**Disclosures: M.A. Goldin:** None. **E.R. Harrell:** None. **L. Estebanez:** None. **D.E. Shulz:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.13/CC14

**Topic:** D.04. Somatosensation: Touch

**Support:** National Science Centre (Poland) Grant DEC-2012/05/D/NZ3/00994 to AP

**Title:** Visualizing learning-induced cortical plasticity in the barrel cortex with intrinsic signal optical imaging

**Authors: *A. M. POSLUSZNY**[1], R. ZAKRZEWSKA[1], M. KOSSUT[1,2]
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**Abstract:** Intrinsic signal optical imaging (ISOI) was found to be a good tool for visualizing cortical plasticity in the models of vibrissae deprivation (Polley et al., 1999; Drew & Feldman, 2009). We investigate changes in the barrel cortex after fear learning in which stimulation of vibrissae is paired with a tail shock. Previously, we mapped changes in cortical representation of vibrissae involved in behavioral training with 2-deoxyglucose (2-DG) and found enlargement of functional cortical representation of the row of vibrissae stimulated during training. We wanted to replicate the 2-DG results with ISOI. The aim of the experiments was to find a protocol of vibrissae stimulation for the ISOI from the barrel cortex, which could provide constant signal in temporally separated recordings and enable visualization of plastic changes after learning. Two subsequent ISOI were performed with 6 days interval. Training consisted of 3 daily sessions of 40 trials of manual stimulation of row B vibrissae on one side of the snout (three 3-s smooth strokes with a fine brush) coupled with a mild tail shock. 24 hours after the last conditioning session the second ISOI was performed. Two protocols of vibrissae stimulation during ISOI were tested. In the first protocol 5-ms deflections of B1 vibrissa were applied for 1s with frequency of 5Hz. In the second protocol 5-ms deflections of B1 vibrissa were applied for 6s with frequency of 10Hz. Additionally, after second ISOI a 2-DG mapping was performed in order to confirm plasticity in the somatosensory cortex. Using 2-DG method a comparison was performed between left (stimulated during learning) and right (not stimulated) vibrissae representation. The repeated ISOI in which the first protocol (1s/ 5Hz) of vibrissae stimulation was applied, revealed no change of the B1 vibrissa representation after learning, a result that is inconsistent with 2-DG maps. ISOI using the second vibrissae stimulation protocol (6s/ 10Hz) showed expansion of cortical representation of vibrissa stimulated during learning, which was confirmed by 2-DG results. Our results indicate that different paradigms used for induction of experience-dependent plasticity in the barrel cortex require specific protocol for visualizing plastic changes by ISOI. This discrepancy could result from the effect described before, that the activation area seen in images produced by ISOI can be larger than or closer to the area of cellular cortical representation of stimulated whisker depending on the frequency of the stimulation or the number of the stimulation trials (Sheth et al., 1998; Polley et al., 1999).

**Disclosures: A.M. Posluszny:** None. **R. Zakrzewska:** None. **M. Kossut:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.14/CC15

**Topic:** D.04. Somatosensation: Touch

**Support:** DFG CH1232 1-1

DFG SCHW577 16-1

**Title:** Cortical control of sensory gating in the rodent whisker system

**Authors: S. CHAKRABARTI**[1,2], *C. SCHWARZ[2]
[1]Werner Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Univ. Tuebingen, Tuebingen, Germany

**Abstract:** Sensory gating, in which the neural responses to a sensory stimulus are reduced during motion, has been documented across several species and modalities. In the rodent whisker system, it has been repeatedly shown that cortical and thalamic responses to whisker stimulation are reduced during active whisking. Previous studies have further suggested that such gating is present in the brainstem and the abundance of cortical projections to the brainstem trigeminal sensory nuclei strongly suggest a cortical role in such gating. However, no study has demonstrated sensory gating in the brainstem or the dependence of such gating on cortical projections. In the present study we address these issues using awake behaving rats in an active whisking behavioral task. First, we show that sensory gating in which neural responses during whisking are reduced in amplitude, indeed occurs in the neurons in the trigeminal principal nucleus (Pr5). Second, we demonstrate using aspiration lesions of S1 and S2 cortex that such gating is abolished implicating the corticofugal fibers in a causal role. Third, we show that the initiation of whisking results in an increase in neural activity which is strongly reduced following cortical lesions. This increase in activity does not precede whisking onset, arguing against it being a pure motor signal. Finally we recorded from neurons in the trigeminal interpolaris nucleus (Sp5i) which is known to send inhibitory projections to the Pr5 and is therefore a likely candidate mediating the cortical evoked gating of Pr5 neurons. However, we did not find increased activity during whisking consistent with an intra-trigeminal inhibitory circuit nor did we detect whisking evoked activity as in Pr5. We therefore question whether Sp5i mediates the sensory gating of Pr5. Taken together our data point to a cortical origin of the sensory gating phenomenon in which corticofugal projections to Pr5 mediate tactile gating in the brainstem.

**Disclosures: S. Chakrabarti:** None. **C. Schwarz:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.15/CC16

**Topic:** D.04. Somatosensation: Touch

**Support:** New York Stem Cell Foundation

NIH DP2 32162

**Title:** Bidirectional rate coding through the core descending intracortical microcircuit

**Authors: *S. PLUTA**, H. ADESNIK
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**Abstract:** Neurons encode sensory stimuli through both increases and decreases in firing rates. This is particularly true of layer 5 (L5) cortical projection neurons that often exhibit high spontaneous firing rates in the absence of any external stimulus. The neural circuits that account for these bidirectional changes, however, are largely unknown. Prior *in vitro* work in somatosensory cortex demonstrated that descending input from L2/3 generates heterogeneous balances of excitation and inhibition in L5 pyramidal cells that potentially mediate both increases and decreases in touch evoked activity. To test this hypothesis, we optogenetically manipulated L2/3 activity in awake, actively sensing animals while simultaneously recording activity in L5. Optogenetic activation of L2/3 increased the firing rate only of L5 units facilitated by active touch, while it simultaneously decreased the firing rate of all L5 units suppressed by touch. Similarly, optogenetic deactivation of L2/3 reduced the activity of units displaying touched evoked increases in firing rate, while it simultaneously diminished the suppression of units displaying touch evoked decreases. Additionally, L2/3 deactivation significantly reduced the spatial selectivity of L5 projection neurons, both within single units and across the population. Surprisingly, the spatial selectivity of putative fast-spiking interneurons in L5 was unchanged, despite L2/3 deactivation significantly altering the firing rate of a minority of units. In conclusion, these data demonstrate that the core descending pathway of the intracortical microcircuit enhances the representation of touch by bidirectionally distributing the firing rates of cortical projection neurons.

**Disclosures: S. Pluta:** None. **H. Adesnik:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

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**Program#/Poster#:** 584.16/CC17

**Topic:** D.04. Somatosensation: Touch

**Support:** Adelis Foundation

Prince Center

**Title:** Whisker activation evokes network desynchronization and formation of functional small-world subnetworks in the primary and secondary somatosensory cortex of the rat

**Authors: M. KHATEB**, J. SCHILLER, *Y. SCHILLER
Technion Med. Sch., Haifa, Israel

**Abstract:** Neural synchrony is crucial for sensory and conscious processing, perceptual integration, attention selection, working memory and more. In this study, we investigated the effect of whisker activation on neuronal synchrony in the SI and SII somatosensory cortexs. To monitor neuronal synchrony we calculated the pairwise correlations during spontaneous activity and whisker activation in anesthetized and head restrained rats, and defined functional connections between neuronal pairs in case their firing was significantly correlated. Our results demonstrated a significant decorrelation of neuronal pairs in both S1 and S2 cortex during evoked activity. In S1, the average connectivity per unit (percent of units significantly correlated with the reference unit) decreased from 39.5 ± 5 % in the spontaneous pre-stimulus state to 25±4 % during whisker activation (p-value<0.01). A similar reduction was also observed in the S2 cortex, where connectivity per unit dropped from 26.5 ±5% during the spontaneous pre-stimulus state to 11.8 ±4 % during whisker activation (p-value<0.01). This decrease was observed in all recorded putative cortical layers of S1 and S2 (layers 2-5). At the end of whisker activation networks gradually increased their inter-neuronal synchronization, with an average time needed to return to the pre-stimulus connectivity values of 1.23 ± 0.17 seconds.
Interestingly, loss of functional connections (as measured by the existence of significant correlations) during whisker activation was not random, but rather followed specific spatial and functional principles: 1) Connections with spatially distant units where preferentially lost, while maintaining connections with nearby units. In so doing whisker activation transformed the S1 and S2 networks to a more "small-world-network" configuration. 2) Preferential maintenance of correlative connections with units that had similar texture selectivity. In addition we show that hubs (defined as the 10 % of units with the largest number of correlated connectivity) were most likely stimulus non selective units in both S1 and S2.
To conclude, Our data demonstrate stimulus evoked activity results in decorrelation/desynchronization of the cortical networks of primary and secondary

somatosensory cortex, and transformation of these networks to a more small-world network configuration. These findings suggest that during evoked activation the cortical network is segregated into smaller functional networks that may process different aspects of the sensory input.

**Disclosures:** **M. Khateb:** None. **J. Schiller:** None. **Y. Schiller:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.17/CC18

**Topic:** D.04. Somatosensation: Touch

**Support:** Ruth L. Kirschstein National Research Service Award F31NS093925

National Institute of Neurological Disorders and Stroke grant DP2NS087725-01

H.A. is a New York Stem Cell Foundation Robertson Investigator

**Title:** Functional impact of subtypes of somatostatin-expressing interneurons in cortical circuits

**Authors: *A. S. NAKA**[1], J. VEIT[1], H. ADESNIK[2]
[1]UC Berkeley, Berkeley, CA; [2]Univ. of California, Berkeley, Berkeley, CA

**Abstract:** Inhibitory neurons of the cortex are anatomically and molecular diverse. This implies that distinct subclasses might differentially contribute to specific aspects of neural computations. Dendrite-targeting somatostatin (SST) expressing interneurons exhibit an extreme level of morphological and molecular heterogeneity, and can be broken into multiple subtypes with distinct patterns of local connectivity. Emerging evidence suggests that different subtypes of SST cells are differentially coupled to sensory inputs and behavioral contingencies, yet how different sub-classes of SST cells functionally influence cortical dynamics and computation is essentially unknown. We optogenetically manipulated the activity of different subtypes of SST cells while measuring synaptic inhibition and spiking activity across the different cortical layers in primary sensory cortex. Our results suggest that SST subtypes are specialized to modulate different components of the cortical microcircuit and may thereby play distinct roles in cortical computation.

**Disclosures:** **A.S. Naka:** None. **J. Veit:** None. **H. Adesnik:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.18/CC19

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH Grant R01NS078168

NIH Grant R01NS079737

Scholar Award McKnight Endowment Fund for Neuroscience

**Title:** Neuronal and non-neuronal factors mediate behavioral-state dependent development of functional hyperemia in the somatosensory cortex of juvenile mice

**Authors: *K. W. GHERES**[1], Q. ZHANG[2], P. J. DREW[3]
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**Abstract:** In the adult somatosensory cortex, behavior-driven increases in neural activity evoke large, spatially restricted changes in blood volume due to arterial and venous dilations (1, 2). However, as rodent studies have shown neurovascular coupling can be inverted in the juvenile brain (3), we sought to understand how the behavior-evoked hemodynamic response matures during the postnatal period in mice. Using two-photon microscopy, intrinsic optical signal (IOS) imaging, and electrophysiology, we investigated how functional hyperemia is effected by neuronal and non-neuronal mechanisms during the postnatal period in the awake mouse. Stimulation of the vibrissae in the awake mouse caused biphasic (initial dilation followed by a constriction) hemodynamic responses over barrel cortex that developed into adult-like responses within two weeks of eye-opening (P30). In contrast, locomotion evoked decreases in cerebral blood volume in juveniles, rather than the increases in blood volume seen in adults, and only become adult-like four weeks after eye opening. Further analysis suggests these changes are due to decreases in venous blood volume that occur at the onset of locomotion. Electrophysiological recordings show increases in local field potentials as well as multi-unit spiking in response to locomotion during this period, suggesting a decoupling of the neurovascular relationship. This work suggests that age and behavioral-state dependent changes in the neurovascular relationship need to be considered for proper interpretation of hemodynamic signals in the developing brain.

**References:**

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2. Huo, B.-X., Greene, S. E., & Drew, P. J. (2015). Venous cerebral blood volume increase during voluntary locomotion reflects cardiovascular changes. *NeuroImage*, *118*, 301–312.
3. Kozberg, M. G., Chen, B. R., DeLeo, S. E., Bouchard, M. B., & Hillman, E. M. C. (2013). Resolving the transition from negative to positive blood oxygen level-dependent responses in the developing brain. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), 4380–5.

**Disclosures: K.W. Gheres:** None. **Q. Zhang:** None. **P.J. Drew:** None.

**Poster**

**584. Barrel Cortex**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 584.19/CC20

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** IBS-R015-D1

**Title:** The differential patterns of the sensory evoked-hemodynamic response of mouse barrel cortex at two- and six-weeks post soft- cranial window installation

**Authors: *H. PARK**[1,2], N. YOO[1,3], B.-M. KANG[1,3], C. HEO[1], M. SUH[1,3,4,5]
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**Abstract:** Recently, we developed new soft-cranial window for rodents with the soft, flexible, transparent, and biocompatible silicone-based polydimethylsiloxane (PDMS) (Heo et al. 2016). This soft-cranial window allows not only an easy access into tissues with electrodes or needles but also long-term monitoring for a large cortical area. But, this soft-window installation may affect the physiology of brain tissue because full craniotomy is a necessary step for it. Here, we investigated the effects of soft-cranial window installation on sensory-evoked cerebral hemodynamics at different post-op periods. C57bl/6 mice were used in this study. At 2 weeks and 6 weeks post soft-cranial window installation, we optically recorded cerebral blood volume (CBV) changes during a single whisker stimulation (C2 area) for either 4 seconds or 16 seconds and electrophysiologically recorded local field potential (LFP) for 0.1ms of the same stimulation. The animals at 6 weeks post soft-cranial window installation exhibited different CBV response patterns compared to that of the animals 2 weeks post-op. The animals at 6 weeks post-op showed more focalized CBV changes during a single whisker stimulation than the animals at 2 weeks post-op. Furthermore, the maximum CBV changes at the response center at 6 weeks post-op were larger than the CBV changes at 2 weeks post-op. LFP recording during a single whisker

stimulation also showed a similar pattern. The animals at 6 weeks post-op showed larger dendritic summation of neuronal membrane potential changes than the animals at 2 weeks post-op. In immunohistochemical studies, we found that the heightened expression level of GFAP of reactive astrocyte and activated microglia from the animals at 2 weeks post soft-cranial window compared to that of animals at 6 weeks post-op and normal control animals. Also, the animals at 2 weeks post-op exhibited higher brain-blood barrier (BBB) permeability than the animals at 6 weeks post-op and normal animals. This suggests that the soft-cranial window installation procedure with full craniotomy affects overall physiological conditions of brain tissue and may cause traumatic injury for a short term. Further investigation is necessary to understand the exact mechanism for the decreased hemodynamics alongside with decreased neuronal activation at initial periods after the soft cranial window installation.

**Disclosures: H. Park:** None. **N. Yoo:** None. **B. Kang:** None. **C. Heo:** None. **M. Suh:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.01/CC21

**Topic:** D.06. Audition

**Support:** Irish Research Council, GOIPPG/2015/1656

**Title:** Decoding the cortical representation of auditory motion using EEG

**Authors: *A. BEDNAR**[1], E. C. LALOR[2]
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**Abstract:** It is of increasing practical interest to be able to decode the spatial characteristics of an auditory scene from electrophysiological signals. This has potential applications in cognitively controlled hearing aids and for the evaluation of virtual acoustic environments. However, the cortical representation of auditory space is not well characterized - particularly as it relates to auditory motion - and the possibility of decoding cortical signals using non-invasive neuroimaging techniques remains unclear.
In previous work (Bednar et al 2017) we showed that we can classify evoked electroencephalographic (EEG) responses to discrete static sound stimuli as a function of their location in space. In the present study, we extend this work with the aim of decoding the trajectory of continuous, moving auditory stimuli.
Subjects listened to white noise over headphones, which had been spectro-temporally modified to be perceived as randomly moving on a semi-circular trajectory in the horizontal plane. Participants were asked to respond to infrequent tremolo targets which were embedded in the

stimuli. While subjects listened to the stimuli, we recorded their EEG using a 128-channel acquisition system. The data were analyzed by 1) deriving a linear mapping, known as a temporal response function (TRF), between the stimulus and a training set of EEG data, and 2) using the TRF to reconstruct an estimate of the time-varying sound source azimuth from a test set of EEG data.
Preliminary results show that we can decode sound trajectory with a reconstruction accuracy significantly above chance level. We find that reconstruction accuracy is highest when the sound trajectory is estimated using alpha-filtered EEG (8-12hz). Moreover, the subsequent analysis of the reconstruction model parameters reveals strong alpha-power lateralization at the occipital regions of the scalp.

**Disclosures: A. Bednar:** None. **E.C. Lalor:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.02/CC22

**Topic:** D.06. Audition

**Support:** St. Olaf College Faculty Development Grant

**Title:** Psychophysics of free-field sound localization using a novel pointing system

**Authors: D. BRICHETTO**[1], B. M. CARLSON[2], M. GASTON[1], T. OLSON[3], *J. L. LOEBACH[4]
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**Abstract:** The ability to localize sound effectively is critical, not only for determining the directional origin of the sound, but also for segregating overlapping sound sources in complex environments. Much of our ability to localize sound in the vertical plane come from head related transfer functions derived from the filtering functions due to the shape of the pinna, while horizontal localization depends on auditory centers in the brain specialized for calculating intensity and timing differences between the two ears. In order to study sound localization, a group of St. Olaf undergraduate students, faculty, and staff developed a free-field stimulus presentation device known as the SoLoArc (Westerberg et al., 2015). The SoLoArc can systematically present auditory and visual stimuli in a 180 degree free-field environment in both the horizontal and vertical planes. A current challenge for psychophysical studies of free field sound localization is in how the participant indicates where they think the sound is coming from. Finger-pointing and head-pointing can be inaccurate and require movement of the participant, thereby altering the acoustic cues that they are using to localize the sound. For that reason, we

developed an automated system to capture participant responses as they indicate the source location on the SoLoArc. A potentiometer coupled to a servo motor is used to point a laser at the arc. The voltage derived from the potentiometer is converted into angular location, allowing the participant to indicate source localization without any movement of the head or body. Preliminary results indicate that this system accurately reports participant's perception of auditory stimuli in space allowing for more accurate estimates of source localization in both azimuth and elevation than finger pointing devices.

**Disclosures: D. Brichetto:** None. **B.M. Carlson:** None. **M. Gaston:** None. **T. Olson:** None. **J.L. Loebach:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.03/DP08/CC23 (Dynamic Poster)

**Topic:** D.06. Audition

**Title:** Auditory recalibration in Virtual Reality

**Authors: *M. GONZALEZ-FRANCO**[1,2], C. C. BERGER[3,1], D. FLORENCIO[1], Z. ZHANG[1,4]
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**Abstract:** Inside Virtual Reality (VR), different modalities can be presented dynamically in synchronous or asynchronous ways to retrain and recalibrate the senses. This allows for scenarios in which subjects can develop further sensorial expertise or acuity. Generally, auditory perception has good temporal resolution, and humans are naturally good at distance estimation through audition. However, the spatial acuity of audition is poor compared to vision, and being able to discretize sounds in space is necessary to segregate distinct auditory sources in one's environment and enable selective attention. Research has revealed that repeated exposure to spatially and temporally aligned audiovisual stimuli, can lead to cross-modal plasticity and improved performance on source localization in the auditory domain. In Virtual Reality, auditory stimuli are often presented through a simulated spatialization that is calculated using head tracking devices. This is enabled by Head Related Transform Functions (HRTF), which can be generalized or calibrated on a per user basis. Although personalized HRTFs are known to improve one's accuracy for the direction of sounds, these calibrations are arduous. Therefore, HRTFs present a challenge for the adoption of the technology. Here, we hypothesized that personalized calibration of HRTFs might not be necessary if a recalibration of the participants' auditory capabilities is possible via cross-modal plasticity mechanisms. **Methods** Participants (n=16) undergo an auditory calibration inside a VR headset equipped with a generic HRTF. The calibration lasts for 60 seconds in which an audio sound bounces around the scene 10 meters in

front of the participant. In the cross-modal condition a sphere is attached to the sound. The control condition does not provide visual clues. In order to account for adaptation and plasticity effects, we ask participants to perform an audio localization task (25 trials) before and after the exposure. At each trial, the participant indicates the direction the audio is coming from by pointing with a virtual cylinder attached to their head and pressing a button. All the trials are randomly located in a 1D line perpendicular to the sight of the participant also 10 meter in front. The sounds come from 5 predefined locations 2.5 meters apart and all locations are presented at least 5 times without sequential duplicates. **Results** Looking at the actual pre- and post- exposure performance, we find that participants in the cross modal condition exhibited an improvement (Wilcoxon paired test, mean improvement=0.18, $p<0.1$). While participants in the control condition did not show improvement (mean improvement=-0.09, $p=0.6$).

**Disclosures:** **M. Gonzalez-Franco:** A. Employment/Salary (full or part-time):; Microsoft. **C.C. Berger:** None. **D. Florencio:** A. Employment/Salary (full or part-time):; Microsoft. **Z. Zhang:** A. Employment/Salary (full or part-time):; Microsoft.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.04/CC24

**Topic:** D.06. Audition

**Support:** “973” of China 2014CB943002

NSFC of China U1301225

NSFC of China 31529003

NSFC of China 31671083

**Title:** Neuronal discharging to ipsilateral acoustic stimuli in central nucleus of inferior colliculus from the activation of contralateral cochlear via bone-conduction

**Authors:** *J. WEI, ***J. WEI**, C. XIAO, Z. XIAO
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**Abstract:** Binaural integration remains unclear. The mechanism underlying binaural integration have been extensively explored in central nucleus of inferior colliculus (ICC). ICC neurons receive inputs from contralateral ICC and bilateral lower nuclei, are driven by contralateral and/or ipsilateral sound stimuli. Most of them are activated by contralateral stimuli and facilitated or suppressed by ipsilateral stimuli, that is, EE and EI neurons. However, it is unknown whether or not the EE and EI responses of ICC neurons are uploading from the lower

nuclei. In other words, what is the response of ICC neurons to contralateral or ipsilateral sound stimuli, respectively. Here, we adopted in vivo cell-attached recording to compare the response characteristic to contralateral or ipsilateral sound stimuli in normal hearing, middle ear blocked and cochlear damaged mice with enclosed sound delivery system. Furthermore, we performed in vivo whole-cell recordings to investigate the synaptic activity to ipsilateral sound stimuli in contralateral cochlear damaged mice with or without lidocaine in contralateral ICC. Data showed that responses to ipsilateral stimuli in normal was consistent with those in ipsilateral cochlear damaged mice and in contralateral middle ear blocked mice. It demonstrates that the responses to ipsilateral stimuli were formed by the activation of contralateral cochlear via bone-conduction rather than contralateral cochlear via air-conduction or ipsilateral cochlear. Moreover, no spike was evoked by contralateral acoustic stimuli in ICC of contralateral cochlear damaged mice, but stronger inhibitory postsynaptic currents (IPSCs) and weaker excitatory postsynaptic currents (EPSCs) to ipsilateral stimuli were recorded. After the ICC on the same side of the damaged cochlear was silenced with lidocaine, the EPSCs to ipsilateral stimuli faded away, the IPSCs decreased about 10-20%. The results suggests that ICC neurons only process contralateral auditory information, their discharging to ipsilateral stimuli is from the activation of contralateral cochlear via bone-conduction, they integrate subthreshold excitation from contralateral ICC and inhibition from contralateral ICC and directly ipsilateral lower nuclei when responding to ipsilateral stimuli.

**Disclosures:** **J. Wei:** None. **C. Xiao:** None. **Z. Xiao:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.05/CC25

**Topic:** D.06. Audition

**Support:** NIH Grant DC013906

**Title:** Stimulus dependent versus cell specific computational strategies in localization of two simultaneous sounds

**Authors: *S. M. WILLETT**[1], V. C. CARUSO[2], S. T. TOKDAR[1], J. M. GROH[1]
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**Abstract:** Coding of multiple simultaneous sounds is a known problem for broadly tuned neural representations of auditory space. Previous reports suggested neurons in monkey inferior colliculus (IC) implement time-division multiplexing, or alternation between firing rates corresponding to each stimulus (Caruso et. al., BioRxiv, 2017; Willett et. al., Soc Neuro Abstr 2016). These studies found a variety of fluctuating and stable response patterns across the set of

tested conditions. Here, we investigated whether the same neurons tested with different stimuli tended to demonstrate the same vs. different types of response patterns.
Single unit activity was recorded in the IC of a rhesus macaque performing single- and dual-sound localization tasks involving one or two saccades to the location of each stimulus. Spike counts on dual sound trials were modeled in relation to the Poisson distributions of spike counts observed on single sound trials. Responses were classified as deriving from (a) a mixture of the two single-sound Poissons, (b) an intermediate Poisson distribution with a rate between those of the two single sounds, or a Poisson distribution that (c) matched or (d) was outside the range of the single-sound Poissons. We then determined whether the winning model was consistent for all conditions an individual neuron was tested with.
The results were heterogeneous. Roughly 45% of cells appear to use a cell specific computation, with the majority of winning models being consistent across the tested conditions. Another 37% of cells exhibited distinctly different behavior across different stimulus conditions. Together, these results indicate that IC neurons can either play a consistent type of role in the population response to dual sounds or they can be flexible and respond differently to different combinations of stimuli.

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**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.06/CC26

**Topic:** D.06. Audition

**Support:** NIH Grant DC013906

**Title:** How many sound locations can humans distinguish at a time? Implications for neural processing of auditory space

**Authors: L. FARRELL**[1], *J. M. GROH[2]
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**Abstract:** • How the brain encodes multiple sounds is unclear, particularly given recent evidence that sound azimuth is encoded in the level of activity in a neural ensemble rather than via an auditory space map of circumscribed receptive fields. One possibility is that the brain takes advantage of frequency differences to sort different sound sources to different regions of tonotopic maps (Willett et al. Soc Neuro Abstr 2016). Alternatively, the brain may use time division multiplexing to encode multiple sounds via fluctuating activity rates corresponding to individual sounds across time (Caruso et al., biorxiv, 2017).
• Insight into which possibility or possibilities may apply and how they operate can be gained

from knowing how many sounds humans are capable of distinguishing at one time. In this study, 8 different bandpass filtered noise stimuli (with different center frequencies) were combined and presented simultaneously from between 1 and 8 different locations. For example, all 8 bandpass filtered stimuli were combined and played from 1 speaker, all were separately played from 8 speakers, or any combination in between. Human participants were asked to report how many different locations they heard on each trial.
• We found that the reported number of locations scaled with actual number up to about 4 sounds. However, participants tended to underestimate the actual number and were accurate only for 1-2 locations. Since the frequency information was held constant, we interpret these results chiefly under the time division multiplexing theory. We conclude that the time division multiplexing component of multiple stimulus encoding may operate on a duty cycle that permits representation of a maximum of four sound locations at a time.

**Disclosures: L. Farrell:** None. **J.M. Groh:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

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**Program#/Poster#:** 585.07/CC27

**Topic:** D.06. Audition

**Support:** NIH DC00436

NIH P30 DC0466

**Title:** Interaural time difference sensitivity in turtles

**Authors: *K. L. WILLIS**[1,2], C. E. CARR[2]
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**Abstract:** The hearing range of turtles is only about 50-1000 Hz. Hearing limited to low frequencies can constrain sound localization abilities, particularly in Red eared slider turtles, which have a small head, isolated middle ears, and an amphibious lifestyle. To determine if the turtle's nucleus laminaris NL were sensitive to interaural time differences (ITD), we investigated the physiological responses of the brain stem auditory nuclei, nucleus magnocellularis (NM) and NL. We recorded extracellular single and multi-unit responses in NM and NL to dichotic sound in an isolated head preparation. NM and NL neurons responded to frequencies from 100-600 Hz with thresholds from 60-80 dB SPL. NM neurons phase locked reliably with vector strengths of 0.82 ± 0.07 (n = 123). NL vector strengths were comparable to NM (0.70 ±0.17, n = 113). NM projected bilaterally to NL, which was sensitive to ITD. Measures of characteristic delay revealed best ITDs around ± 200μs. Turtle NL neurons typically had characteristic phases close

to 0 (0.12 (± 0.23, n=45), consistent with binaural excitation. Thus turtles encode ITDs consistent with their physiological range.

**Disclosures: K.L. Willis:** None. **C.E. Carr:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.08/CC28

**Topic:** D.06. Audition

**Support:** "973" of China 2014CB943002

NFSC of China U1301225

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NFSC of China 31671083

**Title:** The responses of neurons in central nucleus of inferior colliculus to binaural acoustic stimulation is either from ipsilateral or contralateral rather than binaural integration

**Authors: *Y. LIU**, J. WEI, Z. XIAO
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**Abstract:** Animals have two ears. They are thought to process binaural auditory information. Neurons in central nucleus of inferior colliculus (ICC) receive inputs from contralateral ICC and bilateral lower nuclei. Most of them are activated by contralateral stimuli and facilitated or suppressed by ipsilateral stimuli, called, EE and EI neurons. The synaptic mechanism underlying EE and EI neurons is thought to be the integration of binaural excitation and inhibition. However, it is lack of consolidated evidences.
Using in vivo whole cell recording in this study, we compared the synaptic excitation and inhibition of ICC to binaural stimulation with that to contralateral or ipsilateral stimulation by an intensity-intensity scan, in which the sound intensity for contralateral and ipsilateral stimuli were randomly delivered from 70 to 10 dB referred to no stimulus for contralateral or ipsilateral. When no sound was delivered in a unilateral, the responses presented to the contralateral or ipsilateral sound stimuli, otherwise to interaural level difference (ILD). At some intensity for an ipsilateral stimulus the postsynaptic excitatory (EPSCs)/inhibitory currents (IPSCs) were dependent on the contralateral sound intensity until to a point, to which the response was stronger than that to the ipsilateral intensity, beyond this point the binaural response was consistent with the ipsilateral response. So does that for an contralateral stimulus. In other words, the response to ILD was level-dependent on ipsilateral or contralateral stimulus, that is, the

response to binaural stimulus was selected either to ipsilateral or to contralateral stimulus. To confirm this selectivity, we used dichotic paired tone stimulation with a interaural time difference (ITD: 0, 1, 2, 3, 5, 10, 20, 40, 80, 160 ms) and directly assessed if the response to a unilateral stimulus is integrated with the another. When the acoustic stimuli were presented simultaneously to both ears (0 ms ITD), the binaural response was consistent with the contralateral or ipsilateral. When the ipsilateral acoustic stimulus was presented before or after the contralateral acoustic stimuli, the EPSCs/IPSCs to contralateral stimulus was separated from that to ipsilateral stimulus. Our findings suggest that the responses of ICC neurons to binaural acoustic stimulation is either from ipsilateral or contralateral acoustic stimulus rather than binaural integration.

**Disclosures:** **Y. Liu:** None. **J. Wei:** None. **Z. Xiao:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 585.09/CC29

**Topic:** D.06. Audition

**Support:** NIH Grant DCD000436

**Title:** Pressure difference receiving ears influence ITD coding in American alligators

**Authors: *L. KETTLER**, C. E. CARR
Univ. of Maryland - Dept. of Biol., College Park, MD

**Abstract:** The difference between the timing of sounds at both ears (interaural time difference, ITD) is a key feature for sound source localization. However, ITD coding strategies may vary across taxa, head sizes, and frequencies. Two of the proposed mechanisms are a place code of neurons with different best ITDs within an iso-frequency band (Jeffress-model), and a population code that utilizes the slope of the rate-ITD curves of the entire neuron population over the physiological range, even while best ITDs lie outside the physiological range. A place code is less accurate with increasing noise, when head sizes are small, and for low frequencies where the peaks of ITD tuning curves become very broad. Thus, a rate-code may provide more robust encoding of ITD at low frequencies. In archosaurs, the detection of ITD is assumed to be consistent with a Jeffress-model-like place code, which consists of coincidence detectors and delay lines. An anatomical structure that resembles the Jeffress model has been found in the nucleus laminaris (NL) of archosaurs, including alligators and birds. However, an additional factor, namely pressure difference receiving ears, may influence ITD detection in archosaurs. Alligators, like most birds, are sensitive to ITDs at low frequencies, where coupled ears allow transmission of sounds through the dorsal and ventral sinuses that connect both middle ears and

create pressure difference receiving ears. Internal coupling increases the range of interaural time differences, and thus compensates for small head sizes. Thus, a place code might be efficient despite a low frequency hearing range. We performed in vivo electrophysiological experiments with American alligators to investigate the roles of evolutionary history and anatomical constraints on optimal ITD coding. Like in chicken and owls, best ITDs were broadly distributed in alligators but often outside the physiological range. Unlike in mammals, the slopes of the tuning curves did not uniformly cross 0μs. Models of the physiological range that included pressure difference receivers predicted that the range of ITD was increased with decreasing frequency, and aligned with the limit of best ITDs given by the period of the stimulus tone (pi-limit). The increase in ITD range with frequency, thus, circumvented the constraint of best ITDs lying outside the physiological range. Alligators and other archosaurs, therefore, may share a place code of ITD detection that co-evolved with internal coupling of ears. Since mammalian ears are not coupled, small early mammals may not have been able to compensate for their narrow ITD range, and may have evolved a population coding strategy.

**Disclosures: L. Kettler:** None. **C.E. Carr:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

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**Program#/Poster#:** 585.10/CC30

**Topic:** D.06. Audition

**Support:** 973 program 2014CB943002

**Title:** Excitatory inputs in cortical layer IV reinforced by ipsilateral acoustic stimuli through the inhibition from contralateral auditory cortex to ipsilateral layer VI

**Authors: *X. HAITING**[1], X. ZHANG[2], Z. XIAO[3]
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**Abstract:** Binaural information processing is still unclear. The auditory cortex is the advanced central of auditory system, and should have significant function in binaural integration. However, our understanding of binaural integration in auditory cortex mainly remains in that cortical neurons are activated by contralateral stimuli and facilitated or suppressed by ipsilateral stimuli, that is, EE or EI neurons. It is unknown whether the EE and EI neurons distribute in layer specificity in auditory cortex, and what are their functions. With cell-attached recording and enclosed sound delivery, we found that the EE neurons mainly exist in the layer IV of auditory cortex while the EI neurons mainly exist in the layer VI. This presentation might upload from the subcortical nucleus. Whole-cell recording could separate the excitatory and inhibitory

inputs from the subcortical nucleus. Data showed that the excitation responding to bilateral acoustic stimuli was bigger or smaller than that to contralateral acoustic stimuli, while the inhibition responding to bilateral acoustic stimuli was always smaller than that to contralateral acoustic stimuli in layer IV. In layer VI, both excitation and inhibition responding to bilateral stimuli were smaller than those to contralateral stimuli. However, the inhibitions responding to bilateral stimuli in layer IV were not different from those in layer VI. When normalized the excitation to inhibition (E/I) for bilateral stimuli, the ratio for the layer IV neurons (1.44±0.48) was higher than that for the layer VI neurons (0.83±0.11). These results suggest that the binaural integration does exist in auditory cortex rather than uploading from the subcortex. A recent study reports that there is an inhibition from contralateral auditory cortex via layer II/III to ipsilateral layer V and VI in slice. When the contralateral auditory cortex was damaged or inhibited with lidocaine, the binaural integration in the layer IV and VI disappeared with the cell-attached and whole-cell recordings. When the ipsilateral layer VI was damaged or inhibited with lidocaine, the EE phenomenon, i.e., the enhancement of excitation relative to inhibition disappeared. Therefore, present study reveals a circuit underlying the binaural integration in auditory cortex, that is, the excitatory inputs in cortical layer IV is reinforced by ipsilateral acoustic timuli through the inhibition from contralateral auditory cortex to ipsilateral layer VI.

**Disclosures:** **X. Haiting:** None. **X. Zhang:** None. **Z. Xiao:** None.

**Poster**

**585. Auditory Processing: Sound Localization and Binaural Interactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** D.06. Audition

**Support:** NIH Grant NIDCD000436.

**Title:** Response types of the superior olivary nucleus of the barn owl

**Authors: *A. KRAEMER**[1], C. CARR[2]
[2]Biol., [1]Univ. of Maryland, College Park, MD

**Abstract:** Inhibition is important for auditory processing. In chickens, the superior olivary nucleus (SO) provides inhibitory feedback to first order nuclei, and has been hypothesized to improve ITD coding (Fukui et al., 2010; Burger et al., 2011; Tabor et al., 2012). Few studies have characterized SO response types in barn owls. Moiseff & Konishi (1983) recorded from the owl SO in vivo, and found the majority of units were only excited by the ipsilateral ear. All units recorded from the SO were ILD insensitive, although the authors noted their search criteria might have increased the percentage of monaural response types (Moiseff & Konishi 1983). The SO is morphologically heterogeneous, however, and projects to multiple targets. Some SO

neurons send inhibitory projections to two or more ipsilateral brainstem nuclei, while projections to the contralateral SON may originate from a separate population of neurons (Burger et al., 2005). Other SO neurons project to the inferior colliculus (Takahashi et al., 1988). Given the heterogeneity of SO cell types and projections, it seems likely to contain multiple response types. In order to examine auditory responses in SO, and to study the effects of descending inhibition provided to the first order auditory nuclei, we characterized barn owl SO responses in vivo. Electrolytic lesions confirmed recording locations. We measured tuning curves, tested for phase locking, and analyzed peristimulus time histograms, latency, regularity and PSTHs in addition to measuring rate-level function responses to noise stimuli. We also analyzed binaural measures of sensitivity to interaural level difference and interaural time difference, to categorize each response type. SO response types analyzed include primary-like, chopper, and onset, which are also found in the nucleus angularis (Koppl & Carr 2003). A majority of recorded SO single units were broadly tuned off-responses, similar to mammalian SPON units (Kulesza et al., 2003) and more responsive to noise than tones. We conclude that the SO displays similar response types and heterogeneity in response types to nucleus angularis, but more SO units preferred broad band stimuli, and many were binaural. These broadly tuned responses may serve to regulate the overall firing rate of other auditory brainstem nuclei and/or the contralateral SON.

**Disclosures:** **A. Kraemer:** None. **C. Carr:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.01/CC32

**Topic:** D.06. Audition

**Support:** NSF Award 1344065

**Title:** Perceptual Classification of Sounds: Why three auditory cortical fields are better than one

**Authors: *H. L. READ**[1,2,3], A. OSMAN[2], C. M. LEE[5], M. A. ESCABI[1,2,4]
[2]Biomed. Engin., [3]Behavioral Neurosci., [4]Electrical Engin., [1]Univ. of Connecticut, Storrs, CT; [5]Mol. and Integrative Physiol., Univ. of Illinois, Champaign, IL

**Abstract:** Auditory cortex is essential for mammals, including rodents, to behaviorally detect temporal cues in sound but it remains unclear what different cortical fields contribute to temporal cue discrimination (Threlkeld et al, 2008; Lomber & Malhotra, 2008). Previously, we found temporally precise spiking patterns change proportionally with temporal shape cues in the sound envelope for spike outputs from primary (A1) and non-primary Ventral and Suprarhinal (VAF, SRAF) auditory fields of the rat (Lee et al., 2016). Here, we extend these findings and report that the response peak delay as well as total response duration mirror changes in sound shape

parameters in all three cortical fields. We tested how each cortical field might contribute to behavioral discrimination between paired sounds or classification of nine different sound shapes using neural discrimination indices or a Naïve Bayesian classifiers applied to neural population recordings. Remarkably, we find discrimination and classification performances are reduced when the temporal precision of cortical neuron responses are degraded. This suggests that auditory cortical neurons do not classify these sound cues based on a spike rate alone. Accordingly, temporally precise spiking and not spike rate output provides the best signal for classification of sound shape in all three cortical fields. Moreover, we find sound burst durations need to be about two-fold greater than the reference sound duration for effective neural discrimination much like observed behaviorally (Kelly et al., 2006). An interesting new twist we discover is that there is not a single optimal time scale for best performance as time scales for temporally precise output and optimal discrimination increase in rank order with A1 < VAF < cSRAF. When we tested the scenario where neural population responses are drawn cumulatively from all three cortical fields, we find a more robust signal for classifying many sound shapes than observed with population outputs from any one field alone. Together these results support the concept that heterogeneous and complementary neural response time scales from these three auditory cortical fields provide a mechanism for animals to discriminate and classify a large number of sound shapes with a high degree of accuracy.

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**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.02/CC33

**Topic:** D.06. Audition

**Support:** R01DC015138

**Title:** Dynamic neuron to neuron correlation statistics can contribute to sound category identification

**Authors: *M. SADEGHI**[1], I. STEVENSON[2], M. ESCABI[1]
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**Abstract:** Real-world sounds including, natural or man-made, background or vocalized, vary dynamically in time and frequency and exhibit unique high-order statistical regularities in their spectrograms. Humans can detect and use such high-order statistical structure to perceive and ultimately identify sounds (McDermott & Simoncelli 2011). Numerous studies have also demonstrated that neurons in the central auditory pathway, including the auditory midbrain and cortex, respond to high-order sound statistics selectively and in an information theoretic optimal

manner. Yet how and if neural response statistics contribute directly to sound recognition phenomenon is largely unknown. Here we used an auditory cochlear model to test whether dynamic neuron-to-neuron correlation statistics can contribute to sound category identification phenomenon. Using the time-frequency outputs of a cochlear model, we measured the dynamic spectral-temporal correlation structure of sounds to test the hypothesis that non-stationary high-order statistics obtained from computational auditory cochlear model can contribute to sound recognition phenomenon. Specifically, we measured the model neuron-to-neuron correlation statistics between frequency organized cochlear channels at times-scales comparable to perceptual and cortical integration times (100-400 msec). Using a database of sounds from 10 natural and man-made categories, we applied dimensionality reduction to the dynamic correlations and constructed a high-dimensional statistical likelihood functions. A Naïve Bayes classifier was used to identify sounds in a ten-alternative forced choice task. The dynamic correlation statistics from the cochlear model outputs achieve robust sound category identification approaching 80% accuracy (10% chance level). When tested independently, both spectral and temporal correlations of the cochlear outputs appear to contribute equally since separately they achieve a similar level of performance. Finally, the classifier performance improves with increasing sound duration and plateaus at ~ 6 seconds mirroring human data performance for texture identification (McDermott & Simoncelli 2012). (Supported by NIDCD and CRCNS: R01DC015138)

**Disclosures:** **M. Sadeghi:** None. **I. Stevenson:** None. **M. Escabi:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Topic:** D.06. Audition

**Support:** R01DC015138

**Title:** Using neuron-to-neuron correlation statistics to categorize sounds in the mammalian auditory midbrain

**Authors: *M. A. ESCABI**[1], F. KHATAMI[1], M. SADEGHI[2], H. L. READ[1], I. STEVENSON[1]
[1]Univ. of Connecticut, Storrs, CT; [2]Univ. of Connecticut, Storrsa, CT

**Abstract:** Barlow and others have suggested that organisms and the neural networks that underlie sensoryperception are optimized to capture statistical regularities in sensory signals. Accordingly, high-order statistical regularities in natural sounds are critical for perceptually discriminating and categorizing sounds (McDermott and Simoncelli, 2011; Geffen et al., 2011). Though neural responses in the central auditory system vary with modulation and correlation

sound statistics (Attias and Schreiner 1998; Escabi and Schreiner, 2002; Woolley et al 2005; Rodriguez et al., 2012) it remains unclear whether sensitivity to high-order statistics could be used to discriminate sound category and how this might be instantiated. Here we characterize single neuron activity of central auditory neurons in the inferior colliculus of awake rabbits in response to an ensemble of sound textures including water, fire, birdsong chorus, snake sounds, and speech babble. Using texture synthesis (McDermott and Simoncelli 2011), we synthetically manipulate each sound by selectively adding or removing high-order statistics or by combining statistics from different sound to create chimeric sound mixtures. We find that correlated spiking between pairs of neurons or multi-unit recording sites is strongly modulated with the sound category and the synthetically manipulated statistics. Using neurometric and ideal observer analyses we demonstrate that the neuron-to-neuron response correlation statistics can be used to discriminate sounds. Systematic removal of the high-order sound statistics decreases the neural-based sound classification performance. Conversely, systematic increase in the number of neurons used or the sound duration increases neural-based sound classification performance. This study indicates that neuron-to-neuron correlation statistics in the inferior colliculus have the capacity to capture statistical regularities in sounds that are critical for sound categorization. These findings are significant as they support the concept that statistical regularities are major drivers of sensory systems in general. Moreover, our findings support the concept that mammalian systems have the capacity to optimize sensory categorization through correlated activity in neuron populations. (Supported by NIDCD and CRCNS: R01DC015138)

**Disclosures:** **M.A. Escabi:** None. **F. Khatami:** None. **M. Sadeghi:** None. **H.L. Read:** None. **I. Stevenson:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.04/DD2

**Topic:** D.06. Audition

**Title:** A freely-moving operant system for auditory discrimination in common marmosets

**Authors: *M. J. METKE**
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**Abstract:** Here we demonstrate a novel operant conditioning system developed for testing free-moving marmoset monkeys (Callithrix jacchus) that complements more traditional head-fixed approaches in nonhuman primates. The operant chamber is an acrylic and wire mesh box with three response ports arrayed horizontally across one wall. Each port consists of an IR LED proximity sensor for detecting subject responses, a tube for delivering juice rewards, and an LED for assisting training.

We have applied this novel system to examine periodicity discrimination in marmosets. The representation of periodic stimuli in primate auditory cortex relies on two distinct mechanisms: a stimulus-synchronized temporal code for slower stimuli and a non-synchronous rate code for frequencies beyond the maximal neuronal firing rate. Previous studies have suggested that largely separate neuronal populations utilise these distinct encoding schemes. However, these have been limited in their reliance on single-unit electrophysiology and head-restrained subjects. Marmosets were trained in a two-forced choice task in which they were presented two pure-tone stimuli with sound pips of varying frequency. Subjects were rewarded for correctly identifying whether or not the stimuli frequencies matched. Manipulating the frequency of the stimulus we create ROC curves to demonstrate the perceptual sensitivity to frequency.
In combination with implanted microelectrode arrays we can simultaneously record single and multi-unit activity throughout all fields of auditory cortex while monkeys perform the task. These data allow us to determine how each region of auditory cortex encode complex periodic stimuli. Further, manipulating the tonal makeup of the sound pips allows us to parse the contribution of each of the regions to the cortical representation of the stimulus. This work lays the foundation for future studies using optogenetics to manipulate auditory cortex during a task to modulate behavioral responses.

**Disclosures: M.J. Metke:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.05/DD3

**Topic:** D.06. Audition

**Support:** NIH R01 DC012087

**Title:** A comparison of marmoset frontal cortex neuron responses to acoustic stimuli in multiple behavioral contexts

**Authors: *V. JOVANOVIC**[1], C. T. MILLER[2]
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**Abstract:** Primate vocal communication is characterized by the production and perception of vocalizations within the context of active social behaviors. Within this complex system, what is communicated by a vocalization is heavily dependent on the immediate social context in which it occurs. However, much of what is known about the neural basis of primate communication comes from traditional studies involving subjects that are either passively listening or trained to respond to vocalizations with a conditioned behavioral response. Because of the active nature of communication, each of these more conventional approaches divorces the signal from the core

context in which it naturally occurs. Here we sought to test this critical issue by recording the responses of frontal cortex neurons in marmosets across multiple behavioral contexts. Specifically, we presented the same vocalizations (phee calls, twitters) and a variety of white noise stimuli to marmosets while they were head-fixed and freely-moving using identical stimulus presentation protocol. During each of these test sessions, we also recorded the same neurons while subjects engaged in their natural antiphonal calling behavior, which involves the reciprocal exchange of phee calls. Preliminary analyses revealed that (1) white noise elicited the most consistent and robust responses across all test stimuli, (2) only half of neurons responsive to an acoustic stimulus in the head-fixed condition exhibited a similar response to the same stimulus in the freely-moving condition and (3) phee calls elicited the weakest responses of all stimuli in all conditions. These findings suggest that neural responses under head-fixed, passively listening conditions may not be predictive of more natural contexts. We are currently examining a broader set of stimuli in order to test the hypothesis that distinct neural processes may underlie vocal signal processing during natural, active communication and these other behavioral contexts.

**Disclosures: V. Jovanovic:** None. **C.T. Miller:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.06/DD4

**Topic:** D.06. Audition

**Support:** NIH/NIDCD Grant K08-DC014299

**Title:** Association between marmoset vocal variability and social context in naturalistic social environments

**Authors: *J. TSUNADA**, S. ELIADES
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**Abstract:** Vocal communication plays a key role in maintaining group cohesion and social bonds for group-living primates. Successful vocal communication requires signalers to inform receivers about the social context of the produced vocal signals (e.g., vocal signals are directed toward specific receivers). Marmoset monkeys (*Callithrix jacchus*) exhibit many human-like social behaviors, including interactive vocal communication. It is unclear, however, the degree to which the natural variability of marmoset vocalizations is deliberate and informative versus a result of random fluctuation. Previous experiments using isolated pairs of marmosets have shown that marmosets produced acoustically different phee calls during vocal interaction than during spontaneous calling. However, vocal communication in naturalistic social environments involves

many types of vocalizations and many group members. Therefore, it remains unknown if marmosets produce acoustically different calls depending on details of the social context, and whether such vocal acoustics influence the responsiveness of others. We recorded vocalizations from an entire group of marmosets housed in a colony environment and measured their vocal interactions in order to determine which animals responded to other's vocalizations and in what order. We particularly focused on the marmoset trill call, frequently produced in the housing environment, and found that acoustical properties (e.g., duration) of a produced vocalization influenced the probability of evoking a vocal response. Furthermore, such response probabilities differed between different producer-responder pairs, suggesting an association between the acoustic properties of produced vocalizations and their intended receivers. Additionally, we further tested whether marmosets are sensitive to vocal acoustic variability by playback of computer-controlled vocal sounds in the housing environment. We found that playback of vocalizations evoked responsive vocal production from different individuals within colony. Together, these results further elaborate our understanding of the complex social mechanisms used by marmosets during vocal communication that can potentially be exploited to understand the neural basis of social behaviors.

**Disclosures:** **J. Tsunada:** None. **S. Eliades:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.07/DD5

**Topic:** D.06. Audition

**Support:** NIH Grant DC014299

**Title:** Auditory cortical activity predicts feedback-dependent vocal control in marmoset monkeys

**Authors: *S. ELIADES**[1], J. TSUNADA[2]
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**Abstract:** Human speech is a sensory-motor process involving auditory self-monitoring to control vocal production and ensure accurate communication. Monitoring auditory feedback during vocal production allows one to quickly adjust speech to compensate for perceived changes in vocal output. Many animal species share similar feedback-dependent vocal control, however, the underlying neural mechanisms are unknown. Previous studies have demonstrated both a suppression of auditory cortex neurons during vocal production, and a sensitivity of such neurons to externally-perturbed auditory feedback. The role of this neural activity in vocal control is not known. We therefore investigated the responses of auditory cortical neurons in

marmoset monkeys during vocal self-monitoring and feedback-dependent vocal control. Recent work has shown that, when presented with real-time frequency-shifted auditory feedback, marmosets will compensate for altered feedback by rapidly changing the acoustics of their ongoing vocalizations. We used implanted electrode arrays to record neural activities during self-initiated vocal production while simultaneously altering vocal feedback and observing the resulting vocal compensation. We found that neural activities in auditory cortical neurons were sensitive to altered feedback during vocalization, consistent with previous results. We further found that auditory cortical responses predicted subsequent changes in vocal production. These results demonstrate the function of auditory cortex in self-monitoring during vocal production, and suggest a role for the auditory cortex in feedback-coding and vocal motor control.

**Disclosures: S. Eliades:** None. **J. Tsunada:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.08/DD6

**Topic:** D.06. Audition

**Support:** NIH 5R01DC009810-07

**Title:** Behavioral significance and auditory cortical representation of harmonic vocalizations

**Authors: *N. L. SO**[1,3], S. M. N. WOOLLEY[2,3]
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**Abstract:** Vocal communication relies on the brain’s ability to detect and process vocal sounds in the environment. Auditory systems that are optimized for vocalization processing must robustly encode acoustic features that distinguish vocalizations from other sound categories. One acoustic hallmark of speech and other animal vocalizations is harmonic structure. Sounds that are harmonic contain energy concentrated at integer multiples of a fundamental frequency, and higher harmonicity correlates with greater pitch salience. Our studies address two main questions. First, how does variation in harmonic structure alter the behavioral significance of vocal sounds? Second, where does selectivity for harmonic structure arise in the auditory processing pathway? We use zebra finches (*Taeniopygia guttata*) to address these questions. These songbirds use harmonic calls and songs for social communication and have well-defined, experimentally accessible auditory systems.
Zebra finches exchange distance calls, a highly harmonic class of vocalizations, to maintain contact with each other when visually separated. Using a behavioral paradigm in which we present distance calls and assess birds’ vocal responses to these stimuli, we found that behavioral

responsiveness is sensitive to the degree of harmonic structure in stimulus calls; birds respond less to spectrally degraded versions of calls compared to natural versions of the same calls. To test whether the responses of auditory cortex (AC) neurons are similarly sensitive to harmonic structure, we recorded the responses of single AC neurons to natural and spectrally degraded distance calls, in awake, restrained zebra finches while they listen to the calls. We recorded single unit responses from: 1) the intermediate region (Field L2), which receives information from the thalamus; 2) the superficial regions (Field L1/CM), which receive input from the intermediate region; and 3) the deep region (Field L3), which receives input from both intermediate and superficial regions. Preliminary results suggest that neuronal responses in the intermediate and superficial regions do not differ with variations in calls' harmonic structure. In contrast, deep region neurons respond more to calls with high harmonic structure than to spectrally degraded versions of those calls. Together, results suggest that harmonic structure contributes to the behavioral salience of vocal communication sounds, and that neural selectivity for this feature arises in the deep region of primary AC.

**Disclosures: N.L. So:** None. **S.M.N. Woolley:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

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**Program#/Poster#:** 586.09/DD7

**Topic:** D.06. Audition

**Support:** Pennsylvania Lions Hearing Research Foundation

Samuel and Emma Winters Foundation

**Title:** Optimal features for auditory recognition

**Authors: S. LIU**[1], X. WANG[3], *S. SADAGOPAN[2]
[1]Bioengineering, [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

**Abstract:** A central challenge in auditory neuroscience is to understand how observed patterns of neural activity in the auditory system relate to behavior. For example, neurons in primary (A1) as well as higher auditory cortical areas exhibit highly nonlinear and surprisingly specific tuning properties, but our understanding of these responses is only at a descriptive level, and the critical question of how these responses might support behavior remains unresolved. Here, we show that nonlinear A1 responses encode essential features for the classification of ethologically-relevant sounds such as conspecific vocalizations (calls). In vocal animals, increasing neural resources are committed for the processing of calls as one ascends the auditory processing hierarchy.

Therefore, the categorization of call types is a reasonable computational goal for the auditory cortex in these animals. We asked, using a theoretical information-maximization approach, how this goal can be best accomplished. We used marmoset vocalizations as our experimental model. First, we transformed the vocalizations into spectrotemporal patterns of auditory nerve activity (cochleagrams) using a highly realistic model of the auditory nerve. Based on an earlier model for visual classification, we then randomly generated a large number of features, or spectrotemporal snippets, from these cochleagrams. We used a greedy-search algorithm to choose the most informative and least redundant feature set for call categorization. We found that call categorization could be accomplished with high accuracy using just a small number of features. Highly informative features tended to be of intermediate size and complexity. Most interestingly, the responses of model feature-selective neurons predicted nonlinear neural responses in marmoset A1 in astonishing detail. These results demonstrate that the auditory cortex uses a mid-level feature based strategy for the recognition of complex sounds. These results further suggest that the tuning properties of neurons in higher auditory cortical stages are likely the result of goal-directed optimization.

**Disclosures:** **S. Liu:** None. **X. Wang:** None. **S. Sadagopan:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Program#/Poster#:** 586.10/DD8

**Topic:** D.06. Audition

**Support:** Pennsylvania Lions Hearing Research Foundation

Samuel and Emma Winters Foundation

**Title:** Emergence of selectivity and invariance in primary auditory cortex

**Authors: *P. MONTES LOURIDO**[1], S. LIU[2], S. SADAGOPAN[3]
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**Abstract:** Humans and vocal animals use vocalizations to communicate and interact with members of their species. Real-world environments add noises, echoes and other sounds to the intended message, degrading its acoustic content. However, we can maintain stable sound perception independent of listening conditions. We aim to determine the neural mechanisms by which stable sound perception can be achieved. To address this question in the context of natural behaviors, we use Guinea pig (GP) vocalizations as an experimental model. Previous studies in GPs have shown that at the level of the inferior colliculus and thalamus, few neurons show selective responses for individual vocalization categories. In primary and secondary cortical

areas, more neurons become selective for particular vocalization categories. It is not known, however, at which stage of the auditory hierarchy this selectivity arises, and how it is preserved or changed in the presence of real-world distortions. Here, we first tested if GPs can perceive vocalizations presented in a wide range of noisy environments using pupillometry as a behavioral readout. This allowed us to determine the GP's threshold for detecting a vocalization in noise. We then recorded single-unit activity in the medial geniculate body (MGB) and auditory cortex (A1) of awake GPs passively listening to vocalizations in different listening conditions. We discovered that neurons in MGB and thalamorecipient A1 layers (A1 L4) have low selectivity for vocalization categories and are more susceptible to acoustic distortions. In contrast, superficial layers of A1 (A1 L2/3) were highly selective for vocalizations and more invariant to distortion. These data demonstrate that both vocalization selectivity and invariance to listening conditions co-emerge in A1 L2/3. These results suggest that a dense representation of complex sounds in A1 L4 is transformed into an invariant and sparse representation in A1 L2/3.

**Disclosures:** **P. Montes Lourido:** None. **S. Liu:** None. **S. Sadagopan:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.11/DP09/DD9 (Dynamic Poster)

**Topic:** D.06. Audition

**Support:** NIH Grante DC001641

**Title:** Aural contrast in midbrain responses to speech: Effects of noise and hearing loss

**Authors: *L. H. CARNEY**
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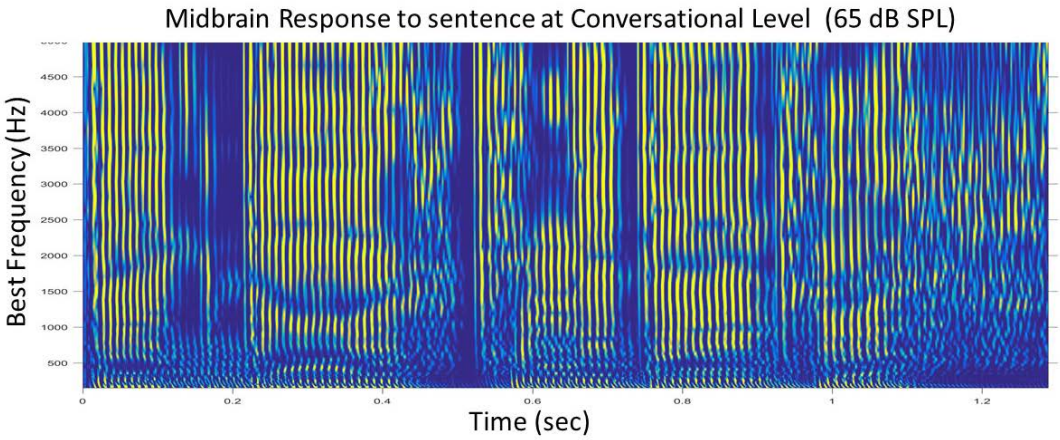
**Abstract:** Responses to complex sounds in the auditory midbrain provide a representation, rich in contrast, that projects to the thalamus and cortex. Midbrain representations differ substantially from the stimulus spectrum. For example, voiced speech sounds are characterized by strong fluctuations at the fundamental frequency (F0), with the largest amplitude fluctuations in frequency channels near spectral peaks. However, auditory-nerve (AN) responses to voiced speech have the largest F0 fluctuations between spectral peaks and reduced F0 fluctuations near spectral peaks, where responses are dominated by a single harmonic close to best frequency. At conversational speech levels, the low-threshold, high-spontaneous-rate AN fibers, which provide the major input to the ascending auditory pathways, have saturated average discharge rates. However, the contrast in fluctuation amplitudes across AN frequency channels is ultimately represented in both rate and temporal responses in the midbrain, where average rates are sensitive to fluctuation amplitude. For unvoiced sounds, midbrain responses are driven by

fluctuations associated with spectral slopes and by temporal characteristics of the stimuli, such as transients. Aural contrast refers to the patterns of midbrain activity that encode the location of spectral peaks, slopes and transients which are important cues to the identity of speech sounds. Computational models for populations of AN fibers and midbrain neurons are used to illustrate and examine the contrasts associated with phonetic features. Aural contrast depends upon the gradual saturation of the inner hair cells (IHCs) over the range of levels in speech sounds and on cochlear amplification, which influences the operating point on the IHC nonlinearity. Background noise and sensorineural hearing loss affect this operating point and reduce aural contrast. Linear amplification does not restore the contrast cues. The representation of speech sounds at the level of the midbrain suggests novel contrast-based signal-processing strategies to improve aural contrast.




**Disclosures: L.H. Carney:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.12/DD10

**Topic:** D.06. Audition

**Title:** The emergence of neural representations of auditory objects and the influence of acoustic features

**Authors: *M. OGG**[1,2], C. NEUFELD[3], T. A. CARLSON[4], L. R. SLEVC[2,1]
[1]Neurosci. and Cognitive Sci. Program, [2]Psychology, [3]Linguistics, Univ. of Maryland, College Park, MD; [4]Univ. of Sydney, Sydney, Australia

**Abstract:** Auditory objects correspond to sound sources in the listener's environment that allow one to orient themselves or respond to potential threats. A critical constraint on these percepts is that they require time to develop. Concurrently, auditory objects must be processed quickly, to allow for a rapid response. Therefore, a listener's auditory system must quickly transform acoustic information into mental representations of objects, which, in turn, support complex cognitive tasks. Relatively little is known about what acoustic features are extracted and used to construct auditory object representations early in perception. To better understand this process, we decoded natural sound tokens (speech, musical notes from different instruments, and everyday items) from the neural responses of human listeners recorded using MEG. We modeled pairwise decoding accuracy as a function of differences between stimuli along various acoustic dimensions (including aperiodicity, pitch, and spectral centroid) and representations (spectrotemporal modulation spectra and cochleograms) during the first 500 milliseconds following sound onset. We found that decoding accuracy peaked at 150 milliseconds, capturing the rapid emergence of the neural representation of auditory objects. Decoding results at 150 milliseconds were supported by differences in spectrotemporal modulation and aperiodicity between individual stimuli. Decoding after 150 milliseconds, however, came to rely on more frequency dependant features such as pitch and differences in spectral envelope in addition to aperiodicity. These results point to a set of acoustic cues that support the emergence of auditory object representations early in auditory perception.

**Disclosures:** **M. Ogg:** None. **C. Neufeld:** None. **T.A. Carlson:** None. **L.R. Slevc:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Topic:** D.06. Audition

**Support:** NIH R01-DC012379

NIH F32-DC014192-01

**Title:** Cortical responses in human superior temporal gyrus that differentiate intonation contours in speech are a response to pitch, not fundamental frequency

**Authors: *C. TANG**[1], L. S. HAMILTON[1], E. F. CHANG[2]
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Neurosurg., UCSF, San Francisco, CA

**Abstract:** Speech intonation is an important component of prosody in all spoken languages. It conveys sentence-level linguistic meaning, such as sentence type (statement vs. question) or focus (which word was emphasized). In our previous work using electrocorticography (ECoG),

we showed that specific neural populations in the human superior temporal gyrus (STG) respond differentially to speech stimuli with distinct intonation contours, realized through digital manipulation of the fundamental frequency (f0). Because those stimuli were synthesized to control for intensity and duration of syllables, the only acoustic difference across intonation conditions was f0 over time. Although the f0 of a sound generally determines its perceived pitch, sounds with acoustic energy at the fundamental removed, called 'missing fundamental' stimuli, can also produce a pitch percept. To determine whether STG responses that differentiated intonation contours in speech are a response to pitch or to f0, we created a set of non-speech stimuli that preserved each intonational pitch contour but did not contain f0 and played them to participants while we recorded cortical activity using ECoG. To test whether neural responses to pitch contours in the missing f0 stimuli were similar to neural responses to f0 contours in speech, we used linear discriminant analysis to fit a model predicting the intonation contour from neural responses to speech. We then tested this model on the missing f0 data to see whether the model could discriminate between responses to the missing f0 stimuli. We found that in almost all electrodes (47/49 electrodes from 3 participants), neural activity patterns to the different intonation contours were the same between speech and missing f0 stimuli. This result indicates that the cortical activity in neural populations that differentiate intonation contours in speech can be explained as a response to the perceptual attribute of 'pitch', rather than energy at the fundamental frequency.

**Disclosures: C. Tang:** None. **L.S. Hamilton:** None. **E.F. Chang:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Topic:** D.06. Audition

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**Title:** Context dependence of ultrasonic vocalizations of mice

**Authors: *S. AGARWALLA**[1], S. BANDYOPADHYAY[2]
[1]ELECTRONICS AND ELECTRICAL COMMUNICATION ENGINEERING, INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR, India; [2]IIT Kharagpur, Kharagpur, India

**Abstract:** Social cues are known to modulate communication related behavior in a range of species including humans. In mice, ultrasonic vocalisations (USVs) are considered to be of communicative significance. Recordings of vocalizations of male mice (WT, C57BL6J) were made under four different contexts. The contexts are: (i) the male mouse alone, (ii) the male mouse with a female present in view but separated by a mesh, (iii) the male mouse alone in the same cage were it was exposed to a female mouse and (iv) male and female together without any mesh in between. The USV vocalizations were parsed as syllables and bouts of syllables. The sequence of USV syllables were obtained and analyzed based on distributions of syllables and transitions of various steps. Structure in vocalization sequences was studied using information theoretic approaches. Based on significant differences in each context it is concluded that different structures or patterns are present in vocalization based on context. Predictability of the sequences also varied depending on context. The current study hence provides not only further evidence that structures exist in vocalization sequences of mice but also suggests that the structures in vocalization change in a context dependent manner.

**Disclosures: S. Agarwalla:** None. **S. Bandyopadhyay:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Program#/Poster#:** 586.15/DD13

**Topic:** D.06. Audition

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JST PRESTO program Grant Number JPMJPR14D8

**Title:** Adaptive spectral changes in pulses of echolocating bats during group flight with multiple conspecifics

**Authors: *K. HASE**[1], Y. KADOYA[1], Y. MAITANI[1], T. MIYAMOTO[1], K. I. KOBAYASI[1,2], S. HIRYU[1,2,3]
[1]Grad. Sch. of Life and Med. Sci., [2]Fac. of Life and Med. Sci., Doshisha Univ., Kyotanabe, Japan; [3]JST PRESTO, Kawaguchi, Japan

**Abstract:** Because echolocating bats actively emit ultrasounds to scan surrounding environments, acoustic interferences occur when multiple conspecifics occupy same spaces. Although many studies tried to understand their solution to extract their own faint echoes from

backgrounds, little is known about whether and how echolocating bats adaptively alter acoustic characteristics of their emitted pulses because it is difficult to separately measure echolocation pulses of group flying bats. In the present research, we successfully recorded echolocation behavior of group flying Eastern bent-winged bats (*Miniopterus fuliginosus*) which emit downward frequency-modulated (FM) echolocation pulses. The bats were flown singly (Single flight 1), within groups of four bats (Group flight), and then flown singly again (Single flight 2). Echolocation pulses emitted by each individual of the groups were captured with individually mounted on-board microphones. This allowed us to investigate pulses emitted by each animal during group flight without the Doppler effect and atmospheric attenuation. As a result, the bats significantly expanded inter-individual differences in mean terminal frequency (TF) of the emitted pulses from 0.7 ± 0.6 kHz in single flight 1 and 0.6 ± 0.5 kHz in single flight 2 to 1.2 ± 0.7 kHz in group flight (Tukey's HSD test, $P < 0.05$). To understand how changes in acoustic characteristics of FM echolocation pulses reduce interferences, we calculated cross-correlation between an FM signal that mimicked echolocation pulses of *Miniopterus fuliginosus* and an acoustic characteristic (TF, initial frequency, and duration) of which was gradually changed from -10% to 10%. The cross-correlation vales were decreased the most when TF of the signal was manipulated. The half width at half maximum of the values was obtained when TF was changed by only 2%, corresponding to about 1 kHz (mean TF was about 48 kHz). Moreover, cross-correlation values between bats in same groups were significantly decreased in group flight in comparison with single flight 1 and 2 (Tukey's HSD test, $P < 0.05$). These results suggest that echolocating bats reduce similarities between their echoes and interfering sounds in the presence of multiple conspecifics, and changes in TF contribute the most to decrease the similarities.

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**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Topic:** D.06. Audition

**Support:** 5R01-DC013826

Helen Hay Whitney Foundation

Burroughs Wellcome Fund

**Title:** Motor cortex suppresses auditory cortical responses to self-generated sounds

**Authors: *D. M. SCHNEIDER**[1], R. D. MOONEY[2]
[1]Dept. of Neurobio., Duke Univ., Durham, NC; [2]Duke Univ. Hosp., Durham, NC

**Abstract:** One often wants to ignore the sounds of their own movements, such as the sound of their own footsteps, to focus on environmental cues. Filtering out self-generated sounds requires that the brain forms an internal model that selectively suppresses neural responses to sounds that are reliable consequences of certain movements. Consistent with such a predictive mechanism, indirect and population-level recordings suggest that auditory cortical responses to self-generated sounds are selectively attenuated. Moreover, correlative studies suggest that this predictive suppression may be mediated by motor cortical inputs to the auditory cortex. Although motor cortical inputs are capable of generically suppressing auditory cortical activity during a wide variety of movements, whether these inputs selectively suppress auditory cortical responses to self-generated sounds, and the synaptic mechanisms through which this predictive suppression may arise, remain unknown.
To address these questions, we developed an acoustic virtual reality (aVR) platform to study how the mouse cortex learns to predict the sounds associated with a movement. Following several days of aVR experience during which locomotion triggered a series of tones with fixed pitch, auditory cortical excitatory neurons became largely unresponsive to predictable self-generated sounds, yet they retained their responsiveness to locomotion-triggered sounds at other frequencies. Mice exhibited a perceptual blind-spot for tones matching the self-generated pitch when they were locomoting, but not for other sound frequencies and not when they were resting. In contrast to excitatory neurons, auditory cortical inhibitory neurons responsive to the training tone displayed enhanced movement-related activity after several days of aVR experience. Notably, long-range excitatory projections from the motor cortex are an important source of movement-related recruitment of auditory cortical inhibitory neurons. One possibility is that aVR experience selectively strengthens motor cortical drive onto auditory cortical neurons that are responsive to the locomotion-associated pitch. Consistent with this idea, we found that aVR experience strengthened connections between the motor cortex and auditory cortical interneurons that were responsive to the self-generated pitch, resulting in the selective motor-related recruitment of a tuned-subset of inhibitory neurons. These findings are consistent with a framework in which motor-related signals, and their interaction with local auditory cortical circuitry, can predictively suppress neuronal responses to self-generated sounds.

**Disclosures: D.M. Schneider:** None. **R.D. Mooney:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.17/DD15

**Topic:** D.06. Audition

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NSF Grant IOS 1456743

**Title:** Dopamine modulates the peripheral auditory system of a vocal fish

**Authors: *J. PERELMUTER**[1], J. A. SISNEROS[2], P. M. FORLANO[3]
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**Abstract:** Dopamine appears to be a conserved neuromodulator in the peripheral auditory system of vertebrates, having been identified in the ears of both mammals and fish. In rodents, it is hypothesized that dopamine protects the cochlea from noise trauma, however other functions remain unexplored. The plainfin midshipman (*Porichthys notatus*) is a seasonally breeding marine fish that coordinates its summer mating behavior through acoustic communication. In time for the breeding season, females undergo a steroid-dependent increase in peripheral hearing sensitivity which enhances their ability to find a mate. This coincides with a reduction of dopaminergic innervation in the saccule, the principal end-organ of hearing in midshipman fish. We hypothesized that the reduction of dopamine provides a release of inhibition which contributes to this increase in sensitivity. We recorded auditory evoked potentials from populations of saccular hair cells after the application of dopamine, dopamine receptor agonists and antagonists in summer reproductive females. Exogenous dopamine increased auditory thresholds (reducing sensitivity), confirming an inhibitory effect. A D2 receptor agonist produced a similar inhibitory effect; however, a D1 receptor agonist had no effect on sensitivity. Co-application of dopamine and a D2 receptor antagonist abolished the effect of dopamine. Exogenous dopamine also produced an inhibitory effect in females in the winter, when they do not reproduce and hearing sensitivity is naturally reduced. This suggests that dopamine receptor expression is relatively stable across seasons and that dopamine fiber plasticity alone may account for release of inhibition. Our results point to a novel biological function for dopamine, adaptive regulation of peripheral auditory sensitivity, and raises the possibility that this function could be conserved across vertebrates.

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**Poster**

**586. Auditory System: Cortical Processing and Perception**

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**Program#/Poster#:** 586.18/DD16

**Topic:** D.06. Audition

**Support:** JSPS KAKENHI 16H01683

**Title:** Temporal and rate coding of sound envelope and temporal fine structures of vocalizations in the primary auditory cortex of marmoset monkeys

**Authors: *T. BANNO**[1,2], W. SUZUKI[2,3], N. MIYAKAWA[2,4], T. TANI[3], N. ICHINOHE[2,3]
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**Abstract:** Human speech and animal vocalizations are characterized by slow modulations in the sound envelope (ENV) and rapid fluctuations in temporal fine structures (TFS). Previous psychophysical studies demonstrated the ENV and TFS convey information of different aspect of speech; the ENV cues are critical for speech intelligibility, whereas the TFS cues are important for segregating the speech sound from the background noise. Although both of the acoustic cues are crucial for vocal communications, it has not been fully elucidated how the auditory system process this information. To address this issue, we used marmosets as a model for human speech perception and conducted electrophysiological recordings from the primary auditory cortex (A1) of anesthetized animals while presenting acoustic stimuli consisting of various combinations of amplitude (AM) and frequency modulations (FM). The fundamental frequency and the depth of the modulations were matched to the marmoset vocalizations so that the stimuli sounded similar to their calls when the AM and FM were equated to the temporal parameters of the vocalizations. We found the spike timing of the A1 neurons precisely followed the modulation frequency of the stimuli if the AM and/or FM were slower than ~10 Hz, suggesting the neurons temporally encode the slow rhythms in both the ENV and TFS of the sounds. A greater number of spikes were observed in the sounds with the slow AM and FM combinations, but the firing rate showed more obvious tunings for FM than AM as a population. The information carried by the firing rate was further examined by mutual information analysis, and we confirmed the general tendency that the A1 neurons encoded a higher amount of FM information. Interestingly, the neurons could have a high amount of information either for AM or FM but not for both, implying the A1 neurons were optimized for encoding the animal vocalization sounds. These results suggest the A1 neurons process information about ENV and TFS cues differently by the temporal and rate codings in a way that they efficiently encode the temporal structures in the conspecific vocalizations.

**Disclosures: T. Banno:** None. **W. Suzuki:** None. **N. Miyakawa:** None. **T. Tani:** None. **N. Ichinohe:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.19/DD17

**Topic:** D.06. Audition

**Title:** Direct effects of social vocalizations on serotonin in the auditory midbrain

**Authors: *K. HOOD**, L. M. HURLEY
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**Abstract:** Neuromodulatory systems like the serotonergic system can represent salient features of behavioral context within sensory systems, tuning sensory neurons to relevant behavioral events. During sexual interactions, female house mice (*Mus musculus*) produce broadband vocalizations (BBVs) that correspond to rejection behaviors, while males produce ultrasonic vocalizations (USVs) that correspond to increased investigatory and sexual behaviors. High BBV production by a female in the first phase of a sexual interaction is inversely correlated with male mounting success. Serotonin in the inferior colliculus (IC), an auditory midbrain nucleus, parallels these events. Serotonin increases in males interacting with females, but is also inversely correlated to female rejection behaviors, including BBV production, indicating that serotonin reflects both the presence and behavior of the female partner. Despite this correlation, whether female vocalizations directly influence serotonergic dynamics in the auditory system is unknown. To address this issue, we used slow-scan voltammetry to measure serotonin in the IC of males whose access to a female partner was limited by a perforated barrier. In this condition, males produce 'courtship' USVs, but females do not vocalize. We compared changes in male IC serotonin in response to 1) limited access to a female or 2) limited access to a female and BBV playback. Compared to baseline levels, males with limited access to a female in preliminary trials did not show a change in IC serotonin. When males were presented with limited access to a female and BBV playback, however, serotonin levels declined. This preliminary finding favorably compares to behavioral studies using the same paradigm and stimuli, in which BBV playbacks also change male sexual behavior by decreasing USV production. Both male courtship behavior and serotonin in the auditory system may therefore be directly influenced by female vocalizations.

**Disclosures: K. Hood:** None. **L.M. Hurley:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

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**Program#/Poster#:** 586.20/DD18

**Topic:** D.06. Audition

**Support:** NIH Grant 5R01-DC013826-03

**Title:** Mechanisms of movement-related changes to auditory detection thresholds

**Authors: *J. SUNDARARAJAN**, D. M. SCHNEIDER, R. D. MOONEY
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**Abstract:** Sensory stimuli can arise from our own movements as well as from the environment. For example, when walking along a leaf-covered path, our brain must distinguish the sounds generated by our footsteps from those of a stalking predator. How the brain makes this distinction is not clear. With respect to audition, studies in humans and other animals have demonstrated that self-generated vocalizations as well as other movements suppress auditory cortical responses to acoustic stimuli relative to responses to the same stimuli measured at rest. An influential but largely untested idea is that this cortical suppression works to minimize responses to predictable acoustic consequences of movements, while enhancing sensitivity to novel stimuli. How this cortical suppression influences auditory perception and whether this suppression functions predictively, as widely theorized, remain unknown. To explore these issues, we used operant methods to train head-fixed mice to lick in response to tone pips of varying intensities while at rest or running on a quiet treadmill. Using this approach, we observed that: 1) the overall threshold for auditory detection is elevated during movement compared to rest, 2) inactivating the auditory cortex elevates tone detection thresholds, indicating that the auditory cortex is a component of an auditory detection circuit in the mouse, 3) optogenetic activation of motor cortical terminals in the auditory cortex of resting mice increases detection thresholds in a manner similar to movement, and 4) movement-related elevation of auditory threshold is specific to predicted sounds following experience with predictable movement-related auditory feedback. These findings support a model in which motor cortical inputs to the auditory cortex function as part of a mechanism for selectively suppressing predictable acoustic consequences of movement while enhancing sensitivity to novel stimuli.

**Disclosures:** **J. Sundararajan:** None. **D.M. Schneider:** None. **R.D. Mooney:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

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**Program#/Poster#:** 586.21/DD19

**Topic:** H.01. Animal Cognition and Behavior

**Support:** DFG SFB-TRR 62

**Title:** Serial reversal learning in humans and gerbils: Data and network model

**Authors: *A. L. SCHULZ**[1], M. L. WOLDEIT[1], A. BRECHMANN[2], C. JARVERS[3], T. BROSCH[3], M. LOMMERZHEIM[1], H. NEUMANN[3], F. W. OHL[1]
[1]Systems Physiol. of Learning, [2]Special Lab. Non-Invasive Brain Imaging, Leibniz Inst. for Neurobio., Magdeburg, Germany; [3]Fac. of Engineering, Computer Sci. and Psychology, Inst. of Neural Information Processing, Ulm University, Germany

**Abstract:** Cognitive flexibility allows adjusting behavior in response to dynamic environmental contingencies and is one of the most important aspects of adaptive goal-directed behavior. An established experimental method for measuring this flexibility is the serial reversal task. In this task the behavioral contingencies between two stimuli and two corresponding trained behaviors are changed after subjects reached a certain performance. Subjects reach their performance level faster after multiple reversals indicating that subjects do not relearn contingencies every time anew but apply previously acquired patterns. However, this capability is sensitive to several parameters such as stimulus types, reinforcement and schedule. Rewarding reinforcement is used in almost all studies. In this study we employed a comparable multiple reversal task for human subjects and Mongolian gerbils employing auditory discrimination of upward and downward frequency-modulated tones. Human subjects were trained in a two-way alternative force choice task with positive and negative feedback and gerbils in a Go/Nogo discrimination task in a shuttle box with electrical foot shock as negative reinforcement. We found, that humans and gerbils alike reached a specific performance threshold faster after the second contingency reversal compared to the initial learning phase and first reversal. With multiple reversals the gerbils tended to generalize the two different stimuli and developed a shock minimization strategy rather than shock avoidance. Based on these results we propose a hierarchically organized network architecture capturing the shorter time period after a contingency reversal for until previous discrimination performance was reestablished (Jarvers et al. 2016). The network consists of multiple reinforcement learning subsystems which acquire behavioral strategies and a dynamic control network subsystem controlling the strategy selection. The model architecture is conceptually similar to other multiple-expert architectures (Jacobs et al. 1991, Graybiel 1998). However, in our model the dynamic control subsystem requires no additionally external cue but learns from the trace reinforcement only. In conclusion, we show that animal as well as human

subjects reached their performance level faster in an auditory serial reversal task after the second reversal, even gerbils received only negative feedback. Based on the behavioral data we propose a hierarchically organized network model resembling expert model architectures. Unlike previous modeling approaches we suggest that each expert subsystem selects strategies based on the trace of the past reinforcements.

**Disclosures:** **A.L. Schulz:** None. **M.L. Woldeit:** None. **A. Brechmann:** None. **C. Jarvers:** None. **T. Brosch:** None. **M. Lommerzheim:** None. **H. Neumann:** None. **F.W. Ohl:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

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**Program#/Poster#:** 586.22/DD20

**Topic:** D.06. Audition

**Support:** NIDCD Grant DC008854

**Title:** Neural discrimination of novel acoustic signals improves with repeated stimulus presentation

**Authors: *E. SOYMAN**, D. S. VICARIO
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**Abstract:** Organisms encounter numerous novel sensory signals throughout life. Thus, the sensory representations in the adult brain undergo dynamic changes to adapt to the complexity of the external world. One such dynamic encoding mechanism is the process of stimulus-specific adaptation (SSA), in which initially robust auditory responses to novel sounds gradually decrease as a function of stimulus repetition. The current study investigated the effects of SSA on neural recognition and discrimination of novel auditory signals in the songbird auditory forebrain. Songbirds use complex, learned acoustic signals for social communication with many parallels to human speech. Furthermore, the neural responses in an auditory structure in the songbird brain, Caudal Medial Nidopallium (NCM), undergo a long-term form of stimulus-specific adaptation, providing an excellent substrate to probe the neural underpinnings of adaptive sensory representations. Electrophysiological activity in NCM in response to novel songs was recorded bilaterally in awake, restrained zebra finches. A Euclidean distance-based metric was used to compute the dissimilarity between the spike trains in response to different stimuli at corresponding stimulus presentations. Strikingly, the temporal profiles of neural responses to different signals became more and more dissimilar from each other with stimulus repetition, despite decreases in overall firing rates with SSA. To test whether this increasing contrast among different stimuli improved decoding of stimuli from neural responses, a Bayesian decoding procedure was used. As predicted, the accuracy of neural recognition of stimulus identities

improved with stimulus repetition. Furthermore, the neural decoding process reached a specified confidence level for the correct stimulus at earlier points in the stimulus duration profile with repeated stimulus presentation. The improvements in neural decoding performance were also reflected in mutual information estimations. Mutual information, estimated for the first and the second half of the experiment separately, was compared to test the effect of stimulus repetition on the informativeness of neural responses. In line with above findings, mutual information estimations in the second half of the experiment were greater than those in the first half. Taken together, these findings demonstrate that SSA may represent a dynamic encoding process, which not only makes neural representations sparser - using fewer spikes - but also increases the contrast between complex signals for rapid stimulus recognition and discrimination.

**Disclosures: E. Soyman:** None. **D.S. Vicario:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 586.23/DD21

**Topic:** D.06. Audition

**Support:** NIH Gratnt 5R01NS088649-03

**Title:** Corticostriatal plasticity underlying a sensory-motor association in an auditory discrimination task

**Authors: *S. GHOSH**[1], F. CARNEVALE[2], A. M. ZADOR[3]
[1]Watson Sch. of Biol. Sci., [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Cold Spring Harbor Lab., Cold Spg Hbr, NY

**Abstract:** Perceptual decision-making often involves pairing sensory discrimination with appropriate motor actions. The brain circuits involved in such decision-making, and how these circuits change during learning of such associations remain largely unexplained. Previous studies in rats trained on an auditory discriminatory task (the ‘tonecloud’ task) have established a critical role for the connections between auditory cortex and auditory striatum in task performance (Zador and Znamenskiy, 2013). Moreover, learning the tonecloud task results in a specific pattern of plasticity in this pathway, which is determined by the frequency tuning of the neurons projecting to the recording area and the corresponding motor response, e.g., ‘turn left’ or ‘turn right’ (Xiong et al., 2015). We are now investigating the striatal substrates of this plasticity and its effects on striatal circuitry.
The striatum is primarily composed of inhibitory medium spiny neurons (MSNs) that express either D1 or D2 type dopamine receptors and constitute the ‘Direct’ or ‘Indirect’ pathway, respectively. Stimulating D1 neurons facilitate contralateral movements whereas activating D2

neurons inhibits movement. Thus, the balance between these two seemingly antagonistic pathways is considered critical in movement control (Kreitzer and Malenka, 2008). We hypothesize that learning the tonecloud task induces changes in strength of corticostriatal synapses onto a given MSN depending both on its frequency tuning and the pathway it belongs to.
To test our hypothesis, we have adapted the tonecloud task to train mice. This allows us to use genetic markers to assess the learning induced changes in synaptic strength in the D1 vs. D2-MSNs. We have established that the corticostriatal projections in the mice are arranged in a tonotopic manner, as seen in rats. Moreover, training mice on the tonecloud task induces a specific pattern of plasticity that reflects the tone-response association. Also, mice trained on opposite training contingencies faithfully show opposite plasticity patterns. We are now using whole cell patch-clamp method on acute slices prepared from brains of trained transgenic animals (D1/A2A-CRE x Ai14) to measure the synaptic strength of the cortical afferents onto D1 or D2-MSNs. These experiments will help elucidate how the two major striatal pathways are recruited during such task-learning and thereby further our understanding of the role of striatal plasticity underlying arbitrary sensory-motor associations.

**Disclosures: S. Ghosh:** None. **F. Carnevale:** None. **A.M. Zador:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

**Location:** Halls A-C

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**Title:** Encoding of modulation frequencies and depths by surface and micro electrodes for a cochlear nucleus auditory prosthesis

**Authors: *M. HAN**[1], D. B. MCCREERY[2]
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**Abstract:** For persons with deafness who cannot benefit from cochlear implants, some hearing can be restored by an array of stimulating electrodes implanted on the surface of their cochlear nucleus (CN). However, these Auditory Brainstem Implants (ABIs) usually do not restore hearing comparable to that provided by cochlear implants. A clinical trial of a hybrid ABI

containing macroelectrodes on the surface of the CN and microelectrodes that penetrated into the nucleus showed that the surface electrodes best conveyed the loudness of the electrically-encoded sound but poorly conveyed its pitch, while the penetrating microelectrodes best conveyed the encoded sound's pitch, but with only limited range of loudness. In this study, we have chronically implanted arrays of penetrating silicon-based microelectrode arrays into cats' cochlear nucleus. These are three-dimensional, four-shanked devices with five to eight microelectrode sites on each shank. This allows access to the tonotopic organization of the ventral cochlear nucleus while also minimizing the number of shanks penetrating into the brain. The silicon shanks were fabricated using the Bosh-process deep reactive ion etching technique followed by mechanical shaping of the probes' tip region, yielding a mechanically sturdy shank and a sharpened tip to reduce insertion force into the cochlear nucleus (Han, 2012). The cochlear nucleus device also included macroelectrodes that reside on the surface of the nucleus. The neuronal activity induced by modulated electrical stimuli applied though both types of electrodes was recorded by another array of silicon microelectrodes implanted in the central nucleus of the cats' inferior colliculus. Results showed that the temporal modulation of the neuronal activity induced by the modulated stimuli applied in (penetrating) or on the surface of the CN was very similar across a range of modulation frequencies and modulation depths, especially for transient modulation simulating the periodicity of speech. This concordance should greatly facilitate development of an ABI using surface electrodes and penetrating microelectrodes in an integrated manner, in order to convey both the loudness and pitch of sound.

**Disclosures:** **M. Han:** None. **D.B. McCreery:** None.

**Poster**

**586. Auditory System: Cortical Processing and Perception**

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Burroughs Wellcome Fund Career Award (MNG)

**Title:** Robust discrimination of sounds embedded in noise by adapting cortical gain

**Authors: *C. F. ANGELONI**, K. C. WOOD, M. N. GEFFEN
Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Natural acoustic environments are variable; to function in them it is necessary to reliably differentiate important sounds from one another despite the presence of a noisy background. Recent work demonstrated that neurons in auditory cortex modulate their response gain in an adaptive manner to account for changes in the spectrotemporal statistics of acoustic stimuli (Rabinowitz et al., *Neuron*, 2011; Natan et al., *Cereb. Cortex*, 2016). In the current study, we model how such changes in neural gain facilitate robust discrimination of stimuli embedded in different types of noise. Using a linear-nonlinear probabilistic model of neural activity, we simulated neural responses to sounds embedded in noise backgrounds of different contrast levels. Then, using techniques from signal detection theory, we estimate behavioral detection and discrimination from the predicted neural responses and how behavioral performance changes depending on the gain of the neuron and the loudness of the embedded signals relative to the mean noise level (ie. their signal to noise ratio, SNR). We find that detection of a signal embedded in noise is always facilitated by high gain, but does not change appreciably as the neuron adapts to a new noise context. However, discrimination in different noise contrasts depends on the gain state of the neuron when differentiating between targets embedded at different SNR levels. Namely, target stimuli with high SNR are readily discriminable in high contrast noise, but not low contrast, while target stimuli with low SNR are more discriminable in low contrast than high contrast. In this framework, we also observe differences in the time course of discrimination performance, such that during high to low contrast adaptation, discriminability decreased earlier and faster for higher SNR targets, while the opposite effect is seen in low to high contrast. These findings provide a predictive framework in which we can model detection and discrimination tasks in the presence of a noise background, the subsequent neural responses to target stimuli as a function of gain adaptation, and how those neural responses predict behavioral performance.

**Disclosures: C.F. Angeloni:** None. **K.C. Wood:** None. **M.N. Geffen:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.01/DD24

**Topic:** D.06. Audition

**Title:** Sound symbolism - Related brain area relative to language processing

**Authors: *S. ITAGAKI**, S. MURAI, K. I. KOBAYASI
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**Abstract:** Sound symbolism is an idea that sounds itself has certain impression. In most of the previous psychology and linguistics researches, stimuli were presented visually with alphabets, and subjects directly answered the impression of the sound. Our previous study confirmed that sound symbolism does occur even when the sound stimulus was presented aurally. The purpose of this study is to elucidate neural substrate for sound symbolism. In the experiment, we focused on sound symbolism in visual size. Subjects were all right-handed Japanese native speakers, and they did not have knowledge about sound symbolism and this experiment. They were required to answer visual size difference between standard and target stimulus. Visual stimuli were a gray circle on black background LCD screen, and had 1 type of standard and 2 types of target. Target size was either smaller or bigger than the standard by ±40%. Sound stimuli were voice sounds ( /bo/ and /pi/ ) and noise and click sound (control). Voice sounds were assumed to have impression of “bigger” and “smaller” respectively, according to previous researches. The subject performed the task under functional magnetic resonance imaging (fMRI) scanning. Congruent trials were defined as those where circle sizes were correlated with the impression of accompanying sounds, and incongruent trials were with opposite stimulus contingency. Additionally, we defined that sound symbolism does occur if reaction time of incongruent conditions is longer than that of congruent conditions. As a result, reaction times in incongruent condition were longer than congruent condition. According to analysis of MR signal, left triangular part of inferior frontal gyrus and left supramarginal gyrus are more activated in incongruent conditions than congruent conditions, and are a part of Broca’s area and Wernicke's area, respectively. The activation could reflect the functional interaction between the sound symbolism and language. The fMRI imaging results also shows that the location and amount of activity differ between subjects, suggesting the neural substrate of the sound symbolism could vary between individuals. We will discuss relationships between the functional brain differences and individual behavioral differences.

**Disclosures:** **S. Itagaki:** None. **S. Murai:** None. **K.I. Kobayasi:** None.

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**587. Auditory Processing: Perception, Cognition, and Action II**

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**Topic:** D.06. Audition

**Support:** NSF Grant BCS1460633

UC MERCI Exchange

**Title:** Mapping out cortical contributions to auditory timing: A causal transcranial magnetic stimulation study of interval and beat-based timing perception

**Authors: *J. M. ROSS**[1], *J. M. ROSS[1], J. R. IVERSEN[2], R. BALASUBRAMANIAM[1]
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**Abstract:** It has been suggested that networks involved with movement planning play an essential role in timing perception, but specific contributions of premotor, supplementary motor and parietal cortices are unknown. The Action Simulation for Auditory Prediction (ASAP) hypothesis proposes that the dorsal auditory stream is involved in bidirectional interchange between musical beat-based timing prediction in motor planning regions and auditory perception via parietal projections (Patel & Iversen, 2014). Regions that are not part of the dorsal auditory stream have also been implicated for duration-based interval timing perception and auditory-motor synchronization, including supplementary motor cortex and ventral premotor cortex. We used a transcranial magnetic stimulation protocol, continuous theta burst stimulation (cTBS), that is known to down-regulate cortical activity for up to 60 minutes following stimulation, to test for causal contributions to interval timing perception and predictive beat-based timing perception. cTBS target areas included dorsal premotor cortex (dPMC) and posterior parietal cortex (PPC), which are part of the dorsal auditory stream, and supplementary motor area (SMA) and ventral premotor cortex (vPMC). Our data support causal involvement of movement planning networks for timing perception, functionally distinct mechanisms for interval timing perception and beat-based timing perception, and contribute a nuanced understanding of cortical network dynamics for interval and beat-based timing perception.

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**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

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**Topic:** D.06. Audition

**Support:** KAKENHI/16K00380 to R.K.

KAKENHI/17H00740 to Y.K

**Title:** Experimental design for investigating the acoustic preference in rats

**Authors: *K. OKAMOTO**[1], K. FUJIMOTO[1], Y. KOMURA[2], R. KAJIWARA[1]
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**Abstract:** For investigating whether rats have acoustic preference or not, many kinds of maze assay are commonly used and the preference level was assessed by tracking the locomotor activity of the animal in the maze. The aim of this study is to design the behavioral task for

extracting the acoustic preference in rats, and to construct apparatus which enables us to control the instrument for carrying out the task. In our system, modified M-shaped-maze (70cmX40cmX45cm) for alternative selection task was placed in a sound-attenuated box. The center lane with an infrared sensor (Panasonic, FX-501), which can trigger the acoustic stimulation from right and/or left lane, was used for the conditioning experiment. At the left and right side of the chamber, a light emitting diode (LED) and a drinking port as well as a speaker for presenting the sound were fixed. The LED was located 22cm above the floor and centered on the panel. The drinking port was fixed 20cm above the floor and also centered on the panel. Sucrose solution was delivered to the rat from the drinking port, which was connected to a plastic bottle through the solenoid valve (Takasago, JTV-2). The LED was also mounted on the panel at the end of center lane. To analyze the activity of the rat, three ceiling mounted digital video camera (logicool,C270) were utilized. The whole system was controlled by original software developed in our laboratory on the LabVIEW platform. The software running on the Windows10 machine communicated with all the equipment via the USB ports. The NI-DAQ Device (National Instruments co.) was also used as an interface controlling the LEDs, solenoid valves, and passing sensor. The video tracking system enabled behavior to be studied in a reliable and consistent way, and over longer time periods than if they are manually recorded. The system received three video signals simultaneously in parallel for the data analysis. To recognize the position of the rat, we defined all connecting pixels that were brighter than a threshold value as the animal, and all other pixels as the background. In addition, the software enabled multiple zones to be defined for each experiment. We divided the chamber into five zones. Also, the software changed the volume of sounds according to each zone where rat stayed in and measure the time spent in right and left zones in the chamber. As for the acoustic stimuli delivered to rats, we used white noise, famous classical music, and arranged-music which are considered to be audible for rats by using CUBASE software. By using such an experimental apparatus, we assessed rats' preferences for two contrasting pieces of acoustic stimuli.

**Disclosures:** **K. Okamoto:** None. **K. Fujimoto:** None. **Y. Komura:** None. **R. Kajiwara:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.04/DD27

**Topic:** D.06. Audition

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FCT PhD Fellowship

Champalimaud Foundation

**Title:** The cost of control in perceptual decision-making

**Authors: *J. R. CASTIÑEIRAS**, J. L. PARDO-VÁZQUEZ, M. VALENTE, A. RENART
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**Abstract:** We consider the optimal strategy in a classic perceptual decision paradigm: the binary categorization of a noisy sensory stream. Reinforcement Learning (RL) can specify the best course of action (optimal policy) that an agent should follow, given certain contingencies, to maximize long-term reward. However, agents in RL are conceived as a *tabula rasa* without any existing behaviors or response tendencies that may interfere with the optimal policy. In the field of optimal control theory, on the other hand, optimal control laws can steer the existing dynamics of an agent in a way that is compatible with her current goals, but considering the cost of control in the optimization problem. We have generalized ideas from Optimal Control (Todorov, PNAS, 2009) to describe situations where an agent is uncertain about the state of the environment ('partial observability'), and applied this theory to construct a normative model of perceptual decision-making in control-limited agents. The goal of these agents is to maximize long-term reward subject to a control cost that penalizes deviations from a default tendency to make random choices at a particular rate. As in previous studies that did not consider the cost of control, the agent needs to perform Bayesian inference on upcoming observations to update her beliefs about the environment. However, we show that the cost of control changes how these beliefs about the sensory world are linked to action. Using latent-variable inference, we solve for the joint probability that the agent will make a particular choice, at a particular time, with a certain belief (decision confidence) and derive testable predictions specifying the phenomenology of how accuracy, reaction-time and decision-confidence depend on each other for different control costs. When control is cheap, one recovers the standard RL solution to the task (Rao, Front. Comp. Neuro., 2010; Drugowitsch et al., J. Neurosci., 2012) which relies on a hard, time-varying bound on belief. However, we identify a novel, high-control-cost regime, in which agents develop stochastic policies, and show that this stochasticity smooths the relationship between pre-choice beliefs and decision confidence. As a result, varying the cost of control, one recovers behavioral phenotypes that interpolate between the predictions of Signal Detection Theory and Sequential Sampling Theory.

**Disclosures:** **J.R. Castiñeiras:** None. **J.L. Pardo-Vázquez:** None. **M. Valente:** None. **A. Renart:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

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Human Frontier Science Program (HFSP) Young Investigator Grant RGY0089

FCT PHD Fellowship

Champalimaud Foundation

**Title:** Contribution and interaction of auditory and posterior parietal cortex during an auditory delayed response task in head-fixed mice

**Authors: *R. STEINFELD**, A. TACÃO-MONTEIRO, A. RENART
Champalimaud Res., Champalimaud Fndn., Lisboa, Portugal

**Abstract:** The ability to internally manipulate or maintain information during brief periods of time is critical for goal-directed behaviour, for instance, in order to associate actions and their delayed consequences, or to plan future actions. Internally generated activity carrying information about recent sensory input during a delay period is thought to be important for this function. We have developed a delayed-response auditory frequency discrimination task for head-fixed mice to study the contribution of different cortical areas to this behaviour. Animals are presented with either of two pure frequency sounds indicating at which of two constantly available spouts water will be available. The mice are required to delay their response until presentation of a visual go cue, presented 400-700ms after sound offset. Premature licking will abort the trial. Performances typically average above 80% across a session. The mice behaviour is almost exclusively explained by the identity of the sound cue on each trial, although, using generalized linear models, it is possible to show that mice have a small tendency (< 5% of variance in the model output explained by the previous-trial predictor) to repeat their previous choice regardless of outcome. Performance is robust against presentation of loud, white noise sound distractors during the delay period. Optogenetic silencing (bilateral hSyn-Jaws activation by LED implants) during different epochs of the task revealed involvement of auditory cortex (ACx) during the sound cue presentation (10-15% reduction in performance; 5% increase in abort rate; stimulation during delay did produce similar effects on abort rate, but did not alter performance). We are currently studying the contribution and interaction between the ACx and the posterior parietal cortex (PPC) during performance of this task. Preliminary experiments

show that it is possible to simultaneously record large populations of neurons (50-80 simultaneous units in each area) with two 64-channel silicon probes in ACx and PPC. We will describe the activity of these two reciprocally connected cortical networks across layers in awake mice during a delayed response task.

**Disclosures: R. Steinfeld:** None. **A. Tacão-Monteiro:** None. **A. Renart:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

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Human Frontier Science Program (HFSP) Young Investigator Grant RGY0089

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FCT doctoral fellowship

Champalimaud Foundation

**Title:** Effect of stimulus intensity versus stimulus identity in perceptual decision-making

**Authors: *A. RENART**, M. VALENTE, J. CASTIÑEIRAS, J. PARDO-VAZQUEZ
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**Abstract:** In most binary-choice perceptual decision-making experiments, subjects need to categorize the value of an unobserved latent variable on the basis of noisy observations. The difficulty of the task can either be controlled manipulating, at fixed intensity, stimulus identity (the distance between the latent variable and decision threshold), or by varying the overall intensity (energy) of the stimulus. To characterize the role of stimulus intensity and stimulus identity, we have developed a binary choice sound lateralization task for rats. The stimulus (delivered through custom-made detachable ultrasonic headphones) is a white-noise burst to each ear. The task of the animal is to report (reaction time - RT - configuration) the lateralization (sign of the inter-aural level difference - ILD: difference in dB SPL of each speaker) of the stimulus by poking in the corresponding lateral port, and the overall stimulus intensity (absolute binaural level - ABL: mean dB SPL of the two stimuli) is fixed at 20, 40 or 60 dB SPL in blocks of 80 trials. After taking rats to psychophysical threshold for ILD discrimination, accuracy in this task is a measure of perceptual sensitivity, in the sense that non-sensory factors don’t contribute

significantly to performance. Manipulations of motivation (either directly by increasing reward magnitude, or indirectly by restricting stimuli to the ILDs closest to the decision threshold) do not lead to increases in performance. Furthermore, generalized-linear modeling shows that the outcome of previous trials plays no role in explaining the outcome of a current trial. There is a speed-accuracy tradeoff as a function of ILD in the task. As expected, animals take longer to decide when the stimulus is close to the decision threshold. Accuracy (psychometric curve slope, or discriminability) does not change for the three ABLs we tested but, surprisingly, animals take longer to decide when intensity is lower. Thus, whereas increasing difficulty through either manipulating intensity or identity both lead to longer RTs, rats can use the extra time to achieve the same level of performance when the stimulus is fainter, but not when it is more close to decision threshold. Remarkably, this differential effect of identity and intensity is also observed at the level of the shape of the RT distributions: lowering ABL leads to a re-scaling of time by the same factor for each of the different ILDs, whereas changing ILD does not lead to temporal-rescaling. We hypothesize that this phenomenology results from the fact that intensity and identity in our task limit performance mainly through external and internal sources of noise respectively.

**Disclosures:** **A. Renart:** None. **M. Valente:** None. **J. Castiñeiras:** None. **J. Pardo-Vazquez:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

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Human Frontier Science Program (HFSP) Young Investigator Grant RGY0089

FCT PhD Fellowship

Champalimaud Foundation

**Title:** Neural activity in the mouse mPFC during a memory-guided spatial task on a treadmill

**Authors: *J. AFONSO**[1], A. TACÃO-MONTEIRO[1], C. GOLDEN[2], S. ROYER[3], P. T. CHADDERTON[2], A. RENART[1]
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Kingdom; [3]Ctr. For Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

**Abstract:** Voluntary behavior is based on the models animals have of what their bodies can do, their environment, the consequences of their actions, and how these relate to their current goals. These models are learned, and give rise to richer behaviors the more flexible and abstract they become. A key factor for this is to be able to break free from the 'immediacy of the present', which in principle permits associations only between spatially and temporally contiguous events. The ability to create overlap between the representations of spatially and temporally distal events, such as causes and effects, or intentions and actions, endows animals with the possibility of establishing more complex associations and plan ahead. The medial Prefrontal Cortex (mPFC) has traditionally been implicated in combining sensory information and contextual rules to generate and maintain active representations used to orchestrate behavior. Here we present a novel head-fixed spatial memory-guided behavioral task developed to explore how populations of neurons in the mouse mPFC guide behavior based on a transient environmental input that is no longer immediately available. In the task, head-fixed mice running on a treadmill with a long belt have to make a binary decision (stop or not stop) upon reaching an area (decision area - DA) with a different texture. The decision is made based on the identity (5 or 12 kHz) of a transient auditory cue, presented a variable distance before the DA. Through behavioral manipulations and data-analysis of the mice speed trajectories in the treadmill, we confirmed that (a) correct performance is dependent on the existence of an actual DA (and thus reflects a decision made at the DA on the basis of information no longer present in the environment, as opposed to being the result of a stereotyped movement trajectory specified at sound onset), (b) performance is flexible, in the sense of being equally good at several randomly presented delay distances, and (c) appears to be based on a representation of the no-longer-present sound, rather than on the speed of the mouse when he reaches the DA. These together point to the existence of an active control mechanism involved in guiding behavior during the delay period, in opposition to an automatic habitual type of strategy. Preliminary recordings using 64 channels silicone probes reveal the existence of stimuli and context responding neurons with varied temporal spiking profiles across the trials.

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**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.08/DD31

**Topic:** D.06. Audition

**Title:** Representations of amplitude modulations in auditory onsets, ramp tones, and speech in the human superior temporal gyrus

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**Abstract:** Making sense of complex auditory inputs requires temporally precise parsing of single events out of the auditory stream. Auditory event onsets are typically marked by an increase in amplitude, and previous animal and human studies identified the dynamics of amplitude rise at onset as a central feature encoded throughout the subcortical auditory pathway. Amplitude modulations, however, also mark changes within ongoing sounds, e.g. in the speech envelope. Yet, it is unknown how the human auditory cortex differentiates between amplitude rises in silence and within an ongoing sound, and whether onset encoding can account for tracking of the speech amplitude envelope. To address this, we designed tone stimuli containing amplitude ramps (.75 sec duration), rising from silence (*RfS* condition), or from an amplitude baseline (*RfB* condition, base amplitude -12dB re peak amplitude). The sound intensity of ramps increased linearly to peak amplitude and returned to silence/baseline, with a parametric modulation of the rate of amplitude change. Crucially, fast-rising ramps in the RfB condition are perceived as onsets, whereas slow-rising ramps are perceived as modulations of the ongoing sound. We recorded local field potentials using intracranial multi-electrode arrays placed over the temporal lobes of five patients undergoing evaluation for epilepsy neurosurgery, as they passively listened to the tones. In both conditions, ramps elicited transient responses in the high gamma frequency range (HG, 70-150 Hz) in posterior (p) and middle (m) superior temporal gyrus (STG), with larger HG amplitudes for fast-rising ramps. We observed a striking double dissociation of response types: pSTG encoded the rate of amplitude change in the *RfS* condition only, whereas mSTG encoded the rate of amplitude change in the *RfB* condition only. Crucially, an analysis of HG responses to continuous speech showed that the rate of amplitude envelope modulation in continuous speech was also represented in mSTG, but not in pSTG. Our results reveal functionally and spatially distinct representations of sound onsets in silence and in background along STG. Moreover, our data suggest that speech amplitude tracking in mSTG may rely on the same neural mechanisms as the encoding of onsets in background.

**Disclosures: Y. Oganian:** None. **E.F. Chang:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.09/DD32

**Topic:** D.06. Audition

**Title:** Neural correlates of single phoneme versus rapid auditory processing in adults with and without dyslexia

**Authors: *T. M. CENTANNI**[1,2], S. D. BEACH[1,3], O. OZERNOV-PALCHIK[1,4], S. MAY[1], J. D. E. GABRIELI[1]
[1]MIT, Cambridge, MA; [2]Psychology, Texas Christian Univ., Fort Worth, TX; [3]Harvard Univ., Boston, MA; [4]Tufts Univ., Boston, MA

**Abstract:** In dyslexia, the double deficit hypothesis suggests that children who struggle to acquire reading exhibit deficits in phoneme processing, rapid automatized naming, or both. Recent work in rodent models suggests that these two deficits may be caused by different dyslexia susceptibility genes and may manifest in unique patterns of brain activation. In children who struggle to learn to read, poor reliability in the brain's response to sound has been verified in at least three independent studies, although not all children with dyslexia exhibit this feature. In the current study, we designed two tasks to differentiate between individuals with dyslexia who have increased neural variability to sound versus those who have difficulty processing rapid auditory stimuli. One task consisted of a ten-step consonant continuum between the sounds /b/ and /d/, with participants pressing one of two buttons to categorize each stimulus. The second task presented three sets of sounds at 4 different speeds, with participants making sense/nonsense judgements; sentences, strings of consonant-vowel-consonant sounds, and amplitude modulated white noise. Adults with and without dyslexia were assessed and then tested on both tasks twice; once in an initial behavioral session and again while magnetoencephalography (MEG) data were acquired. Tasks were administered twice both to measure consistency of the task as well as to validate performance while in the scanner. We present preliminary behavioral and brain data from these participants on the proportion of individuals with dyslexia exhibiting each of the two deficits described above, and the respective neural correlates.

**Disclosures: T.M. Centanni:** None. **S.D. Beach:** None. **O. Ozernov-Palchik:** None. **S. May:** None. **J.D.E. Gabrieli:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

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**Topic:** D.06. Audition

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**Title:** Dissociation of knowledge and performance during sensorimotor learning

**Authors: *K. KUCHIBHOTLA**[1], T. A. HINDMARSH STEN[1], E. PAPADOYANNIS[1], S. OSTOJIC[2], R. C. FROEMKE[3]
[1]Skirball Inst., New York Univ. Sch. of Med., New York, NY; [2]Ecole Normale Superieure, Paris, France; [3]Otolaryngology, NYU Med., New York, NY

**Abstract:** In animal behavioral experiments, we are constrained by the degree to which subjects can report what they know. Factors such as motivation and arousal weigh on behavioral decisions and may impinge on the ability to self-report underlying knowledge. This becomes particularly challenging when tracking behavioral performance and neural dynamics during learning. Is there evidence that animals know more at earlier stages of task learning than their performances suggests? To address this question, we trained awake, head-fixed mice on an auditory stimulus recognition task in which mice lick for a water reward in response to one tone (target, 8 kHz) and withhold licking to avoid a short time-out in response to a different tone (foil, 5 kHz). In every session, we introduced probe trials ("knowledge context", by removing the licktube for 20-40 trials) during predominantly active training ("testing context", presence of licktube, 200-300 trials). Surprisingly, in the knowledge context, mice correctly licked to the reward-linked tone and withheld from licking to the other tone much earlier in training (1700-2000 trials to expert, n=6) than in the testing context (3000-8000 trials to expert, n=6). Importantly, the individual learning trajectories in the knowledge context were far more stereotyped across animals than in the testing context. Thus, the striking differences in learning speed across animals does not arise from differences in baseline learning rates, but rather, due to other factors including internal state and behavioral strategies.
To understand how the two coexisting learning trajectories might arise, we implemented a neurally-inspired behavioral model subject to reward-prediction error (RPE) learning (Bathelier et al., 2013). The learning model consists of a decision unit that linearly sums feed-forward inhibition and excitatory drive from two sensory units, the target and foil tones, whose weights update based on positive and negative reward expectation error. A scaling parameter applied to feed-forward inhibition recapitulated the two context-dependent learning trajectories while scaling of excitatory drive, decision noise, or RPE learning rate parameters were insufficient. Our model therefore points to inhibition as a gate for latent knowledge suggesting that contextual factors may influence behavioral performance during learning via inhibitory networks (Kuchibhotla et al., 2017). Overall, behavioral dissociation between performance in knowledge versus testing contexts suggests that learning trajectories of individual animals may depend on a combination of RPE-based learning and contextual modulations.

**Disclosures: K. Kuchibhotla:** None. **T.A. Hindmarsh Sten:** None. **E. Papadoyannis:** None. **S. Ostojic:** None. **R.C. Froemke:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

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NSF GRFP

ARCS Foundation

**Title:** Feature selective attention shifts noise correlations based on both target and distractor tuning in A1

**Authors: *J. D. DOWNER**[1], K. N. O'CONNOR[2], M. L. SUTTER[3]
[2]Ctr. for Neurosci., [1]UC Davis, Davis, CA; [3]Univ. of California Davis, Davis, CA

**Abstract:** Feature selective attention (FSA) is a critical component of real-world listening. FSA mediates competition between simultaneous, mixed sound features to prioritize the processing of relevant features over irrelevant features. Though much recent work highlights the impact of task engagement on neural activity in primary auditory cortex (A1), how A1 supports FSA remains an open question. To study the effects of FSA on population-based neural coding, we measured noise correlations ($r_{noise}$) within small groups of A1 neurons while rhesus macaques performed a FSA task. Previously, most studies have shown that attention globally reduces $r_{noise}$ in sensory cortex, though recent evidence has shown that attention can shift $r_{noise}$ up or down depending on population tuning to the attended feature. We found that the effect of FSA on $r_{noise}$ depended not only on the population tuning to the relevant (attended) feature, but also on the tuning to the irrelevant (distractor) feature. These results suggest an efficient mechanism by which FSA reshapes high-dimensional population response distributions to simultaneously enhance the representation of relevant features while suppressing the representation of irrelevant features.

**Disclosures: J.D. Downer:** None. **K.N. O'Connor:** None. **M.L. Sutter:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

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**Topic:** D.06. Audition

**Support:** SFI 15/CDA/3316

**Title:** Improved attentional decoding at a cocktail party for audiovisual speech

**Authors: *A. E. O'SULLIVAN**[1,2], E. C. LALOR[2,1,4,3]
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**Abstract:** The brain's ability to selectively isolate and enhance a single speech stream from multiple competing sources allows us to successfully communicate in everyday life. In recent years, a great deal of progress has been made towards understanding this complex process. This has, for the most part, been provided through the use of 'decoders' which transform the recorded neural activity into an estimate of the heard acoustic envelope (Mesgarani and Chang 2012, Ding and Simon 2012). Crucially, these methods allow us to examine the neural dynamics of attention using natural, continuous speech. However, research on this topic to date has been constrained somewhat by the fact that it focuses purely on auditory speech. And we know from our everyday experience that listeners will often look at the talker's face in an effort to aid comprehension - especially in a noisy environment. Indeed, recent work on audiovisual speech has quantified this benefit and shown it is present for speech in quiet (Crosse et al., 2015), and even more so in the presence of background noise (Crosse et al., 2016). Importantly however, listeners will also often switch their attention to a different audio source (eavesdropping), while continuing to look at the face of their conversational partner. Understanding how attention operates under both of these conditions is central to advancing our knowledge on the interactions between top-down attentional selection and the bottom-up visual influence on speech processing. Here, we present subjects with congruent audiovisual (AV) speech in the presence of a competing audio (A) stream. Subjects are required to attend to one speech stream (condition 1: attend A; condition 2: attend AV) while ignoring the other. Our results show that we can better decode attentional selection to AV speech than A speech, and that this improvement is mainly driven by additional contributions from occipital regions. Furthermore, in decoding A speech in the presence of distracting AV speech, we find that the activity in these occipital regions is heavily down weighted. Combining indices of both audio and visual speech processing provides a more robust attention decoder which can successfully operate under both of these conditions. We also examine the underlying temporal dynamics of the interaction between visual speech and audio speech processing, and the extent to which this is influenced by attention. This work may lead to important neuroscientific insights on the interaction between attention and multisensory integration and aid progress towards the goal of an attentionally steered hearing aid.

**Disclosures:** **A.E. O'Sullivan:** None. **E.C. Lalor:** None.

**Poster**

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**Program#/Poster#:** 587.13/DD36

**Topic:** D.06. Audition

**Support:** Uehara Memorial Foundation

**Title:** Dissociation of stimulus and outcome expectations in perceptual decision making

**Authors: *A. FUNAMIZU**[1], F. MARBACH[1,2], A. M. ZADOR[1]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Watson Sch. of Biol. Sci., Cold Spring Harbor, NY

**Abstract:** In perceptual decision making, humans and animals use the prior knowledge of stimulus probability and reward outcome to optimize their behavior. Although a series of studies have shown the neural substrate of outcome expectation in cortico-basal ganglia circuit, the neural substrate of stimulus expectation is unclear. To dissociate the two neural circuits, we are training head-fixed mice on an auditory frequency discrimination task (based on Marbach and Zador, bioRxiv 2016), in which either (i) the stimulus probability or (ii) the reward size for category A and category B trials changes in blocks, and analyze the choice behavior with a belief-updating reinforcement learning model.
We found that both the stimulus probability and reward amount biased mice toward choices associated with high-probability stimuli or large reward, respectively. In the stimulus probability task, a belief updating model, which recursively updated the probability of stimulus category, predicted the biased behavior better than a reinforcement learning model, which updated the expected outcome of each category. In contrast, in the reward amount task, the reinforcement learning model predicted the behavior better than the belief updating model. This suggests that mice updated their stimulus expectation in the stimulus probability task, while they updated their outcome expectation in the reward amount task. We expect that our paradigm combined with two-photon calcium imaging will help dissociate the neural circuits for stimulus and outcome expectations in the auditory cortex.

**Disclosures: A. Funamizu:** None. **F. Marbach:** None. **A.M. Zador:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

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Hoover Fund

**Title:** Cortical processing of spectrally degraded speech revealed by intracranial electrophysiology

**Authors: *M. STEINSCHNEIDER**[1], K. V. NOURSKI[2], A. E. RHONE[3], H. KAWASAKI[4], M. A. HOWARD, III[5]
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**Abstract:** There is considerable variability in speech perception outcomes following cochlear implantation. Auditory cortical function and plasticity are hypothesized to account for much of this variability. Understanding the cortical processing of spectrally degraded speech can help define the roles of different auditory cortical areas in speech perception and contribute to improvements in CI design. A noise vocoder serves as an acoustic model of CI sound processing, allowing for investigation of responses to spectrally degraded speech in normal-hearing listeners. This study investigated electrocorticographic (ECoG) responses within auditory cortex elicited by noise-vocoded speech stimuli and related these responses to behavioral performance in a 2-alternative forced choice (2AFC) identification task. Subjects were neurosurgical patients undergoing chronic invasive monitoring for medically refractory epilepsy. Stimuli were utterances /aba/ and /ada/, spoken by a male talker. The stimuli were spectrally degraded using a noise vocoder (1-4 bands). ECoG data were recorded during performance of the 2AFC task and were obtained simultaneously from Heschl's gyrus (HG) and superior temporal gyrus (STG) using multicontact depth and subdural grid electrodes, respectively. Event-related band power was examined within the high gamma (70-150 Hz) frequency range. All subjects performed at chance-level identification accuracy with speech degraded to 1 or 2 spectral bands, and performed at or near ceiling with non-degraded stimuli. Subjects exhibited a wide variability in accuracy at 3- and 4-band conditions. High gamma responses in posteromedial HG (auditory

core cortex) were similar in magnitude for vocoded and natural stimuli and did not reflect behavioral performance. In contrast, intelligible stimuli elicited larger and broader patterns of high gamma activity on lateral STG. Responses from non-core cortex on anterolateral HG were generally weaker and often exhibited selectivity for natural stimuli. Findings highlight differences in representation of spectrally degraded speech across core and non-core cortical areas. These findings support earlier non-invasive results that demonstrate the relationship between robust non-core auditory responses and speech intelligibility. By modeling the patterns of cortical activity elicited by CI stimulation, intracranial data offer the opportunity to clarify cortical processing of degraded speech and, in future studies, the improvements that occur in CI users with rehabilitation and experience.

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**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.15/EE2

**Topic:** D.06. Audition

**Title:** Neural substrates of behavioral performance in a speech-in-noise task

**Authors: *I. CHOI**[1], S. KIM[1], A. T. SCHWALJE[2]
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**Abstract:** Even young normal hearing listeners show great variance in their ability to understand speech with interfering background noise. The main aim of this study was to investigate the neural correlates of individual differences in speech-in-noise recognition. Cortical models of speech processing involves left-hemisphere inferior frontal gyrus (IFG). However, it is unclear how the IFG activity contributes to the behavioral variance in speech-in-noise performance. We tested primary auditory cortex and IFG activity in twenty-four normal hearing listeners during a 4-AFC monosyllabic word-in-noise understanding task using cortical surface-constrained EEG source analysis. Our results showed better encoding of speech-acoustic features in high performers as reflected in stronger and earlier responses in the auditory cortex than those of low performers. High performance was also related to strong left IFG activity, while low performance was linked with extended right IFG activity. This finding demonstrates that both auditory and bilateral prefrontal cortices are recruited during speech processing while differential lateralization of IFG activity is correlated with behavioral performance. Prolonged IFG activity may also indicate exertion of more listening effort by low performers in noise.

**Disclosures:** **I. Choi:** None. **S. Kim:** None. **A.T. Schwalje:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.16/EE3

**Topic:** D.06. Audition

**Support:** JSPS 15K12069

**Title:** A new behavioral paradigm for investigating auditory perception of untrained animals: Application of novel object recognition procedure to hearing research

**Authors: *Y. TAMAI**[1], T. NOGUCHI[2], S. HIRYU[3], K. I. KOBAYASI[2]
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**Abstract:** Novel object recognition procedure (NOR) is widely used in rodent research because the method depends on animal's natural innate preference for novelty and does not require any training. The purpose of our study was to develop a new version of NOR which can investigate auditory modality. Mongolian gerbils (*Meriones unguiculatus*), one of the most common model animals in auditory physiology, were used to demonstrate the novel sound-object recognition procedure. Six kinds of noise burst trains were used as sound stimuli. Durations of each noise bursts were 125, 250, 500, 275, 375 and 1500 ms, and duty-cycle of each train was 50 %. These sounds were classified into two groups: familiar sounds (noise bursts of 250 and 500 ms) or novel sounds (noise bursts of 125, 275, 375 and 1500 ms). Before behavioral experiment, all subjects were habituated with an empty experimental box. After the habituation, each animal was familiarized with two different objects (rectangular and four-sided pyramidal object) placed inside the experimental box. Repetitive noise bursts (familiar sounds) were presented from both objects. One was noise bursts of 250 ms, the other was that of 500 ms. Animals behavior were recorded by video camera. The contact duration with each object was quantified as exploration. After subjects were familiarized with both objects and sounds, familiar sound (repetitive noise burst of 250 ms) was changed to novel sound (repetitive noise bursts of 125, 275 or 375 ms). As results, the exploration time significantly increased when we changed the duration of repetitive noise burst from 250 to both 125 and 375 ms but it did not when we changed that from 250 to 275 ms. In subsequent experiments, ibotenic acid (10 µg/1 µl), a neurotoxin for selectively destroying cell bodies, was injected into auditory cortex. Subject with the lesion did not show tendency to spend more time exploring novel sound-object (repetitive noise bursts of 375 ms or 1500 ms). However, changing one object to new object (i.e., different shape object) encourage subject to explore the new object. These results suggest that novel sound-object recognition

procedure is suitable behavioral experimental paradigm which can investigate auditory modality including the function of auditory cortex.

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**Location:** Halls A-C

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**Program#/Poster#:** 587.17/EE4

**Topic:** D.06. Audition

**Title:** Real-time tracking of the selective auditory attention from M/EEG via Bayesian filtering

**Authors: *S. MIRAN**[1], S. AKRAM[3], J. Z. SIMON[2], T. ZHANG[3], B. BABADI[1]
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**Abstract:** The ability to rapidly switch attention across multiple speakers in a competing-speaker environment is one of the hallmarks of human auditory processing. The neural mechanisms underlying this phenomenon, a dynamic generalization of the classic cocktail party problem, are for the most part unknown. In the past few years, there have been successful attempts at decoding the attentional state of a listener in a competing-speaker environment using non-invasive neuroimaging data including magnetoencephalography (MEG) and electroencephalography (EEG). To this end, most existing approaches compute correlation-based measures by either regressing the features of each speech stream to the M/EEG channels (the decoding approach) or vice versa (the encoding approach). In the latter case, the regression coefficients for each speech stream are referred to as a Temporal Response Function (TRF). These procedures operate in an offline fashion, i.e., require the entire duration of the experiment and multiple trials to provide robust results. Therefore, they cannot be used in emerging applications such as smart hearing aid devices, where a single trial must be used in real-time to decode the attention state.
Recent results using MEG suggest that the prominent negative peak observed in the TRF with latency ~100 ms, the M100 component, is modulated by attentional state. Therefore, real-time tracking of the M100 component of each TRF in a multiple-speaker environment can yield a robust neural correlate of the selective auditory attention. In this study, we employ this finding in a Bayesian filtering framework in order to construct real-time algorithms for tracking the auditory attention from M/EEG data. We utilize a dynamic TRF estimation procedure to track the M100 component of each TRF. We account for the underlying dynamics of selective attention as well as the TRF components by state-space models. In order to infer the model parameters in real-time, we apply an Expectation-Maximization (EM) procedure together with a fixed-lag adaptive filter. We evaluate the performance of our proposed algorithm on

experimentally recorded M/EEG data. Our results reveal that the proposed real-time algorithm using single trial data performs nearly as accurately as existing offline techniques requiring multiple trials, while providing a significant degree of adaptivity and decrease in computational costs.

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**Poster**

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**Topic:** D.06. Audition

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**Title:** Machine-learning classification of speech-evoked electroencephalographic signals reveals speech intelligibility

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**Abstract:** An important issue of hearing science is individual differences in the central processing for speech understanding. Although previous studies uncovered general cortical pathway for speech perception that involves the interplay between temporal, parietal and frontal lobes, how to quantify differential cortical processing among individual listeners is unclear. Here, we propose to use a machine learning-based classifier of speech-evoked electroencephalographic (EEG) signals to quantify fidelity of cortical speech processing. We hypothesize that important time-varying characteristics of acoustic and semantic features are extracted more accurately in listeners with better speech understanding and more strongly reflected in their cortical neural representation, which results in more accurate classification of time-sensitive EEG signals. To prove this concept, we compared the accuracy of EEG-signal classifier when it classified natural sentence-evoked potentials and vocoded sentence-evoked potentials. Given that a vocoder degrades speech intelligibility, we expected worse accuracy of EEG-signal classification when it is applied to the vocoded-sentence driven cortical signals. We recorded 64-channel EEG signals from seven young normal hearing adults in response to ten natural sentences and their corresponding vocoded sentences in a passive listening condition. We used an eight frequency-band vocoder with continuous interleaved sampling strategy that mimics cochlear implant signal processing. Each sentence was repeated hundred times. One hundred random draws of ten trials (epochs) were used to compute event-related spectral perturbations

(ERSPs). We divided ERSPs into five frequency ranges and four different time windows (40, 50, 100, and 200 ms), which produced 105-550 features (i.e. time-frequency bins) per epoch. We reduced the feature dimensionality using minimal redundancy maximal criterion. An artificial neural network with one hidden layer was trained with 80% of ERSPs. The other 20% of ERSPs were used for the cross-validation of classification accuracy. Results confirmed our main hypothesis; classification accuracy was higher for natural sentence-driven EEG responses than vocoded sound-driven ones at frontal, central, and parietal electrodes in six out of seven subjects. This result demonstrates that a machine learning-based EEG classification can quantify the fidelity of speech comprehension. Now we are investigating whether this classification method is sensitive enough to reveal individual differences in normal hearing and hearing-impaired listeners' speech understanding.

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**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

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**Program#/Poster#:** 587.19/EE6

**Topic:** D.06. Audition

**Title:** Differential phoneme confusion patterns linked with performance in a speech-in-noise task

**Authors: *A. T. SCHWALJE**[1], S. KIM[2], I. CHOI[2]
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**Abstract:** There is significant variability in the performance of normal hearing listeners in complex listening situations such as speech in noise. The purpose of this study is to investigate phoneme-level effects on behavioral word confusions, along with neural correlates of these confusions, on high and low performers in a speech in noise task. Recently, a method of analyzing EEG signals that are time-locked to individual phoneme onsets (phoneme-related-potential, PRP) was described, and differences in PRPs were seen for different manners of articulation of consonants. In the current study, thirty normal hearing adult subjects completed a 4 AFC consonant-vowel-consonant word-in-noise recognition task while 64-channel scalp EEG recordings were obtained. Preliminary analysis of behavioral phoneme confusions based on manner of articulation of consonants revealed significant differences between high and low performers, with low performers having significantly more plosive-to-affricate and fricative-to-fricative confusions in the low SNR condition, and more fricative-to-affricate and fricative-to-fricative confusions in the high SNR condition. A computational model of peripheral speech-acoustics encoding, phonological neighborhood density, and cortical surface-constrained EEG source analysis were used to predict the behavioral phoneme confusions.

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.20/EE7

**Topic:** D.06. Audition

**Title:** Perceptual manifestations of auditory modulation during speech planning

**Authors: Y. MERRIKHI**[1], R. EBRAHIMPOUR[1], *A. DALIRI[2]
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**Abstract:** Previous studies have shown that auditory system's response to auditory stimuli is modulated (suppressed) during speech movement production in comparison to its response to identical stimuli during rest. In addition, in a series of electrophysiological studies, we have shown that the auditory system's response to stimuli is already suppressed during speech movement planning, well before movement production. Here, we examined the perceptual consequences of the suppression of auditory system during planning using a psychophysical paradigm. The study consisted of three conditions: Speaking, Listening, and Reading. In all three conditions, each trial started with a presentation of a plus sign in white characters. During the presentation of the plus sign, a standard auditory stimulus was delivered (75 dB SPL). Then, a target word in white color was presented. During the presentation of the word, a comparison auditory stimulus with varying intensity was delivered (70-80 dB SPL). Next, the color of the word changed from white to green. In the speaking condition, participants were instructed to produce the target words aloud, immediately after the word changed to green color. In the listening condition, participants listened to a self-produced version of the word, which was played immediately after the word changed to green color. In the reading condition, participants were instructed to silently read the target word. After the completion of the trial, participants were asked whether the comparison stimulus was louder or softer than the standard stimulus.Proportion of correct responses were calculated for each comparison level, condition, and participant. Psychometric functions were fitted to individual data in each condition. Using the fitted psychometric functions, we calculated point of subjective equality (PSE) at 50% correct response, and just-noticeable difference (JND) at 75% correct response. Our results showed that PSE and JND were higher in the speaking condition as compared to those in the listening and the silent reading conditions. These results suggested that one consequence of the suppression of auditory system during planning is an increase in the perceptual threshold when comparing the loudness of two auditory stimuli. Overall, consistent with previous

electrophysiological results, we provided behavioral evidence for modulation of auditory system during speech planning.

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**Topic:** D.06. Audition

**Support:** NSF IOS 1453084

**Title:** Auditory processing of mate choice cues in the female songbird

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**Abstract:** Decision making consists of evaluating sensory signals and using that information to direct motor responses. The first step in this process is identifying and evaluating sensory signals, however, it remains unknown how the brain assigns value to some stimuli but not others. In many songbird species, including the Bengalese finches (*Lonchura striata domestica*) studied here, females recognize individual males by their songs and evaluate the features and quality of those songs to choose one mate from many suitors. A key experimental advantage is that song is a unimodal stimulus that is so effective in driving female preference that females will perform behavioral indicators of mate choice (i.e. copulation solicitation displays and calls) in response to song even if no male is physically present. Studies of female responses to song have implicated auditory cortical regions such as the caudal mesopallium (CM) in the expression of mate preferences. Here we electrolytically lesioned CM bilaterally to investigate: 1) whether CM plays a role in preference for conspecific over heterospecific songs, and 2) whether CM plays a role in higher-resolution preference for one conspecific song over others. Preliminary results confirm a previous report from another species that CM is important for species discrimination. Specifically, using calls as a measure of female preference, we have found that lesioning CM causes female birds to call more to heterospecific songs. Ongoing experiments are also investigating the degree to which lesioning CM alters preference among conspecific songs. This and future studies will determine the role of CM and other areas of the auditory lobule in song preference and mate choice.

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**Topic:** D.06. Audition

**Support:** Office of Naval Research

**Title:** Interaction between top-down and bottom-up predictions in auditory decision-making

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**Abstract:** Auditory perception and decision-making are mediated by bottom-up and top-down processes. Although a perceptual decision is based primarily on incoming sensory stimuli (bottom-up processing), it can be biased by different cognitive and task-relevant pieces of information. For example, prior knowledge and attention can facilitate and modulate this decision process by prioritizing relevant stimuli or features of stimuli (top-down processing). In contrast, changes in stimulus salience, such as regularity violation, can also bias perceptual decision-making through bottom-up attention. However, the effects that top-down and bottom-up processing have on auditory decision-making are not well understood. Here, we investigate how top-down and bottom-up predictions influence auditory decision-making.
Human subjects listened to sequences of high-frequency (2000 Hz) and low-frequency (500 Hz) tone bursts (tone duration: 300 ms with a 10-ms $\cos^2$ ramp; inter-burst interval: 100 ms) embedded in background, broadband noise and played through headphones. Subjects reported whether the last tone in each sequence (i.e., the "test tone") was "low-frequency" or "high-frequency" by pressing the left or right button, respectively, on a gamepad. We titrated difficulty by varying the amplitude of the test tone relative to the noise masker. Top-down processing was manipulated by presenting a visual pre-cue that indicated the prior probability that the test tone would be high or low frequency for the given block of trials (corresponding to ratios of low- versus high-frequency tones of 5:1, 1:1, 1:5). Bottom-up processing was manipulated by varying the sequence of tone bursts presented just before the test tone ("pre-tones"), in terms of: 1) the ratio of high- and low-frequency pre-tones, which was either highly regular (all high or all low) or irregular (randomized); and 2) the number of pre-tones, which were selected from an approximately exponential distribution (range: 2-10) to minimize the ability to predict the timing of the test tone.
Preliminary results indicate that both the top-down and bottom-up manipulations caused choices biases. These biases were largest when stimulus discriminability was lowest, consistent with principles of signal detection theory. These biases also tended to be larger in response to the

bottom-up manipulations. Further analyses of choice and response times will help identify the computations used to incorporate these two sources of information into perceptual decisions.

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2014BP-A00226

**Title:** Neural responses to behaviorally relevant syllable sequences in the ferret auditory- and frontal cortices

**Authors: *D. DUQUE**[1], N. H. JOSHI[2], D. ELGUEDA[1], J. B. FRITZ[1], S. A. SHAMMA[1]
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**Abstract:** Speech unfolds in time, and words and sentences are built around sequential strings of phonemes. As a result, spoken language relies not only on the ability to segregate and recognize individual phonemic elements in ongoing speech, but also in understanding that ordered combinations of such discrete elements form auditory objects. The generation of these auditory objects, known as words, is essential for language since once we create a sound-to-meaning association, oral communication relies on recalling these “word memories” when a complex sound input is processed. Such memories may be stored in association cortices and retrieved when a sound input matches an acoustic memory template generated in auditory cortex.
To explore the neural mechanisms by which the auditory system encodes human words, we train ferrets to discriminate tri-syllabic consonant-vowel words. Ferrets are an excellent animal model to study speech representation because their hearing is similar to humans, their auditory cortex is complex enough to encode all phoneme classes (Mesgarani et al., 2008) and they can be trained to differentiate and recognize syllable sequences (Bizley et al., 2015; Duque et al., 2016). Several ferrets were trained on a conditioned avoidance GO/NO-GO task to lick a spout for water upon presentation of distractor words (e.g. FA-BE-ti) but to refrain from licking the spout after a target word (e.g. FA-BE-LO) was presented, in order to avoid a mild shock. Once the animals learned the task, a head-post was implanted to enable single unit electrophysiological recordings in primary auditory cortex (A1), higher order auditory cortical areas (dPEG) and frontal cortex (FC) while the animals were performing the behavioral task.

Preliminary neurophysiological data indicate that, unlike the stimulus-dependent responses in A1, responses in dPEG reveal differences between target and distractor words based on associated behavioral meaning. This study suggests that neurons in higher order auditory cortical areas can encode specific syllable sequences. These results will help us understand the neural basis for the representation of complex sound sequences, and yield deeper insight into neural mechanisms underlying initial stages in human speech processing.

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**Topic:** D.06. Audition

**Support:** NSF CRCNS 1515678

**Title:** Perceptual alternation in auditory streaming as an evidence accumulation process

**Authors: *J. M. RINZEL**[1], A. NGUYEN[2], R. CURTU[2]
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**Abstract:** Subjects report when listening to brief pure tones, alternating high (A) and low (B), in sequences ABA_ABA_.... spontaneous switching between two percepts: “integration” (Int), a single, coherent stream, the galloping pattern and “segregation” (Seg), two simultaneous distinct streams: A_A_ and B___B___. This triplet stimulus is considered a paradigm for auditory perceptual grouping, stream formation, and for the cocktail party problem. The initial percept is typically Int while Seg ‘builds up’ with a time course of seconds. It’s said that Int is the default while evidence accumulates for the switch into Seg and thereafter, during long presentations, irregular alternations occur between Int and Seg. The psychometric build-up function (from trial-averaging) depends on the difference in A-tone and B-tone frequency, DF, is typically monotonic, and percept durations are gamma/lognormal distributed. Recordings from primary auditory cortex (area A1) of monkeys listening to triplet sequences have been used to construct neurometric functions from a signal-detection model (Micheyl etal, 2005) that resemble psychometric functions. However, that model predicts neither realistic mean percept durations nor percept durations that are gamma-like distributed (Pressnitzer & Hupe, 2006). We developed an accumulation model in the form of a multi-layer feedforward network with neural-like components. The model accepts A1 neuronal responses (including variability) as input. Each B-tone is classified as Int or Seg by binary units (each samples a subset of A1 neuron spike counts).

A noisy accumulator increments its activity based on the classifier output, representing growing evidence against the current percept. A switch and reset occur when a threshold is reached. The model generates build-up functions as well as percept durations with statistics that match data from our behavioral experiments (15 subjects, 30 sec trials) for DF=3,5,7 st (Fig. 1)
Fig. 1: Time course of accumulator model (**A)** yields build up function (**B**) and percept durations **(C)** that match experiment

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**Poster**

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**Title:** Neural correlates of decision-making in primary auditory cortex neuronal populations

**Authors: *N. A. FRANCIS**[1], D. E. WINKOWSKI[1], Z. BOWEN[1], A. SHEIKHATTAR[2], K. ARMENGOL[3], B. BABADI[4], D. PLENZ[5], P. O. KANOLD[3]
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**Abstract:** The detection of sound is essential for communication and survival in natural environments. Auditory task performance enhances the neural representation of behaviorally meaningful sound throughout primary and higher-order auditory cortices. However, it remains unclear how auditory cortex contributes to decision-making. For neurons in primary auditory cortex (A1) layer 2/3, heterogeneous stimulus selectivity and complex intracortical connectivity suggest that task-related information coding may differ across neural subpopulations. To study the neural correlates of decision-making in distinct neuronal subpopulations, we used in vivo 2-photon imaging in A1 layer 2/3 of mice performing tone detection and discriminations tasks. During tone detection, we found that neural responses to target tones were enhanced during correct detections, and the enhancement was independent of neuronal selectivity for tone frequency. Preliminary analysis from tone discrimination experiments suggest similar neural response enhancements. Using a linear neural decoder, we found that neurons tuned away from the target, with moderate noise correlations, and positive attentional gain, were the best predictors of behavioral choice. Population activity during behavioral tasks showed neuronal avalanches, suggestive of critical dynamics. Investigation of neural network activity using Granger causality and avalanche analyses suggest that separate clusters of neural networks within A1 may exhibit unique spatiotemporal activity patterns that depend on behavioral choice. Thus, we find that sensory-based decision-making may depend on inhibitory control of small neural populations in sensory cortex that are driven by the sum of sensory input, attentional gain, and the cost of behavioral choice.

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**Title:** Cortical responses and functional connectivity derived from electrocorticography (ECoG) during speech in noise task

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**Abstract:** Understanding speech in noisy environments can be quite challenging, especially so for hearing impaired listeners. Performance on speech in noise word recognition task cannot be predicted directly from an audiogram alone, and speech degradation has been recently shown to recruit inferior frontal gyrus (IFG) in addition to auditory cortical areas along the superior temporal gyrus (STG). Recent human non-invasive neuroimaging work suggests that STG activity represents stimulus-driven low-level perceptual processing while IFG is recruited for top-down feedback processing to resolve perceptual ambiguity resulting from stimulus degradation.
Using a speech in noise word recognition task based on the California Consonant Test (CCT), we collected intracranial electrocorticography (ECoG) data in normal hearing subjects (n = 5). Subjects were implanted with intracranial subdural and depth electrodes for clinical seizure monitoring. Subjects were tested using a 4AFC word recognition task in which subjects listened to CVC monosyllabic words presented in multitalker babble at +3 and -3 dB SNR. The answer choices were drawn from a set of matching rhyme or cohort answer choices.
We examined the role of gamma and high gamma band responses in Heschl's Gyrus (HG), STG, and IFG during the task. Additionally, we analyzed the functional connectivity within auditory cortex as well as between auditory cortex and IFG. These results reveal the role of IFG as well as the functional architecture of both auditory and frontal cortices during speech perception in difficult hearing situations.

**Disclosures: A.S. Liu:** None. **P.E. Gander:** None. **C.K. Kovach:** None. **H. Kawasaki:** None. **M.A. Howard:** None. **T.D. Griffiths:** None. **I. Choi:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.27/EE14

**Topic:** D.06. Audition

**Title:** Brain sources of the human MMN identified from intracranial recordings

**Authors: *A. O. BLENKMANN**[1], H. N. PHILLIPS[2], T. BEKINSCHTEIN[2], J. ROWE[2], P. G. LARSSON[3], J. IVANOVIC[3], C. MURAVCHIK[4], T. ENDESTAD[1], S. KOCHEN[5], A.-K. SOLBAKK[1]
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**Abstract:** In order to investigate the brain sources of the Mismatch Negativity (MMN) we recorded intracranial EEG in 20 candidates for epilepsy surgery while they listened to one out of two auditory tasks:1- Local-global paradigm (1) consisting of sequences of 5 complex binaural tones. Every sequence has a local pattern making the last tone predictable (STD) or not (DVT) based on the previous 4 tones (pat. 1-6).2- A modification of the local-global task (2), where tone sequences were either monaural or interaural (pat. 7-20).
Electrodes were localized using iElectrodes toolbox (3), from MRI and CT images in the MNI normalized space.
A total of 1249 channel recordings were analyzed. Channels with epileptic activity or noise were discarded. Bipolar montage was used to improve SNR. STD and DVT trials were segmented time-locked to stimuli, filtered (1-40Hz), and bad trials were rejected.
Channels with significant activations on STD or DVT trials compared to a surrogate null distribution were selected. The MMN was obtained subtracting STD from DVT. Non-parametric permutation-based and cluster-based statistics were used to determine significant activations ($p<0.05$) corrected for multiple comparisons.
We found the most consistent activations bilaterally in superior and middle temporal gyrus, precentral gyrus, postcentral gyrus and supramarginal gyrus, in right middle frontal gyrus, and left inferior frontal gyrus.
The earliest activations were observed at least in 2 independent conditions in the temporal lobe at 65/55ms, the frontal lobe at 95/100ms, and the parietal lobe at 80/65ms (for left/right hemispheres).
These results support that MMN sources, evoked by two different tasks and found in multiple patients, are consistently distributed in a fronto-temporo-parietal network. 1. T. Bekinschtein et al., Neural signature of the conscious processing of auditory regularities. Proc. Natl. Acad. Sci. U. S. A. 106, 1672-7 (2009).2. S. Chennu et al., Expectation and Attention in Hierarchical Auditory Prediction. J. Neurosci. 33, 11194-11205 (2013).3. A. Blenkmann et al., iElectrodes: a comprehensive open-source toolbox for depth and subdural grid electrode localization. Front. Neuroinform. 11, 14 (2017).

**Disclosures:** **A.O. Blenkmann:** None. **H.N. Phillips:** None. **T. Bekinschtein:** None. **J. Rowe:** None. **P.G. Larsson:** None. **J. Ivanovic:** None. **C. Muravchik:** None. **T. Endestad:** None. **S. Kochen:** None. **A. Solbakk:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.28/EE15

**Topic:** D.06. Audition

**Support:** 5R01NS088649-03

4R01DC012565-05

**Title:** The roles of specific striatal cell types in an auditory discrimination task

**Authors: *C. J. STONEKING**, A. M. ZADOR
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**Abstract:** Multiple lines of evidence indicate that auditory striatum, by processing inputs from auditory cortex, guides decision-making in rodents performing an auditory discrimination task. However, the activity of auditory striatal neurons during this behavior has not been directly characterized. In particular, the roles of different striatal cell types are unkown. We used a genetically encoded calcium indicator (GCaMP6s) together with a chronically implanted lens and head-mounted microscope to image the activity of specific auditory striatal cell types in freely moving mice. The mice were trained on an auditory 2-alternative forced choice task (cloud-of-tones task). In this task, in each trial, mice initially held a fixed position while listening to a sound stimulus which consisted of a stream of tones. They then were required to move to a lickport located at the left or right, depending on whether the majority of tones were in a low or high frequency band. We found that in trained mice performing this task, D2-dopamine-receptor expressing medium spiny neurons (D2-MSNs) show an increased response to sound stimuli associated with a subsequent contralateral movement. This pattern (contralateral favored) parallels previous findings on movement-associated activity in motor striatum, suggesting that an increase in auditory striatal D2-MSN activity contributes to a subsequent contralateral action.

**Disclosures: C.J. Stoneking:** None. **A.M. Zador:** None.

**Poster**

**587. Auditory Processing: Perception, Cognition, and Action II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 587.29/EE16

**Topic:** F.01. Neuroethology

**Support:** KAKENHI 15K09856

KAKENHI 16H01873

**Title:** Dissociating the neural response to contingency errors: Agency- and prediction-error accounts

**Authors: *M. SUGIURA**[1,2], T. KIKUCHI[1,3], Y. YAMAMOTO[1], Y. SASAKI[1], S. HANAWA[1], A. SAKUMA[4], K. MATSUMOTO[3], H. MATSUOKA[3], R. KAWASHIMA[1]
[1]IDAC, Tohoku Univ., Sendai, Japan; [2]IRIDeS, Tohoku Univ., Sendai, Japan; [3]Dept. Psychiatry, Tohoku Univ. Grad. Sch. of Med., Sendai, Japan; [4]Tohoku Univ. Hosp., Sendai, Japan

**Abstract:** The contingency of sensory feedback to one's actions is essential for the sense of agency, and experimental violation of this contingency is a standard paradigm in the neuroscience of self-awareness and schizophrenia. However, neural responses to this violation have arbitrarily been interpreted either as activation of the system generating forward prediction (agency-error account) or decreased suppression of processing of predictable input (prediction-error account). In this functional magnetic resonance imaging (fMRI) study, the regions responsive to auditory contingency errors were examined if they showed responses to an isolated auditory stimulus and to passive-contingency delay, which the prediction-error account expects. These responses were observed only in the auditory association cortex in the right superior temporal gyrus. Several multimodal and motor-association cortices did not show these responses, suggesting their relevance to the agency-error account. Thus we formulated the coexistence and dissociation of two accounts in neural contingency-error responses.

**Disclosures: M. Sugiura:** None. **T. Kikuchi:** None. **Y. Yamamoto:** None. **Y. Sasaki:** None. **S. Hanawa:** None. **A. Sakuma:** None. **K. Matsumoto:** None. **H. Matsuoka:** None. **R. Kawashima:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.01/EE17

**Topic:** D.07. Vision

**Support:** CIHR project grant

ERA-Neuron grant

FRSQ Vision Reseau grant

**Title:** Duration dependent changes in visual plasticity via monocular deprivation

**Authors: *S. MIN**, A. BALDWIN, A. REYNAUD, R. HESS
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**Abstract:** Background: Short-term monocular deprivation has been recently shown to temporary increase the sensitivity of the patched eye. Many studies have patched subjects for an arbitrary period of 2.5 hours, but for no principled reason. This project explores the duration-dependence of this deprivation-induced plasticity phenomenon.
Methods: Three monocular deprivation durations were tested in nine subjects: 1-, 2-, 3- hours. Three of the nine subjects were patched for 5-hours. Monocular deprivation was achieved by the use of a translucent eyepatch. A session included two rounds of baseline testing of interocular eye balance, patching, and post-patching tests, which are the abridged versions of the baseline testing. Each post-patching test occurred at 0, 3, 6, 12, 24, 48, 60 and 96 minutes after patching in order to track the effects over time. Every subject performed two sessions per condition.
Results: 1-hour patching produced small but significant shifts in eye dominance. Larger shifts occurred from 2-hours patching, but 3-hours patching produced comparable effects to those measured after 2 hours of patching.
Discussions: These results show a saturation of the patching effect beyond 2-hours patching. Hence, we believe that 2-hours patching duration is the optimal duration for eye dominance changes induced by monocular deprivation.

**Disclosures: S. Min:** None. **A. Baldwin:** None. **A. Reynaud:** None. **R. Hess:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.02/EE18

**Topic:** D.07. Vision

**Title:** Contributions of the supragranular layers to the short term monocular deprivation response in adult macaque V1

**Authors: *R. A. MILLER, III**[1], M. BEGUM[1], D. Y. TS'O[2]
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**Abstract:** Proper interocular balance is necessary for normal binocular summation and stereovision. The weights of monocular inputs are dynamic and have an interdependent relationship yielding a single unified visual percept. Previous adult human psychophysical experiments using short term monocular deprivation (STMD) showed surprisingly a strengthening of the deprived eye (DE) post-deprivation, relative to the non-deprived eye (NDE).

We have been studying the neural mechanisms responsible for STMD-invoked monocular gain in adult macaque V1. We have previously shown, using optical imaging, a shift in interocular balance towards the deprived eye in the activities of the V1 ocular dominance columns (ODCs). Recording with multi-electrodes, we have also found a range of V1 single cell responses, often consistent with these STMD-induced shifts. We now have observed that the strengthening of the DE input post-deprivation is more appreciable in cells where its non-dominant eye has been deprived. Often both DE and NDE inputs are enhanced in the post-deprivation period, but the change in gain is greater in the DE relative to the NDE. These trends are more pronounced in the supragranular layers of V1. Preliminary current source density (CSD) analysis has also revealed marked STMD-induced shifts in the supragranular layers. Little or no such shifts are observed in the granular (input) layers of V1. Initial imaging and single-unit studies in macaque V2 have revealed STMD-induced interocular balance shifts post-deprivation similar to those observed in V1. These studies are aimed at dissecting the contributions of different elements of the visual cortical circuit, their role in establishing proper binocular vision and in the STMD-induced shifts of interocular balance.

**Disclosures:** **R.A. Miller:** None. **M. Begum:** None. **D.Y. Ts'o:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.03/EE19

**Topic:** D.07. Vision

**Support:** R01EY016431

**Title:** Role of matrix metalloproteinase-9 (MMP-9) in the response to chronic monocular deprivation and the reactivation of plasticity by light reintroduction following dark exposure

**Authors: *S. MURASE**, C. L. LANTZ, E. M. QUINLAN
Biol., Univ. of Maryland at Col. Park Dept. of Biol., College Park, MD

**Abstract:** The plasticity of ocular preference revealed by monocular deprivation is typically confined to a postnatal critical period. However, we previously proposed that dark exposure can reactivate robust synaptic plasticity in the adult primary visual cortex (V1; He 2007; Montey 2011). We recently reported that it is light reintroduction (LRx) after dark exposure (DE), which induces degradation of extracellular targets by matrix metalloproteinase-9 (MMP-9) that is responsible for the reactivation of structural and functional plasticity in adults (Murase et al., submitted). Although the visual system of MMP-$9^{-/-}$ mice develops normally, the functional and structural plasticity reactivated by LRx in WT adults (>P90) is absent. Moreover, structural and functional plasticity is rescued in MMP-$9^{-/-}$ mouse by exogenous hyaluronidase, an enzyme with

known targets in the ECM. Here we examine the role of MMP-9 in the induction and recovery from amblyopia induced by chronic monocular deprivation (cMD). cMD induces a significant decrease in the strength and selectivity of responses of neurons in the deprived visual cortex, and a decrease in the density of dendritic spines on basolateral dendrites of layer 4 neurons in deprived V1b (68±5.8% of non-deprived V1b, n=20 neurons, p=0.0014, t-test; Montey 2011). However, dendritic spine densities are unchanged in MMP-$9^{-/-}$ visual cortex following cMD (105.7±9.95% of non-deprived V1b, n=19, p=0.72, t-test). Similarly, the robust decrease in the contralateral bias index (CBI, (C-I)/(C+I)) of visually evoked potentials observed in WT mice in response to cMD (control: 0.35±0.054, n=7 subjects; cMD: -0.15±0.14, n=4 subjects, p=0.003, t-test) is absent in MMP-$9^{-/-}$ mice (control: 0.22±0.050, n=7, cMD: 0.25±0.046, n=5, p=0.72, t-test). Thus the visual cortex of $MMP9^{-/-}$ mice is highly resistant to this form of activity-dependent plasticity. Interestingly, immunoblot analysis reveals that DE followed by LRx significantly increases MMP-9 expression and activity in both the deprived and non-deprived V1 of cMD WT mice (MMP-9 level (% of control) deprived: *133.04±9.1%; non-deprived: *131.37±8.9%; $F_{(3,29)=}5.56$, p=0.004; cleavage of beta-dystroglycan (to track MMP-9 activity) deprived: *216.5±37.8%; non-deprived: *194.2±20.3%, $F_{(3,29)=}6.27$, p=0.002, one-way ANOVA, Tukey-Kramer post hoc, *p<0.05). Thus MMP-9 is involved in the response to, and recovery from, cMD. Thus, V1 retains the ability to up-regulate MMP-9 activity in response to LRx following cMD, suggesting that degradation of extracellular targets including ECM at synapses serving the deprived eye may contribute to the LRx-induced recovery.

**Disclosures: S. Murase:** None. **C.L. Lantz:** None. **E.M. Quinlan:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

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**Program#/Poster#:** 588.04/EE20

**Topic:** D.07. Vision

**Support:** NIH R01 EY016431

ARCS (Metro-Washington Chapter) Fellowship (2015-2018)

**Title:** 17-alpha estradiol rejuvenates excitatory synapse density in amblyopic rodents

**Authors: *D. C. SENGUPTA**, E. M. QUINLAN
Dept. of Biol., Univ. of Maryland, Col. Park, College Park, MD

**Abstract:** Reduced synaptic plasticity and excitatory synapse density contribute to age-related cognitive decline. A parallel reduction in circulating sex hormones, particularly Estrogen (E2), in both sexes is thought to exacerbate the age-related changes in cortical structure and function.

Conversely, hormone replacement therapy in aged rodents and non-human primates restores synapse density in the hippocampus (Gould et al 1990; Hao et al 2003) and pre-frontal cortex (Chisolm & Juraksa, 2012; Hao et al, 2006), and improves cognitive function (Gibbs, 2000; Hara et al, 2016). Importantly, short-term E2 administration promotes rapid synaptogenesis, and these newly-formed synapses are stabilized after a chemical LTP protocol (Srivastava et al, 2008). To ask if E2 treatment can be used to promote recovery of sensory function in the aged brain, we use a rodent model in which severe amblyopia is induced by chronic monocular deprivation (cMD). cMD induces a significant decrease in the visual acuity of the deprived eye, a reduction in excitatory synapses density in primary visual cortex (V1), and is highly resistant to reversal in adults (Montey & Quinlan, 2011). Because the presence of E2 receptors in rodent V1 has been controversial, we first demonstrated robust expression of both classical receptor subtypes (ERα and ERβ) in adult male and female binocular control (BC) LE rats. Using a marker for excitatory synapses (PSD95), we demonstrate that cMD induces a decrease in synapse number (BC: 282.55±15.43 (n=4); cMD: 244.30±24.96 (n = 6)) and synapse size (K-S test, $p = 1.03E10^{-8}$). However, treatment of adults with a single physiologically-relevant dose of E2 (17- α E2, 15 μg/kg) induces a significant increase in synapse number (cMD: 244.30±24.96 (n=6), +E2: 305.50±15.40 (n=12); t-test, p=0.03) and size (K-S test, $p =1.99E10^{-6}$) in the deprived V1 (contralateral to the eye deprived from P14 - P190). Using a marker for recently-active synapses (antibody against phosphorylated Serine-831 of the GluR1 subunit of the AMPA receptor), we show that E2 significantly increases the number (cMD: 180.67±25.12 (n=6), +E2: 305.5±15.4 (n=12); t-test, p=0.02) and size of pS831 puncta in deprived V1 (K-S test, p =0.05). Importantly, E2 followed by visual stimulation (100 cycles of 0.05 cycles/degree, 100% contrast full-field gratings reversing @1 Hz) further increases the size of PSD95 (K-S test, p =0.04) and pS831 puncta (K-S test, $p =5.17E10^{-5}$), suggesting that salient stimulation incorporates newly formed synapses into functional circuits. We are currently examining the how repetitive performance in a visual discrimination task impacts the new synapses induced by E2 treatment.

**Disclosures: D.C. Sengupta:** None. **E.M. Quinlan:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.05/EE21

**Topic:** D.07. Vision

**Support:** Picower Institute Innovation Fund

**Title:** Understanding of how retinal inactivation induces recovery from amblyopia

**Authors: *H. J. DE JESÚS-CORTÉS**[1], M.-F. FONG[2], R. W. KOMOROWSKI[2], M. F. BEAR[2]
[1]Picower Inst. for Learning and Memory, [2]Picower Inst. for Learning and Memory, Brain and Cognitive Sci., MIT, Boston, MA

**Abstract:** Our lab recently discovered that experimental amblyopia induced in mice by temporary monocular deprivation is reversed completely when binocular visual experience follows a brief period of bilateral retinal inactivation using tetrodotoxin (TTX). In the present study, we aimed to understand how retinal inactivation is able to induce this rapid recovery. We hypothesized that retinal inactivation promotes recovery by lowering the threshold for experience-dependent synaptic strengthening via metaplasticity. To test this hypothesis, we first recorded multi-unit activity and local field potentials in layer 4 of mouse visual cortex before, during, and after the period of TTX-driven inactivation. We found that retinal inactivation induces an overall reduction of visually-evoked and spontaneous activity within the first several hours after intravitreal TTX injection. As the drug effect wore off, we observed a striking potentiation of visually-evoked activity, evident 24 hours after treatment and persisting for several days. This rebound effect observed after TTX treatment could be accounted for by a decrease in the synaptic modification threshold that allows the same sensory input to elicit heightened sensitivity in visual cortex. We therefore examined common molecular correlates of metaplasticity in visual cortex. We first examined the subunit composition of NMDA-type glutamate receptors, which has been closely linked to shifting the propensity for LTP in cultures and in vivo. Here, we use immunoblotting to show that retinal inactivation rapidly increases expression of NR2B, resulting in a significant reduction in the NR2A/B ratio. Further, we show that retinal inactivation increases phosphoryation of the *grin2b* transcription factor CREB, an effect that is still observed at 24 hours. We are now conducting experiments to determine if the NR2A/B ratio shift is necessary for recovery after TTX-induced retinal silencing. This knowledge will not only provide crucial information about how the visual cortex compensates for loss of sensory input, but also could suggest novel non-invasive clinical interventions for the treatment of amblyopia.

**Disclosures: H.J. De Jesús-Cortés:** None. **M. Fong:** None. **R.W. Komorowski:** None. **M.F. Bear:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

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**Topic:** D.07. Vision

**Support:** NIH grant EY022720

NIH grant EY014882

**Title:** Sensory deprivation promotes cross-modal plasticity at thalamocortical (TC) synapses in the adult mouse brain

**Authors: *G. RODRIGUEZ**[1], H.-K. LEE[2]
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**Abstract:** Sensory loss leads to extensive compensatory adaptations in the brain that impact both deprived and spared sensory areas. In the spared sensory cortices, we previously reported strengthening of thalamocortical (TC) synapses as a result of cross-modal sensory deprivation in adult mice (Petrus, et al. 2014). This was unexpected in light of previous studies demonstrating that TC plasticity is lost after the second postnatal week of development in mice. Here we test the hypothesis that cross-modal sensory deprivation is able to recover synaptic plasticity mechanisms at TC synapses in adults. By combining slice electrophysiology of primary visual cortex (V1) with optogenetic stimulation, we demonstrate that deafening adult mice results in the reemergence of NMDA receptor-dependent long-term potentiation (LTP) at TC synapses in V1. In addition, we show *in vivo* functional changes in V1 as a result of deafening by measuring optical intrinsic signals during presentation of visual stimuli before and after monocular deprivation. We observe a rapid ocular dominance shift after monocular deprivation for 3 days in adult deafened mice that is absent from age-matched normal reared controls. Moreover, we show that this shift is due to accelerated potentiation of the non-deprived eye inputs to V1 in deafened animals. These data suggest that deafening accelerates ocular dominance plasticity in V1 of adult mice by promoting LTP of TC synapses. Furthermore, based on our findings, we propose cross-modal sensory deprivation as a potential method to regain sensory cortical plasticity in adults.

**Disclosures: G. Rodriguez:** None. **H. Lee:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** D.07. Vision

**Title:** Binocular vision restores glutamatergic and GABAergic proteins for regulating experience-dependent plasticity mechanisms to normal level

**Authors: *J. L. BALSOR**[1], B. BESTON[3], D. G. JONES[4], K. M. MURPHY[2]
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**Abstract:** Abnormal visual experience during the critical period (CP) affects visual acuity, ocular dominance and the molecular mechanisms that regulate experience-dependent plasticity. Previously, we found that early monocular deprivation (MD) disrupts the synaptic environment by shifting some plasticity markers towards immature levels (GluN2A:GluN2B), while others shift to mature levels (GluN1:GluA2 and $GABA_A\alpha1$:$GABA_A\alpha3$) (Beston et al., 2010). This abnormal synaptic environment is thought to reduce plasticity and underly why recovery after MD is often poor, especially when reverse occlusion (RO) is used. Recent studies have suggested that binocular vision (BV) or perhaps even some forms of binocular deprivation (BD) promote better recovery after early MD. Here we asked which treatment paradigm, RO, BD, or BV after early MD leads to the best recovery of glutamatergic and GABAergic synaptic proteins that regulate experience-dependent plasticity.

To address this question we reared animals with early MD to the peak of the CP, and then did either RO, BD or BV. The BV was done for 1hr, 6hrs, 1d, 2d or 4d so that dynamic changes in synaptic proteins could be followed. The expression of glutamatergic (GluN1, GluN2A, GluN2B, GluA2) and GABAergic ($GABA_A\alpha1$, $GABA_A\alpha3$) proteins were quantified using Western blot analysis of synaptosomes. We also analyzed the effect of deprivation and recovery on different regions of the visual cortex representing central (CVF), peripheral (PVF) and monocular (MVF) visual fields because previous studies have shown that deprivation causes the largest changes in the CVF representation.

MD causes reduced expression of GluN1 and increased $GABA_A\alpha1$ in the CVF and PVF, and reduced GluN2A and $GABA_A\alpha3$ expression across all of the visual cortex. Treatment with either RO or BD failed to restore expression of those receptor subunits to normal levels. In contrast, treatment with BV restored expression of some proteins to normal levels. After 4 days of BV the levels of GluN2A in CVF, and GluN1 in CVF and PVF had been restored to normal. Only 6 hours to 1 day of BV restored $GABA_A\alpha1$ expression to normal levels. BV also promoted recovery of the GluN2A:GluN2B and $GABA_A\alpha1$:$GABA_A\alpha3$ balance in all three regions of visual cortex. These findings provide further support for the benefits of BV as a treatment for early abnormal vision by showing that it promotes recovery of synaptic mechanisms that regulate experience-dependent plasticity.

**Disclosures:** **J.L. Balsor:** None. **B. Beston:** None. **D.G. Jones:** None. **K.M. Murphy:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.08/EE24

**Topic:** D.07. Vision

**Support:** NIH Grant EY022730

**Title:** Early establishment and stability of cortical network synchronization during development

**Authors: *M. T. COLONNESE**
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**Abstract:** During development neural networks must grow from a state of zero connectivity to the interconnected circuits that underlie complex dynamics in adults. Synchronization of the developing elements is hypothesized to be a critical component of circuit formation. Understanding the nature of this synchronization will constrain models of activity-dependent development. Two broad hypotheses about early synchronous activity exist. In the first, exuberant connectivity and weak inhibition render early networks hyper-synchronous, allowing for rough 'first-draft' connectivity. In this model, maturation of synaptic properties and the integration of new cell classes (eg. inhibitory interneurons) causes activity to become progressively more precise and less-synchronous allowing for synaptic refinement. A contrary hypothesis is that immature activity provides a model of mature function allowing for formation of correct local connectivity from the outset. In this model synaptic maturation and inhibitory neuron integration occurs not to transform network properties, but rather maintain them in the face of increasing connectivity.
To distinguish between these hypotheses I examined early cortical networks for evidence of hyper-synchronization using single-units isolated from multi-electrode arrays in the visual cortex of awake mice from P(ost-natal) day five, through the acquisition of mature activity patterns (P24). Network synchronization was quantified by evaluating the number of co-active neurons active, as well as by pair-wise firing rate co-modulation (Renart et al. 2010. *Science* 327), the distribution of firing vectors (Okun et al. 2012. *J Neurosci* 32) and population coupling (Okun et al. 2015. *Nature* 521). While firing patterns in infant animals during the period of cholinergic retinal waves (P5-9) superficially appear hyper-synchronous because they spend more time in down-states (network silence), when analysis is restricted to active periods synchronization is lower than expected by chance. Adult-like patterns for all measures were established by P10, before eye-opening and during the period of glutamatergic retinal waves. Thus the cortical circuit is exhibiting properties of an asynchronous network before the development of balanced inhibition and before visual experience.
Together the data show that while early thalamocortex produces unique patterns of rhythmic activation and elongated silences, the network properties determining synchronization maybe more similar to the mature brain than expected. Our data provide no support for the hypothesis that early cortical circuits are hyper-connected or hyper-synchronous.

**Disclosures: M.T. Colonnese:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.09/EE25

**Topic:** D.07. Vision

**Support:** NIH R01-EY12124

**Title:** Neuromodulator induced bidirectional changes in ocular dominance in the mouse primary visual cortex

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**Abstract:** Neuromodulatory signals mediated by G protein-coupled receptors are essential for the induction of experience-dependent cortical plasticity. Neuromodulators can exert a push-pull control of Hebbian plasticity, with receptors coupled to Gs and Gq11, promoting the induction of LTP and LTD, respectively, *in vitro*. Importantly, β-adrenergic receptors (coupled to Gs) and 5-HT2c serotonergic receptors (coupled to Gq11) can provide a cellular substrate for reinforced learning, as these receptors can transform the so-called “eligibility traces” into lasting LTP and LTD. Therefore, we tested whether the activation of these receptors *in vivo* can promote lasting bidirectional changes in visual responses. Using optical imaging of intrinsic cortical signals, we found that pharmacological activation of 5-HT2c receptors, in conjunction with brief visual conditioning of one eye, induces LTD-like changes of the responses evoked by the conditioned eye, thus shifting the natural ocular dominance balance. The same visual stimulation, paired instead with the activation of β-adrenergic receptors, results in LTP-like changes and ocular dominance shift in the opposite direction. In addition, blocking β-adrenergic receptors during short monocular deprivation in juvenile mice, which is not expected to change visual responses, resulted in a paradoxical depression of the open eye. These results suggest that the push-pull regulation of Hebbian plasticity underlies the neuromodulation of experience dependent ocular dominance plasticity.

**Disclosures: S.Z. Hong:** None. **S. Huang:** None. **A. Kirkwood:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Title:** An unexpected period of neurochemical imbalance during postnatal development of the rat visual cortex

**Authors: *H. ZHANG**, L. MU, D. WANG, Q. LIU, M. T. WONG-RILEY
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**Abstract:** Last year we reported on a surprising period of synaptic imbalance, with suppressed excitation and enhanced inhibition, in the rat's visual cortex from postnatal day (P) 28 to P33. This period falls within the peak of the critical period of development reported by others in rats and mice. We hypothesized that synaptic changes observable at the electrophysiological level had a neurochemical counterpart. The goal of the present study was to analyze the developmental trends of 5 neurochemicals in the visual cortex of normal Sprague-Dawley rat pups *daily*, from the time of eye opening (P14) to P36, when neurons presumably have reached maturity. The 5 neurochemicals were: cytochrome oxidase (a metabolic marker of neuronal activity), NMDAR1 (the obligatory subunit 1 of the glutamatergic NMDA receptors), $GABA_AR\alpha1$ (the α1 subunit of the $GABA_A$ receptors), BDNF (brain-derived neurotrophic factor), and TrkB (tropomyosin receptor kinase or tyrosine protein kinase B, a high-affinity receptor of BDNF). We found that, against a gradual increase in the expressions of all 5 neurochemicals with age, there was a sudden and significant fall in cytochrome oxidase, NMDAR1, BDNF, and TrkB at P28 and sustained until P33/34, before returning to P27 levels at P34/35. On the other hand, the expression of $GABA_AR\alpha1$ continued to increase with age, with a sharper rise within layers II, III, and VI neurons between P27 and P31, before leveling off at P32. Similar patterns of labeling existed in neurons from layers II to VI for each of the neurochemicals. These results are consistent with our hypothesis that the synaptic imbalance demonstrable electrophysiologically has a neurochemical correlate, with reduced excitatory and metabolic neurochemicals and increased inhibitory neurochemical all occurring from the end of the $4^{th}$ toward the end of the $5^{th}$ postnatal week. Moreover, we found that enhanced GABAergic receptor expression during the period of synaptic imbalance is correlated with a decrease, not an increase, in BDNF and TrkB. This negative relationship is unexpected, as the relationship was thought to be positive during the critical period of visual cortical development in rodents. ($^{\#}$The first 2 authors contributed equally to this work)
Key words: visual cortex, BDNF, critical period

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**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Topic:** D.07. Vision

**Support:** R01EY024918

R01EY 026053

**Title:** Activation of somatostatin inhibitory neurons by Lypd6-nAChRα2 system restores juvenile-like plasticity in adult visual cortex

**Authors:** ***M. SADAHIRO**[1], M. P. DEMARS[1], P. N. BURMAN[1], P. E. YEVOO[1], M. R. SMITH[1], A. ZIMMER[2], H. MORISHITA[1]
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**Abstract:** Globally heightened cortical plasticity during juvenile critical periods declines into adulthood, posing a major challenge for functional recovery following brain injury or disease in later life. The inhibitory cortical network has been long appreciated as a crucial regulator of cortical plasticity, yet contributions of specific interneurons other than Parvalbumin (PV) interneurons in restoring juvenile-like global plasticity in the adult brain have been underexplored. Cortical Somatostatin (SST) interneurons, which represent nearly a third of the cortical GABAergic neurons, are situated as versatile “hubs” due to their ability to integrate multiple inputs, including bottom-up sensory signals and neuromodulation induced by locomotion or top-down regulation. Moreover, SST interneurons highly innervate local PV interneurons, which places them in a potentially ideal position to drive the rapid inhibition known to be instructive to global plasticity. Still, SST interneuron-specific molecular and circuit mechanisms for re-initiating global cortical plasticity in adulthood remain unknown. Here we identified Lypd6, an endogenous SST interneuron-specific positive modulator of nicotinic acetylcholine receptors (nAChRs), as a molecular target for re-initiating cortical plasticity in adulthood. Lypd6 expression in visual cortex decreases into adulthood in concert with declining plasticity. Overexpression of Lypd6 specifically in SST interneurons during adulthood rapidly increases their activity in an experience-dependent manner through the action of α2 subunit-containing nAChRs to in turn inhibit PV interneuron activity and ultimately re-initiate visual cortex plasticity. Importantly, the activity of SST interneurons alone was also found to be an effective target, as transient chemogenetic activation of SST interneurons was able to re-initiate

visual cortex plasticity in adulthood. Identification of the Lypd6-nAChRα2 system and the SST->PV disinhibitory microcircuit as the first SST interneuron-specific plasticity regulator provides potential therapeutic targets for treating disorders with limited recovery due to diminished plasticity such as amblyopia as well as psychiatric disorders with deficits in SST- interneurons.

**Disclosures: M. Sadahiro:** None. **M.P. Demars:** None. **P.N. Burman:** None. **P.E. Yevoo:** None. **M.R. Smith:** None. **A. Zimmer:** None. **H. Morishita:** None.

**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Topic:** D.07. Vision

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R01EY026053 (H.M.)

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March of Dimes (H.M.)

**Title:** Integrative bioinformatics approach identifies that postnatal lead exposure disrupts critical period plasticity in visual cortex

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**Abstract:** Given that thousands of industrial and synthetic chemicals released into the environment have an unknown, but potentially significant capacity to harm neurodevelopment, there is an urgent need for high-throughput systematic approaches to identify chemicals impacting neurodevelopment. Neurodevelopment is marked by periods of plasticity wherein neural circuitry is optimized by the environment. If environmental chemicals perturb these critical periods, development of normal function can be permanently disrupted, and may confer

risk for neurodevelopmental disorder such as autism. To identify chemicals that may disrupt critical periods of neuroplasticity, we applied a high-throughput informatics approach to match a critical period transcriptional signature from primary visual cortex (V1) of juvenile mice at the peak of the critical period for ocular dominance plasticity to approximately 120 known neurotoxic chemical gene sets, derived from the Comparative Toxicogenomics Database. We identified lead (Pb) as the top-ranked chemical whose gene set shows an opposite expression profile to critical period gene expression in V1 (Hypergeometric Test, OR = 4.8, FDR = $3.1x10^{-06}$). Though epidemiological studies have established Pb as a potent developmental neurotoxicant, the functional impact of childhood Pb exposure on critical period plasticity has not been tested. From our analysis indicating that Pb would suppress the critical period gene signature, we hypothesized that juvenile Pb exposure would also suppress experience-dependent plasticity during the critical period. To test this, we administered 50PPM Pb acetate in drinking water to dams and their pups starting at P8, to mimic a childhood exposure to Pb. At the peak of ocular dominance plasticity, we performed monocular deprivation and then assessed plasticity at P28. In mice administered normal water, we observed the expected shift in cortical responsivity. In contrast, Pb treatment suppressed the shift in cortical responsivity, demonstrating that Pb suppressed functional plasticity in vivo.Together, this work indicates that a highthroughput, in silico approach can effectively identify neurotoxicants of critical period plasticity. Given the recent water quality crises in Flint and elsewhere, where elevated levels of Pb were detected, such systematic analyses are critical to identify dangers to childhood neurodevelopment. Future work should identify the mechanisms subserving Pb disruption of developmental plasticity to facilitate therapeutic target discovery to rescue disruption of plasticity and neurodevelopment. P.Y., M.S. equal contribution.

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**Poster**

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Knights Templar Eye Foundation (H.M.)

March of Dimes (H.M.)

**Title:** Integrative bioinformatics approach to systematically identify environmental chemicals that disrupt critical periods of plasticity

**Authors: *M. R. SMITH**[1], P. YEVOO[2], M. PENG[3], M. SADAHIRO[1], B. KIDD[3], J. T. DUDLEY[3], H. MORISHITA[4]
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**Abstract: Background:** Given the thousands of chemicals released into the environment there is an urgent need for high-throughput approaches to identify chemicals impacting neurodevelopment. Central to childhood neurodevelopment are critical periods of plasticity wherein neural circuitry is optimized by the environment. If chemicals perturb these periods, development of normal function can be permanently disrupted.
**Methods:** To identify anti-plasticity chemicals, we computationally matched transcriptional signatures of critical period plasticity (Smith MR et al 2016) to 1742 chemical gene sets from the Comparative Toxicogenomics Database. To gain insight into biology, we applied Chemogenomics Enrichment Analysis (CGEA) to the chemicals across 5191 Gene Ontology (GO) Biological Processes and 96 Library of Integrated Network-based Cellular Signatures (LINCS) ligands. We tested CGEA convergent pathways using qPCR and immunohistochemistry.
**Results:** We identified 50 anti-plasticity chemicals, including antimicrobials, metals, and pesticides. CGEA identified 33 GO Biological Processes and multiple cytokines (FDR < 0.05) enriched among the 50 chemicals, which converged on pathways related to response to pathogen, immune cell chemotaxis, and inflammation via IL1 and TNFa. These pathways mimic a host inflammatory response to infection, including microglia activation. Using the LPS model of inflammation, we found that LPS at a dose that disrupts critical period plasticity (Smith MR et al 2016) activates cortical microglia (>Iba1, $p = 0.02$; >CD68, $p = 0.03$) and decreases the P2y12 purinergic receptor required for critical period plasticity ($p = 0.01$).
**Conclusions:** Anti-plasticity chemicals may induce an inflammatory response, activating microglia to disrupt critical period plasticity. Next steps include directly testing the capacity of anti-plasticity chemicals to induce an inflammatory response to disrupt plasticity.

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**Poster**

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**Topic:** D.07. Vision

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R21EY026702

**Title:** Experience-dependent survival of newly formed spine is gated by Lynx1

**Authors: *M. SAJO**[1], G. ELLIS-DAVIES[2], H. MORISHITA[3]
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**Abstract:** Newly formed spines usually retract within days without transforming into stable spines. However, to what extent genetic and environmental factors regulate this key process is totally unknown. Identification of mechanisms that gate the conversion of new spines into stable spines could provide conceptually novel strategies for restoring function. Here we examined to what extent exposure to new experience impacts the survival of newly formed spines and whether Lynx1, a nicotinic brake for functional plasticity, modulate this key process. To identify newly formed spines, we chronically imaged the apical dendrites of layer 2/3 and 5 pyramidal neurons in binocular region of primary visual cortex of adult Thy1-GFPM-line wild type (WT) and Lynx1 knockout (KO) mice. “Newly formed spines” were defined as ones to first appear in the second imaging session 4 days after the initial imaging session. We then examined the impact of visual experience on the survival of these newly formed spines by performing monocular deprivation (MD) immediately after the 2nd imaging session and then conducting a 3rd imaging session after 4 days of MD. We found that while the survival rate of newly formed spines in adult WT mice is comparable between the non-deprived (no MD) and MD groups in both L2/3 and L5 neurons, the rate is higher in the adult Lynx1KO mice following 4-day MD compared to no MD condition. A previous study in adult WT mice reported that more spines form during MD, selectively in L5 neurons, but these spines do not show experience-dependent change in survival rate over 16 days (Hofer et al 2009). In adult Lynx1KO mice, we found that survival rate of spines formed during MD is also comparable to no MD condition, suggesting that experience-dependent survival is specific to new spines formed immediately before deprivation, but not to those formed during deprivation. Finally, older spines that have existed longer than 4 days show no change in survival rate between no MD and MD group in both WT and Lynx1KO mice. Collectively, our study suggests that experience-dependent spine survival is selective to

new spines formed immediately before MD. Our study reveals new spines just formed before exposure to new experience as key substrates for circuit plasticity, and Lynx1 as the first modulator of experience-dependent survival of newly formed spines.

**Disclosures:** **M. Sajo:** None. **G. Ellis-Davies:** None. **H. Morishita:** None.

**Poster**

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**Topic:** D.07. Vision

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**Title:** Synapse-organizing protein SynCAM 1 controls the cortical critical period closure

**Authors:** ***A. RIBIC**[1], M. C. CRAIR[2], T. BIEDERER[1]
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**Abstract:** The experience-dependent plasticity of brain circuits is high during early postnatal development, but tapers off as the brain matures. The development of proper excitation/inhibition balance, as well as a steady increase in the expression of myelin-associated axonal growth inhibitors, is thought to direct circuit maturation and restrict cortical plasticity. However, the molecular factors controlling circuit plasticity at the level of synapses remain unknown. Synapse-organizing adhesion molecules are potential modulators of circuit plasticity and here we studied their role in visual plasticity. Our analysis has demonstrated a specific activity-dependent regulation of SynCAM/CADM-family adhesion molecules in the visual cortex of mice following monocular deprivation during the cortical critical period. We performed electrophysiological recordings from live, awake mice deficient in SynCAM-mediated synaptic adhesion after monocular deprivation. This revealed that synaptic SynCAM adhesion limits cortical plasticity and that mice lacking SynCAM-adhesion have heightened adult plasticity. SynCAM-deficient mice additionally exhibited impaired development of oscillatory brain activity. Using Cre-dependent recombination and viral-mediated knockdown, we finally demonstrated that synaptic adhesion mediated by SynCAMs limits the plasticity of the mature brain and that cell-type specific knock-down re-opens plasticity. Our work reveals a missing molecular link between the known pathways that control the extent of cortical plasticity and the organization of excitatory synapses. These findings underscore the emerging role of transsynaptic interactions in the wiring and remodeling of functional circuits.

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**588. Visual Cortex: Development and Plasticity**

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VHRN – National /International Networking Program

**Title:** Functional reorganization of visual cortical network following a partial optic nerve injury: A longitudinal wide field calcium imaging study using gcamp6s mice

**Authors: *M. GROLEAU**[1], M. NAZARIAHANGARKOLAEE[2], M. P. VANNI[4], B. A. SABEL[5], M. H. MOHAJERANI[3], E. H. VAUCHER[6]
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**Abstract:** Visual cortical networks have a substantial plasticity and ability to reorganize after a visual deficit. For example, there is considerable spontaneous vision recovery following traumatic optic neuropathy in both rats and humans, but little is known about how the cortical network reorganization takes place during this time. In this study, we monitored the residual visual function and plasticity of the cortical networks following a monocular partial Optic Nerve Crush (ONC) across time. To this end, neuronal responses to a monocular flash illumination in each eye were measured in different areas of the visual cortical pathways of awake mice (Thy1-GCaMP6s) using *in vivo* bilateral wide-field calcium imaging, before and 1 hour, 1, 3, 5, 7, 14, 23 and 30 days after the ONC. Moreover, visual acuity of the mice was measured unilaterally in both sides using the optokinetic reflex before and 1, 3, 8, 15, 22 and 29 days after monocular vision loss. The visually-induced cortical response was decreased in V1 and in the secondary visual areas (V2) following the partial optic nerve crush. A recovery of the cortical activity was noted between 3 and 5 days post-crush which remained stable for at least one month after the injury. Despite this recovery, the number of surviving retinal ganglion cells one month after the optic nerve injury was still very low suggesting that the residual function observed by calcium imaging arises from cortical plasticity mechanisms. ONC led to a loss of visual acuity of the injured side which was not followed by recovery to pre-crush levels. However, we observed an increase of the visual acuity of the non-injured eye, indicating compensatory mechanisms and cortical plasticity. In conclusion, our results show a reorganization of the connectivity between

visual and associative cortical areas following optic nerve injury which is indicative of visual cortical plasticity. These results open an interesting avenue to help understand and manipulate recovery or restoration of vision after visual system damage.

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**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Topic:** D.07. Vision

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**Title:** Functional organisation of the visual cortex in a unique case of achiasma

**Authors:** ***K. AHMADI**[1], A. FRACASSO[2,3,4], A. D. GOUWS[5], A. B. MORLAND[5,6], S. O. DUMOULIN[2,4], M. B. HOFFMANN[1,7]
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**Abstract:** Purpose: Congenital absence of the optic chiasm is a rare disorder preventing normal crossing of the nasal retinal axons to the contralateral hemisphere. Consequently, in achiasmia retinal afferents project entirely to the ipsilateral primary visual cortex resulting in overlaid representations of the left and right visual hemi-fields with bilateral population receptive fields. Here, we report an unusual malformation of visual pathways in an achiasmatic patient who deviated from the known misrouting pattern of achiasma.
Methods: Ultra-high resolution (0.65 $mm^3$, isotropic) functional and structural data were acquired using a 7T Siemens scanner in an achiasmatic participant. Visual stimulation with checkerboard pattern reversal (7.35° vertically and 12.89° horizontally) was performed in a block design for two conditions: (i) bilateral stimulation alternating between the eyes and (ii) left and right hemi-field stimulation of the dominant right eye.
Results: Superimposed hemi-field representations from the ipsilateral eye were found on each hemisphere as typical for achiasma. Using signal detection theory, we also found in a region of calcarine sulcus two neuronal populations responding to either left or right hemi-fields in the

ipsilateral hemisphere across lamina. However, in addition to this pattern we observed for anterior V1 extra input from the contralateral eye indicating that a small portion of nasal fibers decussate at the optic chiasm.
Discussion: The misrouting pattern observed in this participant does not comprise the exclusive hallmark typical for achiasma. The additional representation of the ipsilateral eye can be accommodated by reassigning the ocular dominance columns to hemi-field columns as shown by Olman et al. (2016). However, the process through which the visual cortex incorporates the extra input from the contralateral eye remains a question for further research. Remarkably, similar to other visual pathway abnormalities, relatively normal visual function is preserved by reorganisation of intra-cortical wiring.

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**Poster**

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**Topic:** D.07. Vision

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**Title:** Cortical processing of visual inputs in Retinitis pigmentosa

**Authors: *L. BARONCELLI**[1], G. PIETRA[1], T. BONIFACINO[2], T. BEGENISIC[1], M. CENNI[1], A. SALE[1], G. BONANNO[2], L. GALLI[1]
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**Abstract:** Retinitis Pigmentosa (RP) is a family of inherited disorders caused by the progressive loss of retinal photoreceptors. There is no cure for RP, but research aimed at preventing further photoreceptor loss, or substituting new light-responsive elements of biological or artificial nature, is generating hope for these patients. These strategies require that the visual system downstream of the photoreceptors is capable of elaborating visual signals. Anatomical and functional studies have shown that retinal and thalamic structure are well preserved with RP, but the effect of photoreceptor degeneration on the visual cortex is still unknown. Here, we studied how visual cortical processing changed during the course of progression of RP, and whether the visual cortex retained the capability of plastic remodelling. Binocularity is a key property of primary visual cortex (V1) neurons that is widely used to study synaptic integration in the brain and plastic mechanisms following an altered visual experience. We used visual evoked potential to test whether cortical processing of visual inputs is altered in RP. We found a robust shift in

eye preference in RP animals, due to a selective increase of ipsilateral eye-driven responses. The disruption of the binocular properties of neurons in the primary visual cortex was associated with behavioural deficits in depth perception. We also performed in vitro electrophysiological recordings of field excitatory post-synaptic potentials in V1. Basic synaptic transmission, as assessed by response vs stimulus amplitude, showed a significant shallower response in RP mice. Biochemical analysis suggests that this synaptic deficit could be related to the alteration of absolute levels of inhibition and excitation in the visual corte. These results suggest that cortical changes occur in the visual cortex that might further compromise vision by downregulating or suppressing visual processing, as the retinal input progressively deteriorates.

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**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Topic:** D.07. Vision

**Support:** R01AA022455

R01AA013023

**Title:** Phosphorylation of CREB at serine 142/143 is required for visual cortex plasticity

**Authors: N. S. PULIMOOD**[1], M. CONTRERAS[2], T. BLANPIED[2], *A. E. MEDINA[3]
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**Abstract:** cAMP Response Element Binding protein (CREB) is a transcription factor that has strongly been implicated in long term potentiation, activity-dependent neuronal plasticity, as well as learning and memory. Recent work from our lab showed that phosphorylation of CREB at serine 133 (Ser133) is required for both the depression and potentiation components of ocular dominance plasticity (dc-ODP and pc-ODP) in the visual cortex *in vivo.* While phosphorylation at Ser133 is well known to be critical for activation of CREB function and expression of its downstream targets, this single phosphorylation event is often not sufficient for the expression of many CREB-dependent genes. This implies that there needs to be some secondary event(s) in addition to Ser133 phosphorylation to activate transcription of specific sets of genes. Serine 142 and 143 (Ser142/143) are two amino acids on CREB whose phosphorylation is driven by increased synaptic activity *in vitro,* but their involvement in plasticity has never been explored. Therefore we tested the role of these two phosphorylation sites in the ODP model of activity-dependent neuronal plasticity *in vivo*. We hypothesized that phosphorylation of CREB at Ser133

alone is not sufficient for ODP - additional phosphorylation at Ser142/143 is required. We infected animals with a Herpes Simplex viral (HSV) vector expressing a dominant negative form of CREB that cannot be phosphorylated at Ser142 and 143 (CREBdn-S142A/S143A). Then we chronically implanted electrodes in the binocular zone of the mouse visual cortex, and recorded Visually Evoked Potentials (VEPs) in awake mice before and after 7 days of monocular deprivation (MD). Our data showed that blocking phosphorylation at Ser142/143 blocked both dc-ODP and pc-ODP *in vivo*. We also found that cortical cultures infected with CREBdn-S142A/S143A showed decreased activity-dependent expression of the early gene Arc, which is required for ODP. These results reveal a novel mechanism of CREB action in activity-dependent neuronal plasticity, which could confer some specificity on the wide array of plasticity-related genes regulated by this essential transcription factor.

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**Poster**

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NWO

Human Brain Project

**Title:** Thalamic inhibition regulates critical period plasticity in visual cortex and thalamus

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**Abstract:** During critical periods of development, experience shapes cortical circuits resulting in the acquisition of functions used throughout life. The classic example of critical period plasticity is ocular dominance (OD) plasticity which optimizes binocular vision but can reduce responsiveness of the primary visual cortex (V1) to an eye providing low-grade visual input. The onset of its critical period is thought to involve maturation of inhibitory synapses within V1, specifically those containing the GABAA receptor alpha1 subunit. Surprisingly, we find that removing alpha1 from mouse thalamus, but not from cortex, disrupts OD plasticity in V1.

Furthermore, we show that thalamic relay neurons in the dorsolateral geniculate nucleus undergo OD plasticity, which requires thalamic inhibition. Our findings demonstrate that in critical period regulation, thalamic inhibitory circuits are central. This has far-reaching consequences for the interpretation of studies investigating the molecular and cellular mechanisms regulating critical periods of brain development.

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**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Title:** Exercise affects human ocular dominance plasticity

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**Abstract:** Short-term patching of one eye leads to an increase in the strength of that eye's contribution in psychophysical measurements. Previous studies examining the modulatory effect of exercise on this ocular dominance plasticity in normal adults have reported contradictory results. Lunghi *et al.* (doi: 10.1016/j.cub.2015.10.026), using a binocular rivalry paradigm, found that plasticity was enhanced by exercise. Zhou *et al.* (doi: 10.1155/2017/4780876), using a binocular fusion task, found no exercise effect. This study approaches that same question using the dichoptic surround suppression effect, previously shown to exhibit ocular dominance plasticity (Serrano-Pedraza *et al.*, doi: 10.1167/15.12.379). Our goal was to see if the plasticity measured by this task was modulated by exercise.
Twelve healthy adults were tested. Their dichoptic surround suppression was measured to obtain a baseline. They then underwent one of two, 2 hour treatments, combining either the wearing of an eyepatch with exercise, or the wearing of an eyepatch with rest. Measurements of dichoptic suppression were made at 5 time points after treatment (0, 15, 30, 45 min). All subjects performed both conditions on separate days. Exercise consisted of cycling for 30 minutes at 60% of baseline $VO_2$ max and a 1.5 hr rest period.
In the baseline measurements, dichoptic surrounds elevated contrast detection thresholds by a

factor of 3.5×. This elevation factor was modulated by our treatments. Without exercise, the patching reduced the threshold elevation for detecting a target in the patched eye by a factor of 1.5×. With exercise, this increased to a factor of 1.7×. Exercise made the patching effect stronger. In the unpatched eye we also saw a reduction in dichoptic surround masking. Threshold elevation from the mask in the patched eye was reduced 1.2× without exercise and 1.4× with exercise. These results do suggest an exercise effect for ocular dominance plasticity involving dichoptic inhibitory interactions.

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**Poster**

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**Title:** Development of orientation selectivity without Mexican hat input correlations

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**Abstract:** Miller (1994) proposed a model for development of orientation (ORI) selectivity via activity-dependent competition between ON- and OFF-center inputs. The main ideas were (1) Hebbian plasticity leads individual cortical cells to develop a "maximally correlated" set of inputs; (2) Assume LGN inputs have a "Mexican hat" correlation structure: two inputs of the same center type (ON/ON or OFF/OFF) are more positively correlated than two opposite-type inputs (ON/OFF) at short retinotopic separations, but less positively correlated at larger retinotopic separations (note: ORI selectivity initially develops normally without vision, so this models correlations in spontaneous activity); (3) Then Hebbian plasticity yields a set of inputs to a cell that alternates between inputs of one center type and the other, with a period corresponding to the period of the "Mexican hat" alternation in correlations; (4) This can yield development of ORI-selective simple cells; (5) Local excitatory connections between cortical cells lead nearby cells to develop similar preferred ORI. Point (5) yields a "low-pass" map, but the model did not explain why ORI maps are periodic. Recent results from Lee et al., 2016 and Kremkow et al., 2016 find local spatial phase organization closely resembling predictions of the Miller (1994) model.
Ohshiro and Weliky (2006) measured correlations in LGN of developing ferret, and did not find a Mexican hat structure: same-type pairs were always more strongly correlated than opposite-

type pairs at retinotopic separations with nonzero correlations. How can ORI selectivity develop with such correlations?
We have now explored parameter regimes not studied in Miller (1994) and discovered that ORI selectivity can develop with correlations like those observed by Ohshiro and Weliky, provided that (1) intracortical connections and/or LGN correlations are sufficiently widespread and (2) there is a constraint that each LGN ON-cell and OFF-cell must maintain its total synaptic strength to cortex. We have analytically solved the model to determine conditions for such development, which correctly describes simulation results. We are now exploring whether modifications of the model can also explain the periodicity of orientation maps.

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**Title:** Development of synaptic mechanisms in human extra-striate cortex

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**Abstract:** Development of human cortex is thought to proceed from primary visual cortex (V1) to extrastriate. Three findings support that idea: 1) synapse counts peak first in V1 and later in higher-order areas, 2) neuroimaging finds sensory areas mature first, 3) low-level vision develops before higher-level vision. For example, visual acuity matures in childhood (~8 years) but face perception develops into adulthood (~40 years). One theory to account for this proposes a common trajectory with slightly delayed peaks at successive higher order areas. We tested this by comparing the development of pre- and post-synaptic proteins in human extra striate areas (V3 and V4a) to their development in V1. Is there a consistent delay in the development of synaptic proteins involved in experience-dependent plasticity that contributes to hierarchical development of visual function? We studied V3 and V4a of 14 postmortem human cases (7 female, 7 male) ages 8 to 80 years. We used Western blotting to quantify a set of pre- (Synapsin; Synaptophysin) and post-synaptic proteins (PSD-95; Gephyrin; $GABA_A$ receptor subunits α1,

α2, α3; AMPA receptor subunit GluA2; NMDA receptor subunits GluN1, GluN2A (2A), GluN2B (2B)). We calculated the balances between pairs of functionally related proteins: pre-synaptic index (Synapsin:Synaptophysin), post-synaptic index (PSD-95:Gephyrin), $GABA_A$ receptor indices (α1:α2, α1:α3), AMPA-NMDA receptor index (GluA2:GluN1), and 2A:2B balance, and the sum of Synapsin, Synaptophysin, PSD-95 and Gephyrin expression to compare with the analysis done for V1 (Pinto et al 2015). We found 3 patterns of development in V3 and V4 compared with V1. 1) Similar development to V1 for total expression of Synapsin, Synaptophysin, PSD-95 and Gephyrin suggesting that fundamental synaptic mechanisms develop on a similar trajectory. 2) Delayed development for the α1:α2, α1:α3, and GluA2:GluN1 balances. α1:α2 development was delayed ~10 years in V3 and V4, driven by late development of α1. α1:α3 development was delayed by ~20 years in V4 and ~30 years in V3, driven by late losses of α3. GluA2:GluN1 development was delayed by ~25 years in V4 and ~35 years in V3, driven by delayed development of GluN1. 3) A different pattern of development for the 2A:2B balance. In V1, the balance shifts from 2B to 2A, but in V3 and V4 the change was opposite: in children, there was more 2A, that shifted to more 2B in adults, and back to more 2A in aging. This suggests cortical development is not a simple wave of maturation that starts in primary areas and progresses across the cortex. Different cortical areas have unique developmental signatures that engage plasticity mechanisms at successive stages of the lifespan.

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**Title:** Probing visual field integrity using an anatomical measure of the stria of Gennari at ultra-high field MRI

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**Abstract:** Introduction: The visual input from the retina reaches primary visual cortex at the level of the highly myelinated stria of Gennari. Myelin content and the highly myelinated stria of Gennari represent a marker of input activity from the retina to the primary visual cortex in the calcarine fissure. Using ultra-high field MRI (7Tesla) it is possible to visualize myelin distribution at a sub-millimeter resolution, and visualize the stria of Gennari in-vivo in humans. Many conditions as brain's stroke or tumors along the visual pathways, may cause visual field defects or scotomas. In all of these conditions visual input does not reach primary visual cortex. Here we hypothesize that myelin content co-varies with loss of visual input. We will map myelin in participants with visual field defects or absolute visual scotomas and evaluate whether the clinical symptoms are reflected in cortex myelination in the occipital cortex, providing a proof of concept for an anatomical measure of visual field sensitivity.
Method: Anatomical T1-w images: TD/TI: 6000/1200ms, adiabatic inversion, TR/TE: 8/3ms, flip angle: 8 degrees, voxel size = 500x500x500 $\mu m^3$, FOV: 250x250x180 $mm^3$, 360 sagittal slices, bandwidth 201Hz/px, number of excitations per inversion: 300, linear readout, acceleration using SENSE: 2.5 (anterior-posterior) x 2.5(right-left). Scan duration was 7.5 min (4 to 5 repetitions were acquired for each participant). Proton density scan parameters: 3D turbo-field echo, TR/TE: 6/3 ms, FOV: 250x250x180 mm, voxel size: 1x1x1 $mm^{3.}$ Selction criteria for the participants: diagnosis of unilateral hemianopia or quadrantanopia, with spared (or partially spared) occipital lobe. The event causing the visual field lesion must have happened at least three years prior scanning. These visual deficits give the unique possibility to test within-participants comparisons.
Results: Results are suggestive of a similar myelination content between the affected and non-affected hemisphere, confirming previous results obtained from a between-participant test with congenitally blind participants and normally sighted controls.
Conclusions: Stria of Gennari appears to persist independently of receiving visual input. These results suggest that hard-wired developmental mechanisms forming the stria of Gennari are completed after development, and are not modulated by a sudden change in the functional response of the visual system.

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**Poster**

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**Title:** Development of social-visual engagement in rhesus macaques (Macaca mulatta)

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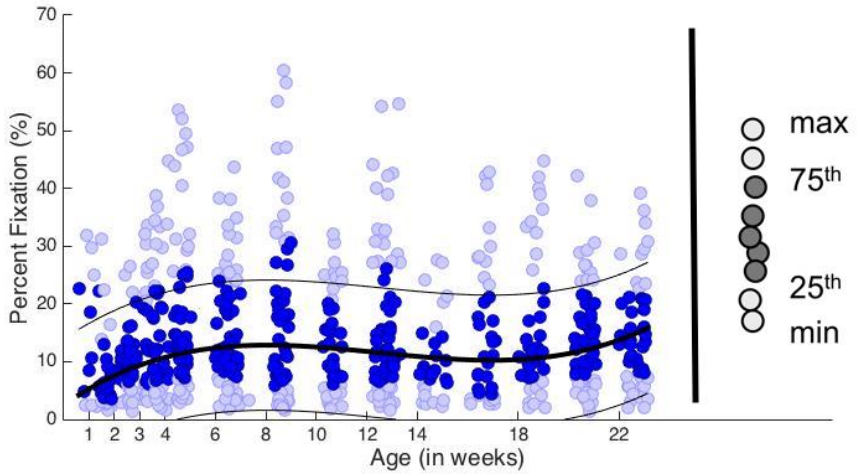
**Abstract:** Autism Spectrum Disorder (ASD) is characterized by altered engagement with the social world and is apparent at early stages of the disorder. A theory in ASD etiology is that neonatal visual function is reflexive, but undergoes an important shift towards voluntary reward-based functioning involving affective neurocircuits. Disruptions in this transition are believed to be involved in ASD pathogenesis (Klin, Shultz & Jones, 2015). Given the critical nature of this transition, illuminating its neurobehavioral underpinnings through a nonhuman primate model will be essential to understanding the pathophysiology of ASD. Twenty-seven mother-reared infant male rhesus macaques living in social groups were eye-tracked longitudinally from birth to 6 months while viewing semi-naturalistic videos of macaque social interactions and full-faced videos of unfamiliar adult and juvenile males and females. Novel stimuli were presented each session with a subset repeated in following sessions to control for novelty across the longitudinal design. Using procedures similar to human studies, 14 sessions were conducted for each infant across the 6 months. The figure demonstrates that monkeys' fixation to the eye region shows an inflection in developmental trajectory, increasing from birth to 8 weeks, decreasing slowly to a trough between 14-18 weeks, before increasing again. Different developmental patterns were observed in fixation on the mouth and body. The results indicate a critical period for social skills refinement around 4-8 weeks of age that parallels the developmental trajectory of eye looking in human infants (Jones & Klin, 2013), and suggest ethologically relevant species differences in the development of mouth and body looking. Importantly, the data suggest the presence of a switch in critical networks supporting these developing social skills. Further analyses of neuroimaging data collected on these animals during this developmental period will allow us to identify neural networks mediating this shift in behavior and will be aid in our understanding of ASD pathophysiology.

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**Poster**

**588. Visual Cortex: Development and Plasticity**

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**Title:** Developmental changes in visual processing of faces: A combined eye-tracking and structural MRI study in infant rhesus macaques

**Authors: *J. STEELE**[1], Z. AMMAR[1], C. PAYNE[3,5], S. MOSS[1], T. JONESTELLER[1], J. WESSON[1], L. LI[3,5], M. STYNER[6], W. JONES[3,5], J. BACHEVALIER[1,2], M. SANCHEZ[1,4], Z. A. KOVACS-BALINT[1]
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**Abstract:** Reading others' facial cues (i.e. trustworthiness) in social interactions is crucial to understand their intentions and emotions, and is impaired in individuals with Autism Spectrum Disorder (ASD). Characterizing the emergence and development of this skill and supporting brain regions may further broaden our understanding of impaired socioemotional development observed in children with ASD. Rhesus macaques, a highly translational nonhuman primate model of early socioemotional development allow for densely sampled longitudinal neuroimaging studies not feasible in human infants. Therefore, this study aims to characterize early development of social and facial feature perception and the underlying brain regions in rhesus infants.

We conducted preliminary analyses of eye tracking and MRI data collected longitudinally in 6 male macaques (1 week - 6 months) living with their mothers in complex social groups. Each session included trials of 2 human faces representing extreme levels of trustworthiness. Looking behavior, including fixation to eye and mouth regions was characterized for trustworthy and untrustworthy faces. Structural MRI scans (T1 MPRAGE sequences (TR/TE = 2600/3.46msec, voxel size: 0.5x0.5x0.5mm)) acquired using a 3T scanner were used to characterize volumetric changes of the amygdala (AMY) and cortical grey matter of the insula, superior temporal sulcus, prefrontal cortex (BA46 & BA9) and inferotemporal area (TE), all areas involved in visual processing of "trust" in faces, and associations with looking behavior were assessed.

At 2 weeks of age, monkeys did not distinguish between the faces, looking equally to them ($p=0.07$), although greater looking to the trustworthy face correlated with smaller right AMY volumes ($r^2=-0.98$, $p=.01$). The ability to distinguish the faces emerged by 8 weeks, when infants looked more at trustworthy faces ($p=0.04$), explained by greater looking to trustworthy than untrustworthy eyes, and greater looking to eyes than mouth of trustworthy faces ($p=0.05$). At this age, there was a trend for an association between looking to trustworthy mouths and increased TE volume (left TE: $r^2=0.79$, $p=.059$; right TE: $r^2=0.79$, $p=.061$). This behavioral pattern was observed at 6 months, with no significant behavior-volume correlations.

Our results show a protracted development in the ability to distinguish between facial features that is predicted by different brain regions across age (AMY and potentially TE). They parallel the developmental trajectories of social visual engagement in human infants (Jones & Klin, 2013), and further validate rhesus monkeys as a translational model of early socioemotional development.

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**Poster**

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**Title:** Developmental changes in visual processing of faces: A combined eye-tracking and resting state functional connectivity study in infant rhesus macaques

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**Abstract:** The ability to attribute intentions and emotions in social interactions based on facial features (i.e. trustworthiness) is impaired in children with Autism Spectrum Disorder (ASD). Understanding the early developmental trajectories of this skill and the underlying brain circuits may further clarify the impaired socioemotional development in children with ASD. Due to limitations of repeated experiments of human infants, we are using a highly translational nonhuman primate model to characterize the early development of social and facial feature perception in parallel with the maturation of the underlying brain networks.
Eye-tracking (ET) data was collected longitudinally (14 times) from 1 week to 6 months in 16 male rhesus infants; here we are only presenting preliminary findings on those with MRI scans (n=6). In each session, monkeys were shown pairs of human faces, one with trustworthy (TW)

and one with untrustworthy (UTW) facial features. Fixations to eyes, mouth and whole face were compared across TW or UTW faces. Structural and resting-state fMRI (rsfMRI) scans were acquired 7 times using a 3T MRI scanner. We mapped developmental changes in functional connectivity (FC) between insula (INS), amygdala (AMY), dorso-lateral prefrontal cortex, superior temporal sulcus and inferotemporal cortex (TE). Associations between ET and rsfMRI data were assessed via Pearson's correlations.
At 2 weeks, there were no significant differences between fixations to TW and UTW regions; however greater looking to the TW faces was correlated with weaker FC between left INS and TE ($r^2$=-0.99, p=.005). The ability to distinguish TW and UTW emerged by 8 weeks, when infants looked more to TW than UTW faces (p=0.04), effect driven by more fixation time to the TW than either UTW eyes (p=0.05) or TW mouth (p=0.05). Greater looking to the TW faces correlated with stronger FC between INS and AMY in the left hemisphere ($r^2$=0.85, p=.003), whereas greater looking to the UTW faces correlated with stronger FC between these regions in the right hemisphere ($r^2$=0.82, p=.047). This pattern was stable through 6 months of age, although at that age greater looking to UTW eyes was associated with weaker FC between right TE and INS ($r^2$=-0.97, p=.002).
Our results suggest a protracted development in social and facial feature perception in infant rhesus macaques and underlying neural networks. These changes parallel typical development of preferential looking abilities in human infants (Jones & Klin, 2013). Results also showed a high conservation of brain regions responsible for facial feature perception in humans and macaques, further validating this model of early socioemotional development.

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**Title:** Plasticity for stimulus selectivity in the visual cortex of adult mice induced by patterned optogenetic stimulation

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**Abstract:** Plasticity in the functional connectivity of cortical neurons is needed for a large variety of adaptive behaviors that allow animals to thrive in a changeable environment. Spike-timing dependent plasticity (STDP) is considered a key biological process governing cortical synaptic plasticity. Although its properties have been extensively characterized in brain slices, it is still largely unknown whether it can change the functional architecture of in-vivo cortical networks of adult animals. In this study we first established an *in vivo* technique for simultaneous imaging and spatially-patterned optogenetic stimulation at the single-cell resolution based on a digital-micromirror-device (DMD). Using adult mice expressing G-CaMP8 and ChrimsonR in the primary visual cortex (V1, n = 6 animals), we paired DMD stimulation of an individual neuron (the “driver”) to a delayed stimulation (10 ms) of tens of surrounding neurons to induce driver-surround STDP (n = 9 experiments). We found that a significant fraction of neurons (268/457 neurons, 59%, $p$ = 4e-05) increased their response amplitude to the orientation orthogonal to the driver’s preferred orientation (166 ± 25% increase in amplitude, mean ± s.e., n = 457 neurons. $p$ = 2e-06), leading accordingly to an overall fractional increase of neurons with an orthogonal preferred orientation relative to the driver (from 26 ± 4% to 41 ± 5%, mean ± s.e., n = 9 experiments, $p$ = 0.02). This effect was not observed in control experiments where the driver and surround stimulation was not paired or where the order of pairing was reversed. Neurons shifting preferred orientation were characterized by weak responsiveness to visual stimulation. Further analysis with a decoding model based on demixed principal component analysis (dPCA) and linear support vector machine (SVM) classifiers revealed that, despite these plastic changes, the network as a whole retained its ability to process stimulus orientations thanks to a core group of unchanged and strongly visually-responsive neurons (average percentage change in correct decoding confidence across orientations: strong neurons, 10.0 ± 5.6%; weak neurons, 26.8 ± 21.2%, mean ± s.d.). In summary, our results show that plastic changes in recurrent connectivity centered solely on the activity of one or a few neurons can dramatically alter the stimulus selectivity of the whole network in a functionally predictable way via processes akin to STDP.

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**Title:** Increased use of peripheral vision is associated with increased functional connectivity between peripheral V1 and functionally specialized visual areas

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**Abstract:** Visual acuity in central vision is vital for everyday tasks such as recognizing faces and reading words. However, with central vision loss, such as in macular degeneration, individuals must rely more heavily on peripheral vision to perform everyday tasks. Previous work from our lab demonstrates that increased reliance on peripheral vision is associated with increased cortical thickness in areas of primary visual cortex (V1) that process peripheral vision. However, the impact of increased use of peripheral vision on the functional connectivity of V1 remains yet to be fully elucidated. Because macular degeneration patients rely heavily on peripheral vision, we hypothesized that areas of V1 involved in peripheral vision would possess enhanced functional connectivity to visual areas that are important for tasks that are typically performed with central vision. For example, Fusiform Face Area (FFA) and Visual Word Form Area (VWFA) are specialized for the processing of faces and written language, respectively, and would both likely possess altered connectivity to peripheral V1 with increased use of peripheral vision. To test our hypothesis, we used fMRI in 10 macular degeneration patients and matched controls in order to measure resting-state functional connectivity between peripheral V1 and functionally specialized visual areas, which were defined based on meta-analyses. Consistent with our hypothesis, macular degeneration patients showed stronger connectivity from peripheral V1 to functionally specialized visual regions, compared to healthy controls. This association likely reflects increased use of peripheral vision for everyday visual tasks. Overall, these results suggest that connectivity between visual cortex and higher order brain regions is influenced by the degree to which certain parts of the visual field are used. Together, these findings demonstrate a stable form of network plasticity that is observable at rest, even when no faces or words are present.

Furthermore, these findings help provide insight into the nature of visual cortical plasticity in the adult brain, a stage well beyond the “critical period” for visual development.

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**Poster**

**588. Visual Cortex: Development and Plasticity**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 588.30/FF17

**Topic:** D.07. Vision

**Support:** NIH Grant 1R01EY024678-01

**Title:** Experience-dependent development of PV neurons and impact on cortical processing in primary visual cortex

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**Abstract:** Activity of cortical inhibitory interneurons is rapidly reduced in response to monocular deprivation during the critical period for ocular dominance plasticity and in response to salient events encountered during learning. In the case of primary sensory cortex, a decrease in mean evoked firing rate of parvalbumin-positive (PV) inhibitory neurons is causally linked to a reorganization of excitatory networks following sensory perturbation. Converging evidence indicates that it is deprivation, and not an imbalance between open and closed eye inputs, that triggers rapid plasticity in PV neurons. However, this has not been directly tested in-vivo. Using two-photon guided cell-attached recording we examined the impact of closing both eyes for 24 hours on PV neuron response properties in mouse primary visual cortex. We found that binocular deprivation induces a 30% reduction in stimulus-evoked mean firing rate, and that this reduction is specific to critical period-aged mice. In contrast to evoked mean firing rate, measurements of trial-to-trial variability revealed that stimulus-driven decreases in variability are significantly dampened by deprivation during both the critical period and the post-critical period. These data establish that open-eye inputs are not required to drive the deprivation-induced weakening of PV neuron evoked activity that defines critical period plasticity, and that other aspects of in-vivo PV neuron activity are malleable throughout life. In addition, we are testing the hypothesis that molecular perturbation of PV neuron development impacts maturation of cortical processing as assessed by two-photon calcium imaging in wildtype and PV-ErbB4 knock-out mice.

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**Poster**

**589. Representation of Objects and Scenes**

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**Topic:** D.07. Vision

**Support:** ERC Grant 240-846300

**Title:** Figure-ground modulation in higher visual areas in mice

**Authors: *H. E. VAN BEEST**, A. BARSEGYAN, M. W. SELF, U. H. SCHNABEL, P. R. ROELFSEMA
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**Abstract:** One of the main questions in visual neuroscience is how an object is segregated from the background. Cells at early stages of the visual hierarchy receive inputs from a small region of the retina, knows as their receptive field (RF). Despite this, the activity of cells at these early stages can be modulated by contextual visual information from far outside the RF. An example of such a contextual effect is figure-ground modulation (FGM), in which the activity of a neuron in the primary visual cortex (V1) is enhanced when its receptive field falls on a figure-area of a stimulus, rather than on a background, even when the boundaries of the figure are well outside the RF. Although this modulation was first observed in monkeys, it has recently been shown by our lab that a similar modulation can be found in mouse V1. FGM occurs irrespective of the feedforward input to the neuron in V1, and it usually starts after the initial visual response onset to the stimulus (after 100ms). This has led to the idea that FGM in V1 is inherited from feedback projections coming from higher visual areas, which have larger receptive fields. The mouse-model provides many new technological possibilities, such as recording from specific cell-populations on a large scale, which makes it possible to identify cortical areas in which FGM occurs. In this study, we imaged through a clear-skull from four awake Thy1-GCaMP6 mice that were passively viewing orientation-defined figure-ground stimuli. Using this method, we could visualize the activity from excitatory cells in superficial layers of the entire cortex. Using population receptive field mapping, we were able to map out the locations of visually responsive areas in each mouse. Areas we could consistently map out in all four mice were V1, anterolateral (AL), lateral (LM), posteromedial (PM) and rostrolateral visual area (RL). As a measure of FGM, we calculated the difference in change in fluorescence (dFF) between the response to an orientation-defined figure compared to the response to a background. In all four mice we found FGM in V1 as expected. Higher visual areas that consistently showed FGM were AL, LM and PM. These findings suggest that FGM is broadly represented in higher visual areas surrounding V1, and does not seem to be limited to areas typically thought to belong to the ventral or ‘object’

pathway. Since GCaMP signals are rather slow compared to neuronal activity, additional proof to test the causal role of higher visual areas in FGM is needed.

**Disclosures: H.E. Van Beest:** None. **A. Barsegyan:** None. **M.W. Self:** None. **U.H. Schnabel:** None. **P.R. Roelfsema:** None.

**Poster**

**589. Representation of Objects and Scenes**

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**Topic:** D.07. Vision

**Support:** ERC Advance Cortic_al_Gorithms_240846300

**Title:** The role of the superior colliculus in figure-ground segregation and saccade planning

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**Abstract:** An important task of the visual system is to segregate objects from their backgrounds to guide behavior. Previous studies showed that neuronal activity in macaque primary visual cortex (V1) is modulated when the receptive field (RF) of a neuron falls on a figure compared to the background, a process called figure-ground modulation (FGM). FGM correlates with the percept of the animal and is modulated by behavioral relevance. We have recently shown that the pattern of FGM in V1 also impacts on the onset and end position of saccades that are planned towards a figure. It is unknown, however, how and through which pathways activity patterns in V1 can influence saccades. The superior colliculus (SC) is a good candidate, as this multi-layer subcortical structure receives visual input from V1 and is connected to motor-related structures. Here, we studied the role of the SC in the planning of saccades towards figure-ground stimuli. We investigated 1) whether FGM occurred in the SC, 2) whether the figure size affected FGM, 3) how the superficial and intermediate layers of the SC interacted to accomplish FGM read-out. Using Tungsten glass electrodes, single and multi-unit activity was recorded from the different layers of the SC of a monkey, while the animal performed a figure-detection task in which he had to make saccades towards the center of a figure. Stimuli were textured figures on a textured background. There were 16 possible figure positions. The RF of a neuron could fall on the background, the center or the edge of the figure. The 16th position was 180 degrees away from the RF and served as baseline. Figure sizes were 0.5, 1, 2 or 4 times the RF size. We found that FGM is present in SC, and that the presence of a figure is clearly signaled by the activity of single neurons. Compared to V1, FGM in SC is stronger and earlier and occurs for figure sizes that are much larger than the RF. The FGM profiles differed per layer. In the superficial layers we found a Gaussian-like profile for small figures (with the largest response for the center

position), while for larger figures the responses are strongest for edge positions and there is filling-in for the center positions. The intermediate layers only showed a center response. This suggests that the superficial layers have a detailed representation of the entire object, while the intermediate layers only represent the figure center, which is the target for the saccade. We conclude that the SC is involved in figure-ground segregation. The timing and the strength of FGM suggests that SC is involved early on and probably interacts with the visual cortex to accomplish FGM quickly after the appearance of a figure.

**Disclosures: A.F. Van Ham:** None. **M.W. Self:** None. **P.R. Roelfsema:** None.

**Poster**

**589. Representation of Objects and Scenes**

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**Topic:** D.07. Vision

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**Title:** Modeling spike synchrony in the visual cortex for figure-ground organization

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**Abstract:** Border ownership indicates which side of a contour owns a border, it is a precursor of figure-ground organization. A large number of neurons in primate cortical area V2 exhibits border ownership selectivity (Border Ownership Selective (BOS) neurons; Zhou et al, J. Neurosci., 2000). Theoretical work shows that modulatory common feedback may underlie the observed synchrony between pairs of neurons with consistent border ownership preferences, i.e. with both neurons in the pair responding to the same visual object (Wagatsuma et al., J. Neurophysiol., 2016). Here we extend this model to explain synchrony observed between neurons with non-consistent BOS (Martin and von der Heydt, J. Neurosci 2015). In our model, the responses of BOS neurons are modulated by the activity of Grouping (G) neurons which receive their input from BOS neurons and whose activity represents the location of visual objects in the scene (Craft, et al., J. Neurophysiol., 2007). The G neurons provide modulatory feedback to BOS neurons according to their preferred radius (Mihalas et al, PNAS, 2011). The BOS neurons have different border ownership preferences, share their receptive field, and interact with one another through inhibitory cells. Simulations of the network model are in overall agreement with the experimental findings.

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**Poster**

**589. Representation of Objects and Scenes**

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**Topic:** D.07. Vision

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**Title:** Neural sensitivity to concavity and convexity of spatial boundary cues

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**Abstract:** Research in both humans and animals has collectively pointed to the importance of spatial boundary cues for an organism to regain its bearings after disorientation; however, little is known about what visual features intrinsic to a boundary can inform our location in an environment. A recent study proposes that mid-level visual features such as junction type and angle are important for scene categorization (Walther & Shen, 2014). In this study, we ask 1) whether there is a neural sensitivity that distinguishes concave and convex boundaries whose parts conjoin at different junction types and angles, and 2) whether sensitivity to junction angles is dependent on the boundary type (concave vs. convex). As these boundary features provide cues to the viewer's position within an environment, we hypothesize that scene-selective brain regions will be sensitive to variations of these properties.
To test this idea, we created artificial scene images of wall boundaries that vary in both Boundary Type (Concave or Convex) and Junction Angle (45, 90, 135). There were 7 stimulus conditions: Concave 45, Concave 90, Concave 135, Flat 180, Convex 135, Convex 90, Convex 45. Participants (N=10) viewed stimuli in blocks of 12s while performing a one-back repetition detection task in the fMRI scanner. We measured both univariate and multivariate response in scene-selective brain areas. Two-way within-subject ANOVA (Boundary Type X Junction Angle) revealed a main effect of Junction Angle in the univariate response of both parahippocampal place area (PPA) ($F(2,18)=4.165$, $p=.044$) and occipital place area (OPA) ($F(2,18)=14.569$, $p=.002$), suggesting a sensitivity to angular changes in both concave and convex boundaries. Importantly, there was a marginally significant interaction between Boundary Type and Junction Angle in OPA ($F(2,18)=3.755$, $p=.054$), showing a greater sensitivity to differences of junction angle (45, 90, 135) in concave boundaries than in convex boundaries. This suggests that OPA is highly sensitive to boundary features that are locally relevant to navigation, as concave boundaries surround the viewer and restrict navigation while convex boundaries don't. Moreover, using a linear SVM classifier on multi-voxel patterns of

activation, we found that OPA shows a significantly higher classification accuracy for Junction Angle within concave boundaries than within convex boundaries ($t(9)=2.451$, $p=.037$). Together, our study suggests that scene-selective brain regions have high sensitivity to boundary types (concave vs. convex) and junction angle, which are properties that can inform the viewer's location relative to boundaries and guide navigation.

**Disclosures: R. Cheng:** None. **D.B. Walther:** None. **S. Park:** None.

**Poster**

**589. Representation of Objects and Scenes**

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**Program#/Poster#:** 589.05/FF22

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

**Support:** Pascal ERC grant

**Title:** Dissecting Infants' comprehension of arrow-like icons, an innate shift from sensory to symbolic system

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**Abstract:** Arrows are one of the most widespread icons across cultures, which is classically considered to be a symbol and has been discussed to be acquired at around 3-5 yrs of age. In a series of eye-tracking studies in 4- and 8-month-old infants we suggest that the role of this icon is possibly not acquired through culture, but on the other hand this icon triggers the attentional system through its unique visual features, even after a long one-second blank delay between icon offset and target. By systematically changing the visual features of this icon, we showed that a triangle alone is enough for reorienting the attention; furthermore by analyzing the spatio-temporal dynamics of the gaze pattern over the different icons and during the delay-period between icon and target, and by comparing it with the identical results from adults, using a generalized linear model, we propose a bottom-up framework for an innate attention-reorientation system based on the gradient of salience in icons, which later accommodates a symbolic comprehension of arrows in toddlers. Furthermore, we are extending the experiment to macaques and an MEG design in adults to acquire a more coherent framework for re-orientation of attention induced by the vertices of triangular-like icons.

**Disclosures: M. Ekramnia:** None. **J. Mehler:** None.

**Poster**

**589. Representation of Objects and Scenes**

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**Topic:** D.07. Vision

**Support:** CIHR

NSERC

**Title:** Contour integration as a function of collinearity, task, and age: An EEG study

**Authors: *A. HASHEMI**[1], J. N. CALI[1], E. ROUDAIA[2], P. J. BENNETT[1], A. B. SEKULER[1]
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**Abstract:** Shape perception requires detecting local orientation information and integrating elements along a contour, segregated from its background. One can assess contour integration by asking observers to detect a collinear set of Gabors embedded within an array of randomly-oriented Gabors. Contour integration is best when contour elements are collinear, and the extent to which deviations from collinearity can be tolerated provides a measure of contour integration ability. Older adults (OA) tend to be worse than younger adults (YA) at extracting contours from dense clutter; however, aging does not affect the sensitivity to collinearity (Roudaia et al., 2013). Collinearity also modulates neural responses to contours: N1 amplitudes are larger to high collinear (HC) contours relative to low collinear (LC) contours (Mathes et al., 2006; Shpaner et al., 2013), although this effect may only exist when the contour is task relevant (Volberg & Greenlee, 2014). Here, we asked if the collinearity ERP effects are modulated by task relevance *per se*, or by a lack of awareness of the contours. We also examined how collinearity ERP effects were modulated by age. Our stimuli were high-density Gabor arrays containing: 1) 8 Gabors positioned to form either a LC or HC contour within distractor Gabors; and 2) one Gabor of noticeably higher contrast than the others. Observers (12 younger, 12 older; mean ages 22.8 and 66.4 years) first completed trials in which they detected the high contrast Gabor without being told about the contour. In the second block, observers detected the tail of the contour, ignoring the high contrast Gabor. In the final block, observers completed the Gabor detection task again, but this time were aware of the presence of the contour. During the contour task we found that N1 amplitude in both age groups was greater for HC than LC contours, although the effects of collinearity were larger and more sustained in OA. When observers were unaware of the contours, we found no significant effect of collinearity on N1 amplitude in either age group. Critically, when the contours were task irrelevant, but observers were aware of them, we found no effect of collinearity on N1 amplitude in either age group, although there were significant age-related collinearity differences in later ERP components. Our results support the view that

the collinearity effect on N1 amplitude results from active top-down processing as opposed to stimulus-driven signals. The larger effect of collinearity in ERPs in older adults may indicate the need for stronger top-down modulation to generate behavioural performance similar to younger adults, indicative of declines in contour integration with healthy aging.

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**Poster**

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**Topic:** D.07. Vision

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Whitehall 2015-12-120

**Title:** 2D vs. 3D shape processing in area V4

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**Abstract:** Area V4 is a pivotal intermediate stage in transforming image information into knowledge about object structure and identity. V4 neurons are known to be sensitive to binocular disparity and 3D orientation. However, previous studies of more complex shape processing in V4 have focused on 2D shape. Here, we studied the relationship between 2D and 3D shape information in area V4. This experiment addresses fundamental questions about the transformation from 2D image signals to 3D shape perception. Are 2D contours and 3D surfaces processed in parallel? Or is shape processing inherently 3D from the start? Does tuning for 2D contours define V4 neurons? Or is it merely a slice through 3D shape tuning? We used microelectrode recording in awake fixating macaque monkeys to analyze the tuning of individual V4 neurons for complex 2D and 3D shape. Shape stimuli were constructed by combining 3D medial axis components with randomized connectivity, orientation, curvature, and surface structure. 3D stimuli were rendered with shading of matte surfaces or specularity of glossy surfaces. 2D stimuli were positive and negative contrast silhouettes with the same contours. Stimuli were flashed in random order inside the receptive field. After an initial generation of random stimuli, subsequent generations included partially morphed descendants of higher response ancestor stimuli. Thus, successive generations evolved toward denser sampling in and near the neuron’s response range. Stimuli evolved within two independent lineages so that tuning

models could be cross-validated between lineages. We parameterized stimulus shapes in terms of local geometric measures on contours, surfaces and medial axes. Dense sampling produced a point cloud description of each stimulus in a multi-dimensional geometric space. We fit multi-dimensional Gaussian models to describe tuning for 2D and 3D shape and predict responses across lineages. Consistent with previous findings, neurons were reliably tuned for local shape fragments with specific intra-object positions, orientations, and curvatures, reflecting parts-based ensemble coding of global shape. Early results suggest that neurons responding to contour fragments also respond to corresponding surface fragments at rotations for which the contour information disappears and only depth cues remain. Thus, 3D shape models might be more explanatory of V4 responses, and responses to 2D contour shapes might be explained by slices through these models. This would suggest that shape processing is already inherently 3D in area V4, two stages removed from primary visual cortex.

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**Poster**

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Alfred P. Sloan Research Foundation

NIH Grant DC014305

**Title:** Cue integration for 3D surface orientation perception in macaque monkeys

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**Abstract:** Three-dimensional (3D) vision is essential for successful interactions with the world. To create accurate and precise 3D visual percepts, the brain integrates perspective and stereoscopic cues. Behavioral studies have shown that humans integrate these cues in a Bayes optimal fashion to estimate 3D surface orientation. The neural basis of 3D vision is widely studied with non-human primates (NHPs), but a tacit assumption of many studies is that the 3D visual perceptual processes of humans and NHPs are analogous. Here we test this assumption through psychophysical experiments with rhesus macaques. Theoretical and human perceptual studies show that the reliability of perspective information increases with orientation in depth (slant) and that the reliability of stereoscopic information decreases with distance. To manipulate

the reliability of perspective and stereoscopic cues, we varied the slant (rotation about an axis perpendicular to the line of sight) and distance of viewed planar surfaces. The animals were trained to report the tilt (rotation about the line of sight) of a center-fixated plane in an eight alternative forced choice (8AFC) task. Tilt ranged from 0° to 315° in 45° steps, and slant varied between 15° and 60° in 15° steps. The planar surfaces were presented at: 37, 57, 77, 87, 107, and 137 cm from the animal. To assess tilt sensitivity based on perspective and stereoscopic cues, as well as the integration of these cues, three sets of planar surfaces defined by random dot patterns were presented: mixed-cue stimuli (with perspective and stereoscopic cues), perspective-only stimuli (monocular presentation of otherwise mixed-cue stimuli), and stereoscopic-only stimuli (binocular presentation of stimuli with no perspective cues). Sensitivity was assessed as the width of probability density functions over the reported tilt. Consistent with previous human studies, sensitivity to perspective cues increased with slant and was independent of distance (after controlling for dot size). Sensitivity to stereoscopic cues decreased with distance from the fixation plane, but increased with slant. Bayes optimal predictions of the mixed-cue sensitivities were computed from the single-cue data, and compared to the measured mixed-cue sensitivities. Strong agreement was found between the measured and predicted sensitivities ($r = 0.97$, $p < 0.01$ Pearson correlation), which were tightly clustered around the identity line. These results indicate that, analogous to humans, accurate and precise 3D visual perception in NHPs is achieved through the integration of perspective and stereoscopic information.

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**Topic:** D.07. Vision

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Alfred P. Sloan Foundation

**Title:** Probabilistic mapping of 3D visual cortical circuits in the macaque monkey

**Authors: *T.-Y. CHANG**[1], N. A. KAMBI[2], E. KASTAR[2], J. PHILLIPS[2], Y. B. SAALMANN[2], A. ROSENBERG[1]
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**Abstract:** The transformation of two-dimensional (2D) retinal images into three-dimensional (3D) visual percepts is a fundamental function of the visual system. However, the cortical pathways supporting this transformation are largely unknown. Here we combine structural and diffusion magnetic resonance imaging methods to probabilistically map putative 3D visual cortical pathways in the macaque monkey. Scans were performed using a GE MR750 3T scanner and either 8- or 16-channel receive-only head coils. First, T1-weighted structural scans were registered with the F99 atlas using surface-based registration in the CARET software to define cortical regions of interest (ROIs). Probabilistic tractography was then performed in a pairwise fashion between 'seed' and 'target' ROIs predominantly located in the dorsal visual pathway using high-resolution diffusion-weighted scans (1 mm isotropic; 60 diffusion directions, b=1000 s/mm$^2$, NEX=10) with the FSL software. The initial seed ROI was the caudal intraparietal area (CIP), which is known to represent 3D object pose. Connectivity was assessed as the percentage of voxels in the seed ROI with tracts terminating in the target ROI. Consistent with previous anatomical data, connectivity between V3A (seed) and CIP (target) was observed (59%). We further found that V3A was strongly connected with the posterior intraparietal area (PIP) (65%) and that PIP was more strongly connected with CIP (68%) than V3A was to CIP. This finding suggests that PIP may be an intermediate stage between V3A and CIP in the 2D-to-3D visual transformation. The current tractography results together with previous anatomical and electrophysiological data suggest that the 2D-to-3D visual transformation is supported by the following hierarchical circuit: V1 -> V2d -> V3A -> PIP -> CIP. Strong connectivity was also observed between CIP, the lateral intraparietal area (LIP), and anterior intraparietal area (AIP). This pattern of connectivity is consistent with a circuit supporting 3D decision making and action. We additionally found strong connectivity between CIP and retroinsular cortex (Ri) (40%), a site of visual-vestibular integration within the visual posterior sylvian (VPS) that is implicated in verticality perception. To our knowledge, this is the first indication of connectivity between CIP and VPS, which may be the basis of gravity-centered visual representations previously found in CIP. The present tractography results suggest that the cortical circuitry supporting 3D vision is broader and more complex than previously recognized, and can guide future investigations into the neural basis of 3D visual perception.

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**Poster**

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**Title:** Object recognition based on informative object components

**Authors: *T. S. ALTAVINI**[1], G. ASTORGA[1], D. HARARI[2], S. ULLMAN[2], G. N. REEKE[1], W. FREIWALD[1], C. D. GILBERT[1]
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**Abstract:** The image properties involved in object recognition include image components that are most informative for distinguishing between objects. The components used for recognition may be reflected in the stimulus selectivity of neurons at different stages of the ventral visual pathway. We therefore endeavored to develop a set of visual stimuli that was curated according to the psychophysics of object recognition and according to the object set that an animal is trained to recognize, with the idea that the features represented in the brain are based on their ethological value. Macaques were trained on a match to sample task: after being shown a cued object, using a touch screen monitor, they responded to presentation of the same or different objects, or object components that either belonged to the cued object or did not, by touching a green “match” spot or red “non match” spot. The object components were generated by making successively smaller crops of the original image from different regions of the object. The animals were readily able to associate even small object components, comprising as little as ~20% of the original image, with the object from which they were taken. As one moves the cropping window around the object image, we find that there are hot spots that are more conducive to recognition of the full object than adjoining regions. Further cropping within these regions, however, leads to a loss of recognition performance, suggesting that there is a minimal level of inclusion of object features that the animals need in order to recognize an object. Images may contain more than one hot spot and the level of cropping that leads to a loss of recognition may vary between them. These findings in a non human primate mirror those seen in human populations, where minimally independently recognizable components (MIRCs) constitute the elements used for object recognition. We anticipate that such components will therefore be useful for studying the shape selectivity of neurons at different levels of the visual pathway.

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**Poster**

**589. Representation of Objects and Scenes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 589.11/GG6

**Topic:** D.07. Vision

**Support:** Leon Levy Fellowship to GA

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**Title:** Adaptive processing and top-down influences in areas V1 and V4

**Authors: G. ASTORGA**[1], Y. YAN[2], W. LI[2], *C. D. GILBERT[1]
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**Abstract:** Neurons in the primary visual cortex (V1) are adaptive processors, carrying different information when one shifts between perceptual tasks. To determine whether this phenomenon extends to other visual cortical areas, and to explore the character of the information that is conveyed in a top-down fashion from higher to lower order cortical areas, we implanted electrode arrays in V1 and V4 in a Macaque trained on a dual task paradigm. Here the animal was cued to perform either of two discrimination tasks with the identical 5 line visual stimulus - a 3 line bisection task or a vernier discrimination task. As we have found previously in V1, neurons in V4 show differential tuning to the identical stimulus attributes (flanking bar position or collinear bar position) in the two tasks, with strong modulation by task relevant inputs and weak modulation by task irrelevant inputs. Interestingly, the difference in tuning is itself very different in correct versus incorrect trials. The arrays were designed with longer electrodes implanted in V4 so we could measure the task dependent properties in deep layers, in order to study the neurons most likely to provide feedback from V4 to earlier visual cortical areas. The receptive fields of the V4 neurons changed their structure with task, showing a non-uniform responsiveness to test probes flashed in different positions within their RFs. These V4 neurons also showed a high level of activity between trial onset and stimulus presentation, potentially providing the prestimulus signal to V1 as to the identity of the task to be executed.

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**Poster**

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**Topic:** D.07. Vision

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R01 NS40596

**Title:** Intrinsic neural spaces from human electrocorticography

**Authors: *G. W. MILSAP**[1], M. J. COLLARD[2], K. RUPP[3], M. J. ROOS[4], C. CACERES[4], C. RATTO[4], M. WOLMETZ[4], N. E. CRONE[5]
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**Abstract:** Recent work in neuroimaging and electrophysiology has demonstrated macroscopic organization of semantic attributes in human cortex. The degree to which these semantic attributes are associated with a given stimulus can be assessed through the use of a semantic space—an embedding of words in a vector space that seeks to maintain spatial proximity of words that are semantically linked. Many such spaces exist, each with its own construction and characteristic dimensionality. However, these spaces are typically not subject-specific; for example, many are built from word co-occurrences in large text corpora. Recently, the notion of an internal state space has provided a fruitful framework for investigating neural population dynamics. We hypothesized that a space generated from a subject's own neural activity—an intrinsic feature space based on patterns of neural responses—might similarly provide a means of meaningfully describing population activity without proscribed semantic axes.
We recorded electrocorticography (ECoG) signals while a subject performed a picture naming task composed of simple line drawings of objects, and used cue-locked traces of high gamma (70-110 Hz; HG) power to construct a stimulus-by-stimulus neural response similarity matrix for each electrode. We then embedded the stimuli into a vector space by applying multidimensional scaling to these similarity matrices. Using data recorded from an independent picture naming task wherein a different set of full color images of objects was presented, we trained a linear model to map from electrode HG power to this intrinsic neural space. To test the model, held-out trials were projected into the intrinsic space and compared to stimuli in the corpus. The mean rank accuracy of held-out objects was significantly above chance, suggesting that the specific organization of intrinsic neural space facilitated generalization of information to stimuli unseen by the decoder in a dataset foreign to the space's construction.

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**Topic:** D.07. Vision

**Title:** Investigating the spatiotemporal dynamics of human visual category processing with intracranial EEG

**Authors: *M. J. BORING**[1], Y. LI[4], N. M. BRUNET[5], M. J. WARD[2], M. RICHARDSON[3], A. S. GHUMAN[3]
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**Abstract:** It has been known for centuries that damage to circumscribed brain regions can cause category-specific deficits in perception. This has led to an extensive search to build maps of category selective regions in the brain. Less is known about the spatiotemporal dynamics of visual category processing and the stages of this information processing. To help elucidate the spatiotemporal dynamics of visual object recognition, 25 patients with intractable epilepsy were presented images of faces, bodies, houses, hammers, words, or scrambled objects while intracranial electroencephalography (iEEG) data was collected from a total of 2,464 electrodes distributed across the cortex. Multivariate classification and time series analyses were applied to these data to produce movies of the dynamics of category sensitivity across the regions covered by these electrodes. Of these electrodes, 195 showed significant decoding accuracy at a conservative multiple comparisons corrected statistical threshold for at least one stimulus category at some point after stimulus presentation. Onset of category sensitivity began around 100 ms with peak sensitivity at 220 ms distributed across the cortex, concentrated in the ventral visual stream. Additionally, many locations in the ventral visual stream continued to show sensitivity beyond 600 ms post stimulus presentation, providing strong evidence of extended information processing dynamics. Object sensitive electrodes had a clear organization with houses represented medially, while words and faces were represented laterally. In addition to this, several electrodes were sensitive to more than one category and some of these electrodes had different time-courses of sensitivity between categories. Further analyses show the functional connectivity dynamics of these object-sensitive regions (time evolving graphs) and use time series approaches to model processing dynamics in a data-driven manner. Taken together, these results illustrate critical principles regarding extended neural information processing dynamics, information flow, and suggests multiple stages of processing underlying visual object processing and recognition.

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**Support:** NIH Grant 1R01MH109954-01

**Title:** Mathematical processing of differential visualizations of numbers

**Authors: *S. R. BAEK**, A. L. DAITCH, J. PARVIZI
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**Abstract:** Our prior observations with intracranial recordings have confirmed the presence of anatomically specific regions along the ventral temporal cortex (VTC) and lateral parietal cortex (LPC) within the human brain that are involved in mathematical cognition (Shum et al 2013; Daitch et al. 2016). However, it remains to be determined if the format of numerical symbols (e.g. numerals such as '5' or number words such as 'five') influences the way these brain regions process mathematical information. To address this issue, we collected data during two mathematical experiments from subjects implanted with electrodes in the ventral temporal cortex (VTC) and lateral parietal cortex (LPC) using electrocorticography (ECoG), taking advantage of its millimeter-scale anatomical precision and millisecond-scale temporal resolution. In the first task, subjects evaluated arithmetic statements with digits (e.g. "2+2=4") or autobiographical memory statements (e.g. "I ate fruit today") in a control condition. In the second task, subjects again evaluated arithmetic statements, but which were presented either in the form of digit numbers (e.g. "2+2=4") or textual numbers (e.g. "two plus two equals four"). From the first task, we replicated the existence of math selective regions in the VTC and LPC as established by previous research, which are selectively active during the math but not the memory condition. We then compared the responses to the two representations of numbers of each of these two math regions and found that they showed activation for math calculations regardless of the number format. Our results illustrate that while there are brain regions sensitive to visual object categories (e.g. numbers versus words), math-selective regions within the VTC and LPC appear to be engaged in quantity calculations regardless of the format in which the quantities are presented. This work provides important information about the mechanisms of arithmetic processing within the human brain.

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**Topic:** D.07. Vision

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**Title:** Abstractness of value representation in orbitofrontal cortex

**Authors: *T. YOSHIMOTO**[1,2], J. CHIKAZOE[1,2], S. OKAZAKI[1,3], M. SUMIYA[1,2], H. K. TAKAHASHI[4], E. NAKAGAWA[1,2], T. KOIKE[1,2], R. KITADA[1,2,5], S. OKAMOTO[6], M. NAKATA[7], H. KOSAKA[8], T. YADA[7], N. SADATO[1,2]
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**Abstract:** Human behavior is shaped by the evaluation of the external environment. Such evaluation is influenced by not only the contents of sensory stimulus but also the internal states of oneself. For example, it is a common experience that hunger makes trivial food look very attractive. The change of metabolic states modulates the value of the very same food, accompanying the change of its neural representation. Although previous fMRI studies have shown that the orbitofrontal cortex (OFC) represents the value of objects, it is unknown whether the OFC representations preserves the individual and concrete content (hereinafter referred to as "stimulus identity"). Because our previous study implied that representation maps increased in abstraction from physical features to object categories to subjective affect along a posterior-to-anterior neural axis, we hypothesized that the OFC might represent abstract value information without preserving stimulus identity. To test this hypothesis, we conducted a functional MRI experiment to estimate the stability of the neural representation of food stimuli in the OFC. Twenty four healthy male adults rated the value of 128 food pictures during the fMRI sessions before and after breakfast. The same experimental procedure was repeated on the separate experimental day (the interval: 17.0±9.0 (mean±SD)). This procedure enabled us to estimate the dissimilarity of the behavioral and neural data between the same metabolic states as well as between different states. If the OFC contains the stimulus identity, the OFC representations should be stable, at least to some extent, even if the value of objects changed, accompanied by the metabolic state change (satiated vs. hungry). If this is the case, neural representations of the same food should be similar both in the different (hungry-satiated) and the same (hungry-hungry or satiated-satiated) metabolic state comparison. The self-reported appetite scores for each food picture significantly decreased after having breakfast. Multivoxel pattern analysis revealed that the OFC activation patterns for the same object were similar only when the metabolic states were similar. In contrast, the fusiform gyrus (FFG) activation patterns for the same object were similar irrespective of metabolic states, suggesting that stimulus identity is represented by the FFG but not the OFC. Furthermore, the change of the activation patterns of the OFC was associated with the change of subjective value accompanied by the metabolic state change. Our findings suggest that the OFC represents abstract value information without preserving stimulus identity.

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**Poster**

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**Title:** Typical real-world locations impact object coding across the visual field

**Authors: *D. KAISER**, M. MOESKOPS, R. M. CICHY
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**Abstract:** Visual information in our everyday environments is structured. Objects often appear at typical locations in space: for example, lamps hang from the ceiling, whereas carpets lie on the floor. As a consequence, these objects repeatedly occupy similar visual field locations. The long-term experience with such spatial regularities prompts the hypothesis that the visual system is tuned to such retinotopic object locations. A key prediction is that typically positioned objects should be coded more efficiently. To test this prediction, we recorded electroencephalography (EEG) while participants viewed briefly presented objects appearing in their typical locations (e.g., an airplane in the upper visual field) or in atypical locations (e.g., an airplane in the lower visual field). Multivariate pattern analysis applied to the EEG data revealed that typically and atypically positioned objects evoked reliably different response patterns. This difference was related to enhanced processing for typically positioned objects: object identity could be decoded more accurately for typically positioned objects, as compared to atypically positioned objects. Crucially, this difference emerged within the first 200ms of visual processing and was most pronounced when the objects were best discriminable, suggesting that early object processing is tuned to typical retinotopic locations. Our results thus confirm the prediction that long-term experience with objects occurring at specific retinotopic locations leads to enhanced processing when these objects appear in their typical locations. The observed processing enhancement may indicate a neural mechanism for efficient natural scene processing, where a large number of regularly positioned objects needs to be processed.

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**Title:** Mental rotation around the cardinal axes

**Authors: *B. P. GEE**, S. CONGDON, C. AQUINO, B. ROMAGNA
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**Abstract:** We heavily rely on perceptual, kinesthetic, and motor faculties to navigate through different environments (Fleishman & Rich 1963). Spatial reasoning is a central cognitive process, which assists in our understanding and interactions with external stimuli. Past studies have indicated a visualization preference of objects when imagined around the vertical axis (Z) compared to other orientations (Battista & Peters 2010). Here, we considered all three cardinal axes (X, Y, Z), both accuracy and speed, and more familiarity with the task to allow for optimal performance. A standard mental rotation task (Shepard & Metzler 1971) was used to test spatial reasoning speed and accuracy. For each trial, a pair of three-dimensional shapes was presented on a computer monitor. Human participants indicated, with a button press, whether the two geometric shapes were identical or mirrored images. The paired stimuli differed in orientation by displacement (disparity angle) around one of the cardinal axes. Disparity angles (40, 80, 120, 160 deg) were randomly varied across trials. A total of 432 trials were divided into six blocks, which were either homogenous (one axis of rotation) or heterogeneous (any axis for any given trial, randomly presented). Odd-numbered blocks were homogenous, and even-numbered were randomly ordered heterogeneous blocks (X, Y, or Z). The results indicated that homogenous trials were easier than heterogeneous blocks. Regardless of block type, subjects demonstrated the best accuracy and speed during Z trials, followed by Y and X, respectively. Across disparity angle, accuracy and speed reliably decremented, except Z trial performance was more resistant to this variable. Superior mental rotation ability with rotations around the Z axis may be attributed to the prevalence of vertically oriented objects in the natural environment (Waszak et al. 2005). This added experience could optimize mental templates and schemas in regards to expectations of what objects should look like (Tarr & Pinker 1989). The current experiment allowed for extensive practice with the task, and all trial types reached asymptotic performance. Nonetheless, vertically-based rotations may reflect real-world experiences, resulting in easier adaptations to viewpoint changes.

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**Title:** A role for parietal area LIP in goal oriented object recognition

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**Abstract:** Visual object recognition in primates is an efficient and reliable cognitive ability. Psychophysical studies have shown that flexibility, efficiency and performance of visual object recognition is achieved by the representation of shape similarities as opposed to the decomposition and representation of parts of shapes. Stable versions of such neural representations have been found in the ventral pathway of non-human primates. However, some aspects of visual object recognition require dynamic comparisons of shape similarity in the context of goal oriented tasks. This form of representation is more likely to appear in an area that can integrate bottom-up sensory with top-down task relevant information. We tested whether neurons in the lateral intraparietal area (LIP) of posterior parietal cortex could fulfill this role by collating information from object specific similarity map representations to allow general decisions about whether a stimulus matches the object being looked for. Specifically, we hypothesized that the responses of LIP neurons should represent the relative similarity of each stimulus to the sample in our match to sample task and this should not depend on stimulus identity. We found that when animals compared two peripheral stimuli to a sample at their fovea, the response to the matching target remained stable, but the response to the distractor depended on how similar it was to the sample: the more similar, the greater the response to the distractor. These results could not be explained by standard decision making activity in LIP or by task difficulty. We propose that LIP integrates incoming visual information, including that from the ventral stream about object identity, to create a dynamic representation that is concise, low dimensional and task relevant, and which signifies the choice priorities in mental matching behavior.

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**Topic:** D.07. Vision

**Title:** Knowledge of physical properties aids visual search for real-world objects

**Authors: *L. GUO**, S. COURTNEY, J. FISCHER
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**Abstract:** Finding a missing earring in a jewelry box can be a frustrating challenge, but it becomes easier if the earring differs in color, shape, or size from other items in the box. These visual attributes and many others help to guide our attention toward items of interest. Does our

knowledge of objects' physical properties - e.g., that the earring is hard, smooth, and dense - also aid visual search? Would we be faster to locate the earring if it appeared among soft objects rather than hard ones? We conducted three experiments to test whether observers can use their physical knowledge about everyday objects to guide their attention in visual search. In Experiment 1, we presented participants with search arrays comprising sixteen objects. The objects were rated on perceived hardness by a separate group of online participants, and in each search array a target object was paired either with 15 distractors of similar hardness (e.g., soft target among soft distractors) or 15 distractors of different hardness (e.g., soft target among hard distractors). Participants ($n$=24) were asked to find the target object among the distractors after viewing a word label of that target for 1s. They pressed a key after locating the target and then indicated the target location with a mouse click. We found that participants were faster to locate a target object when it appeared among distractors of different hardness (1.28s±0.05) vs. distractors of similar hardness (1.64s±0.08; $t(23)=5.04$; $p<0.001$). Critically, this effect was intact after controlling for any influences of image luminance, contrast, color, shape, and semantic content, and was present despite the fact that in follow-up questioning, no participant reported paying attention to the hardness of the objects. In Experiment 2, the same experiment was conducted with a separate group ($n$=24) with the addition of eyetracking, and the reaction time results replicated those of Experiment 1. Further, the eyetracking data revealed that participants fixated on significantly fewer distractors when the distractors were of a different hardness than the target ($t(23)=-6.07$; $p<0.001$) - observers avoided wasting fixations on items of the wrong physical property. In a final experiment ($n$=24), we further controlled for local texture cues by presenting line drawings of objects rather than photographs. We nonetheless observed the same significant effects as in Experiments 1 & 2. These findings collectively demonstrate that observers can use their knowledge of objects' physical properties to guide their visual search toward likely targets, and they point toward an important role of physical knowledge in guiding how we engage with visual scenes in daily life.

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**Title:** Using EEG to compare brain responses to graspable real-world objects versus 2D pictures

**Authors: *F. MARINI**[1], K. A. BREEDING[1], J. C. SNOW[2]
[1]Psychology, Univ. of Nevada, Reno, NV; [2]Psychology, Univ. of Nevada Reno Dept. of Psychology, Reno, NV

**Abstract:** Visual perception of objects has been investigated traditionally using 2D images. Importantly, however, unlike images, real-world objects provide observers with the potential for manual interaction. In line with this distinction, recent behavioral and fMRI research indicates that real objects may be processed and represented differently to 2D images, although the mechanism for these effects remains unknown. Here, we compared electrophysiological brain responses for real objects with matched pictures to examine whether the underlying temporal dynamics of brain activation differed across the two display formats. We hypothesized that the real objects would be associated with stronger motor preparation signals in dorsal cortex than pictures. Using high-density EEG, occlusion spectacles, and a custom-built experimental apparatus, we recorded brain responses to visual stimuli consisting of 96 real-world graspable objects and 96 2D photographs of the same items printed in high-resolution. We found significant reductions of event-related power for real objects versus pictures over bilateral centro-parietal electrodes in the mu frequency band, consistent with enhanced motor preparation processes. The temporal dynamics of recruitment of sensorimotor regions in the dorsal stream are different for real objects than 2D pictures.

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**Title:** The development of action encoding in learning a novel prehistoric task

**Authors: *L. A. WHEATON**[1], A. Y. BAYANI[2], N. NATRAJ[3], N. KHREISHEH[4], D. STOUT[4]
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**Abstract:** Stone toolmaking is a unique human motor skill that dates back to prehistoric times and involves the intentional shaping of a stone into a tool used for daily living. Neuroimaging studies demonstrate brain activation in areas associated with prehension, planning, and

visuospatial processing during execution and observation. Trained individuals exhibited brain activation in areas associated with saccades, visual learning, and visual attention while experts showed additional activation in areas that support stimulus driven visuospatial sequence learning. Due to the recruitment of these brain areas related to visual encoding related with learning, the purpose of this study is to determine if training causes changes in gaze patterns that may reveal the understanding of an underlying action structure and the anticipation of motor intent during observation of the task. All subjects' gaze positions were collected with an eye tracker while viewing an expert creating a stone tool in an egocentric perspective. Gaze behavior was collected in three separate occasions: naïve (0 hours of training), post 1 (50 hours of training), post 2 (100 hours of training). During each session, subjects were instructed to indicate correct and incorrect movements during action observation. With training, the subjects' perception of correct movements coalesced on common time points. Furthermore, subjects' gaze patterns exhibited greater complexity that matched the underlying action structure of the task and visual encoding of motor intent. This work demonstrates a change in action perception, notably the development of action syntax through motor learning

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**Title:** Visual encoding of context and grasp posture throughout adolescent development

**Authors: *A. Y. BAYANI**[1], L. A. WHEATON[2]
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**Abstract:** Learning how to plan and initiate the motor commands based on the visual scene is a skill that is gradually acquired in a variety of situations and contexts. Eye tracking has been shown to be a powerful tool in predicting motor intent through patterns of fixations and saccades when observing or evaluating motor tasks. In a tool-object scene, gaze patterns can be used to classify salient features that shape task facilitation, namely the operant end of the tool (e.g., bowl of a spoon) and objects (e.g., sugar dish). Grasp posture has been shown largely irrelevant in understanding tool-object motor intent. However, most neurobehavioral studies on grasp focus on simple reach and grasp paradigms. End state comfort (ESC), the ability to adapt the initial

hand posture to ensure a comfortable end posture, may reveal novel mechanisms of action intent that promote a deeper understanding of action planning. The purpose of this study is to determine how context and grasp posture affect gaze behavior on task-related features of a tool-object scene in children. We hypothesize that consistent with our previous work, visual encoding will still reveal object-oriented action priming processes, but with greater encoding of grasp intent for the non-ESC and ESC condition in children. Children between 4- to 12-years-old watched tool-object scenes while their gaze patterns were recorded. Each tool-object scene either had correct or incorrect tool-object pairs and had grasps that either afford or does not afford action. Contrary to previous studies, children visually encode not only the salient features of the scenes for object-oriented action priming but also encode grasp intent.

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**Title:** The coding of navigational distance in scene-selective regions of human visual cortex

**Authors: *J. PARK**[1], S. PARK[1,2]
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**Abstract:** To successfully navigate, it is important to detect local boundaries in an environment that impose limits to one's locomotion. Previous studies have shown neural representations of the size and height of a boundary, as well as the perceived egocentric distance in a scene. In this study, we aim to directly test whether scene-selective regions code the *navigational distance* defined by the local boundary by using artificially rendered indoor environments. In our experiment, we took advantage of a glass-wall, which minimizes perceptual change or visibility of the global boundary and presents a local boundary that limits one's navigation. The condition varied in 3 egocentric global distance levels (Near, Middle, Far) and 2 local boundary levels (Glass-Wall, No-Glass-Wall). All environments were rendered to have the same width and height, except for the distance to the back wall, which changed global distance in an environment. In the Glass-Wall condition, a transparent glass-wall was added to each environment. Critically, the navigational distance to the local boundary (glass-wall) was kept the same across all global distance levels. In the No-Glass-Wall condition, navigational distance equaled the global distance because there were no glass walls in the environment. We predicted

that if there is a neural coding of the navigational distance as opposed to the global distance, three different levels of distance (Near, Middle, Far) in No-Glass-Wall conditions will be distinguishable but not in the Glass-Wall conditions as the navigational distance is kept the same across conditions. During fMRI scan, participants (N=7) viewed the stimuli in a blocked design. First, we found a step-wise increment of BOLD response as the global distance increased in OPA for No-Glass-Wall conditions, but not in Glass-Wall conditions. When Near and Far conditions were directly compared, there was an interaction between Glass-Wall and No-Glass-Wall conditions. These results suggest a coding for navigational distance in OPA, rather than a global and visible distance that differed across environments. We found a similar pattern of results in PPA, although weaker at this point. In addition, using multivoxel pattern analysis and support vector machine (SVM), we found an above-chance 3-way classification for near, middle, and far distances in No-Glass-Wall conditions, but not in Glass-Wall conditions. These results provide a novel evidence that scene-selective regions of human visual cortex code for the navigational distance defined by the local boundary that is important for our daily navigation.

**Disclosures: J. Park:** None. **S. Park:** None.

**Poster**

**589. Representation of Objects and Scenes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 589.24/GG19

**Topic:** D.07. Vision

**Support:** KAKENHI JP(25240022)

KAKENHI JP(15J05442)

**Title:** Rapid allocentric coding in the monkey precuneus

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**Abstract:** We have recently shown in our behavioral studies that a target location for reaching, or for a saccade, is represented rapidly and automatically relative to a large rectangular frame in the background (Uchimura and Kitazawa 2013; Chakrabarty et al. 2017). By using an fMRI adaptation technique, such allocentric coding was suggested to take place in the precuneus of the human brain (Uchimura et. al. 2015). To further obtain neural evidence for the rapid allocentric coding, we recorded extracellular activity of single neurons in the monkey precuneus. In each trial, while the monkeys looked at a fixation cross, a red dot was presented in succession at

random locations in and around a rectangular frame that was placed in the background. Positions of the fixation cross and the frame were updated in each trial. To determine whether each neuron encoded truly allocentric information, we compared information on the stimulus position relative to the frame before and after shuffling the frame position across the trials. Of the 782 neurons in the precuneus, 77 neurons (9.9%) encoded significant allocentric information ($p < 0.05$, after Bonferroni correction for multiple comparison at 14 time points). The net allocentric information summed across the allocentric neurons started to increase as early as 50 ms after the stimulus onset, reached its peak at 150 ms and subsided thereafter. These results provide hard neural evidence that rapid and automatic allocentric coding takes place in the precuneus.

**Disclosures: M. Uchimura:** None. **H. Kumano:** None. **S. Kitazawa:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.01/GG20

**Topic:** D.07. Vision

**Title:** Touchscreen-based visual temporal discrimination task in the behaving mouse by the constant method

**Authors: *M. OIKAWA**[1,2], Y. NOMURA[3], A. AMANO[4,5], K. SHIMONOMURA[5,6], Y. SEYA[5,7], C. KOIKE[1,2,3,5]
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**Abstract:** A fundamental principle of vertebrate visual system relies on the functional separation of neuronal signaling into the ON and OFF pathways that generate visual contrast. These two pathways originate in depolarizing ON bipolar cells and hyperpolarizing OFF bipolar cells. Critical fusion frequency(CFF), defined as the frequency at which a flickering light is perceived as a continuous light, is useful for assessing the temporal characteristics of the visual system. Using electrophysiological analysis such as flicker electroretinogram (ERG) and visual evoked potential (VEP), CFF has been studied. It is important to analyze CFF in terms of behavioral performance to understand recognition of flicker frequency. We established a behavioral method for evaluating CFF of mice using the constant method that is one of the psychophysical techniques used for determining thresholds of various sensory functions. C57BL/6 mice were trained to perform a two-alternative forced choice task in which steady (250Hz) light and flickering (4-20Hz) light were presented on a green light emitting diode (green LED, 508nm) screen. The temporal frequency of flickering stimulus was randomly chosen on each trial. Mice

responded to steady light by making nose-poke toward touchscreen. The proportions of correct responses (PCRs) were measured. Results showed that PCRs became lower with increasing temporal frequency of flickering stimulus. In the 20Hz condition, PCRs were close to chance level. From the data obtained, we calculated a thresholdand the results suggest that CFF in behaving mice is approximately 14Hzthat was lower than our previous methods using method of limits. Our behavioral assay of mouse model would contribute to development of diagnosis of human retinal disease by applying genetically manipulated mice.

**Disclosures:** **M. Oikawa:** None. **Y. Nomura:** None. **A. Amano:** None. **K. Shimonomura:** None. **Y. Seya:** None. **C. Koike:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.02/GG21

**Topic:** D.07. Vision

**Support:** NIH Grant HD 054453-08

The John Merck Fund - Developmental Disabilities Translational Research Program

Simons Foundation Autism Research Initiative (SFARI)

**Title:** Network level deficits in primary visual cortex underlie perceptual learning deficits in Fragile X mice

**Authors: *A. GOEL**[1], D. CANTU[1], G. CHAUDHARI[1], A. NEWADKAR[1], B. TODISCO[1], S. COHEN[2], C. PORTERA-CAILLIAU[1]
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**Abstract:** Fragile X Syndrome (FXS), the most common inherited form of mental impairment, is associated with abnormalities in sensory processing. Here, we set out to investigate whether *Fmr1* knockout (KO) mice show perceptual learning deficits and the alterations in neuronal function, at the circuit level, that are responsible for those deficits. Using a go/no-go visual discrimination task for head-restrained mice, we discovered that, compared to wild-type (WT) mice, *Fmr1* KO mice take significantly longer to discriminate between gratings drifting in two orthogonal orientations (median: *Fmr1* KO=6 days vs. WT=4 days; $p=10^{-5}$). To test the hypothesis that abnormal sensory processing of the visual stimuli might explain this delay in perceptual learning we used in vivo two-photon calcium imaging (with rAAV-syn-GCaMP6s) to record network dynamics in layer (L) 2/3 of primary visual cortex (V1). We found that *Fmr1* KO mice show an increase in visual evoked activity (p=0.016) and they have a significantly lower fraction of orientation tuned neurons in V1 than WT mice (*Fmr1* KO=40.7% vs. WT=57.9%,

p=0.012). Hence, we reduced the angle between “go” and “no-go” stimulus from 90$^{o}$ to 7.5$^{o}$ (after mice of both genotypes had learned the basic task) and found that the discriminability index (d’) was significantly lower in *Fmr1* KO than in WT mice (p = 0.006). Delayed learning and reduced cognitive inflexibility are well documented deficits in human subjects with FXS that are potentially mediated by alterations in cortical sensory processing and/or decision making. Parvalbumin (PV) neurons have been implicated in FXS because of evidence of hyperexcitability (Contractor et al., 2015) and the low density of PV neurons in the somatosensory cortex of *Fmr1* KO mice (Selby et al., 2007). PV interneurons exhibit broad orientation tuning (Caroline and Sur, 2012) and provide fast inhibition to sharpen the orientation selectivity of pyramidal neurons that could affect perceptual discrimination (Lee et al., 2012). Thus, we hypothesized that impaired performance of *Fmr1* KO mice on the reduced angle task and the lower proportion of orientation selective neurons in V1 could be due to abnormal activity of PV neurons or to an imbalance in the timing of their activity relative to pyramidal neurons, which ultimately affects the functional output in V1. We are testing this by selectively recording from PV neurons by expressing AAV-flex-GCaMP6s in PV-Cre x LSL-TdTom (ai9) WT and *Fmr1* KO mice.

**Disclosures:** **A. Goel:** None. **D. Cantu:** None. **G. Chaudhari:** None. **A. Newadkar:** None. **B. Todisco:** None. **S. Cohen:** None. **C. Portera-Cailliau:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.03/GG22

**Topic:** D.07. Vision

**Support:** NSFC 31371109

NSFC 31671077

**Title:** Fitting the neural dynamics in V1 during visual perceptual learning

**Authors:** ***L. YU**[1], Y. YAN[2], D. WANG[1,2], W. LI[2,3], M. J. RASCH[2,3,4]
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**Abstract:** Perceptual learning is an important form of adaption in sensory systems and beyond. Changes in the neural dynamics in response to repetitively solving a visual task has been shown to occur in the visual cortex of awake monkeys (e.g. Yan et al., 2014, *Nat. Neurosci.*). Yan et al. chronically recorded multiunit activities of V1 neurons, when two monkeys were trained to perform a contour detection task. They have found progressive strengthening in both facilitation

of neurons (with receptive fields (RFs) nearby the contour) encoding the contour elements and suppression of neurons (with RFs in the surround) responding to the background components during perceptual learning using *the temporal averages of the firing responses*. However, despite these observations, the particular shape of the temporal dynamics of the neural responses to stimuli also changes dramatically during the course of weeks of learning. How such temporal response changes relate to changes in the underlying circuit is difficult to assess without data-driven modeling.
To this end, we here first show that the high-dimensional neural population responses in V1 can be well described by the temporal dynamics of only two main components abstracted from original data using a modified non-negative matrix factorization (NMF) method. The two components are: a facilitatory component involving cells having RFs nearby the contour, and a suppressive component involving cells having RFs in the surround.
We then fitted a number of simple rate-based models to the temporal dynamics of the two components using gradient decent methods. The best model mainly consisted of three populations: two excitatory neuron pools representing the two components, and an inhibitory neuron pool to balance the network. All parameters of the model, such as connection and inputs strengths and facilitation of synapses, were fitted to match the dynamic temporal responses for individual days. We found that the model could well reproduce the data.
We finally asked how the learning affected the fitted parameters, which can be related to a clear biophysical meaning, such as strength of inhibition or inputs. For that we used ranking methods and the t-distributed stochastic neighbor embedding (tSNE) algorithm to reduce the space of parameters and found a largely consistent set of parameters that seems to be mostly affected by learning, including the strength of inhibitory balance, indicating the mechanistic basis and biophysical “turning knobs” that are targeted by perceptual learning in V1.
Our results are thus important for the mechanistic understanding of the possible underlying network changes induced by perceptual learning in V1.

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**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

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**Topic:** D.07. Vision

**Support:** JSPS KAKENHI 26350986

JSPS KAKENHI 24800023

**Title:** Perceptual learning is predicted by enhanced resting-state functional connectivity after training

**Authors: *M. TAGHIZADEH SARABI**[1], R. AOKI[1], K. TSUMURA[2], R. KEERATIVITTAYAYUT[1], K. JIMURA[2], K. NAKAHARA[1]
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**Abstract:** Whether and how changes in resting-sate functional connectivity (rs-FC) in context of visual perceptual learning are associated with later behavioural performance remains under debate. To investigate this question, task-fMRI (t-fMRI) data was obtained in two different days (day1 and day2) and resting-state fMRI (r-fMRI) data was obtained before and after t-fMRI on day1 only. This experimental paradigm allowed us to examine the relationship between post-task versus pre-task functional connectivity and subsequent improvement of task performance. Twenty healthy young adults participated in a perceptual learning task in day1 and day2 and performed a motion discrimination task using randomly moving dots to modulate visual perceptual learning. Moving dots were presented with three coherence levels (20%, 40% and 80%) and with two directions (upward and downward). To identify brain regions indicating activations associated with the coherence levels of moving dots, parametric regression was used in a general linear model. The area MT+ exhibited significant activation ($P < 0.05$, family-wise error [FWE] corrected) with increasing coherence level, while the dorsal anterior cingulate cortex (dACC) and the bilateral insula represented significant activation ($P < 0.05$, FWE corrected) with decreasing coherence level. These findings indicate that the MT+ is sensitive to motion coherence and that the dACC and insula are sensitive to task difficulty. Seed-based rs-FC analysis was performed using the MT+, dACC and bilateral insula as seeds on both resting scans. By contrasting r-fMRI sessions (post-task vs. pre-task), only significant increases ($P < 0.05$, FWE corrected) in connectivity with the MT+ were observed on regions namely: the bilateral postcentral gyrus (POG), the bilateral precentral gyrus (PrG), the left superior temporal gyrus (STG), the left middle temporal gyrus (MTG) and the left superior frontal gyrus (SFG). Further Pearson correlation analysis exhibited that change in accuracy rate from day1 to day2 is significantly correlated with connectivity enhancement from pre-task rest to post-task rest only between MT+ and bilateral PrG (left PrG, $r = 0.56$, $P = 0.01$ and right PrG, $r = 0.5$, $P = 0.023$). Our findings suggest that rs-FC between visual and premotor cortices may reflect the plasticity prior to actual behavioral changes and predict individual differences in subsequent perceptual learning.

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**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

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**Program#/Poster#:** 590.05/GG24

**Topic:** D.07. Vision

**Support:** Whitehall Foundation Research Grant

**Title:** Visual familiarity evoked theta oscillations in primary visual cortex (V1)

**Authors: *S. T. KISSINGER**[1], A. PAK[1], S. MASMANIDIS[2], A. A. CHUBYKIN[1]
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**Abstract:** The primary visual cortex (V1) has been long thought of as a simple feature detector that conveys basic visual information to higher order brain areas. More recent evidence, however, has shown that neural activity in V1 can also depend on previous visual experience. Individual neurons in V1 of awake rodents have been shown to fire persistently out to the timing of reward delivery after presentation of a visual cue, a phenomenon that is dependent on the cholinergic system. Repetitive presentation of phase reversing visual stimuli in awake animals results in significant potentiation of visually evoked potentials (VEPs) in V1, a phenomenon known as stimulus-selective response potentiation (SRP). Potentiation of VEP amplitude has also been shown to report learned sequences of visual stimuli. Persistent theta oscillations (4-8Hz) have been reported in the extrastriate visual cortex of monkeys during visual cue-reward working memory tasks. Interestingly, persistent theta oscillations have also been shown to report the timing of reward delivery after visual cue-reward training in rats. We sought to further explore the specificity of these visually evoked theta oscillations in V1 and the potential mechanisms underlying their generation. Using 64 channel silicon probes acutely implanted in binocular V1, we recorded visually evoked potentials (VEPs) and action potentials from individual neurons (units) in awake mice responding to sinusoidal grating stimuli. After training mice to visual stimuli, oscillations in the theta range emerged in VEPs and individual units. Unit-depth analysis and current source density (CSD) revealed a cortical layer specific distribution of oscillatory activity. Interestingly, these oscillations could be induced by training mice to visual stimuli with vastly different physical properties. Treatment with the muscarinic acetylcholine receptor (mAChR) antagonist scopolamine blocked theta oscillations, suggesting that the cholinergic system is required for their emergence after training.

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**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.06/GG25

**Topic:** H.01. Animal Cognition and Behavior

**Support:** ONR

**Title:** Use of awake fMRI in dogs to model learning of visual and olfactory stimuli

**Authors: *A. PRICHARD**[1], M. SPIVAK[2], G. BERNS[1]
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**Abstract:** Through awake fMRI, we examined individual and group learning curves in dogs to previously neutral visual and olfactory stimuli. Visual stimuli were a toy pineapple and inflatable flamingo, and olfactory stimuli were Isoamyl acetate and Hexanol. During scanning, one stimulus of each pair was associated with food reward and the other with nothing. To generate learning curves for individual dogs, we examined activation within the caudate nucleus per trial following the presentation of each stimulus. The learning curves show that dogs did form stimulus-reward associations within a single MRI scan. Our results demonstrate the speed at which conditioning to stimuli associated with reward occurs in the dog brain, and the potential differences between modalities in learning acquisition.

**Disclosures: A. Prichard:** None. **M. Spivak:** None. **G. Berns:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.07/GG26

**Topic:** D.07. Vision

**Title:** The role of the inferior temporal area TEO in visual categorization

**Authors: *T. SETOGAWA**, M. ELDRIDGE, M. FREDERICKS, R. SAUNDERS, B. J. RICHMOND
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**Abstract:** Inferior temporal cortical area TEO is located between areas V4 and TE in the ventral pathway of the visual system. Our previous studies demonstrated that monkeys with bilateral TE lesions were only mildly impaired in categorization of visual objects based on perceptual similarity. To investigate the relative importance of an adjacent brain region, we examined the effect of bilateral removal of area TEO on the same visual categorization task. We tested a cat vs. dog categorization task with one monkey. In this task, the monkey had to categorize morphed images of cats and dogs into either “cat-like” or “dog-like” to get reward. These stimuli were morphed (blended and warped) cats and dogs ranging between 0 and 100% dog, with a distribution biased around the category boundary (11 levels, 0, 25, 35, 40, 45, 50, 55, 60, 65, 75, 100% of dog). The monkey was trained to touch a bar to initiate a trial, and had to release the bar during one of two intervals; early (signaled by a red central dot) if he identified the stimulus as more “cat-like”, or late (signaled by a green central dot) if the stimulus was identified as more

"dog-like". If the monkey responded correctly, the color of the central dot turned into blue and liquid reward was given. We assessed performance on this task before and after bilateral TEO lesions. We analyzed the behavioral data for 4 days before the lesions, and four days afterwards. The percentage of response as "dog-like" for each morphed level was calculated and used as indicator of categorization accuracy. Our preliminary data suggest that the TEO lesions impaired categorization accuracy only transiently. In particular, the discriminability in the first day after lesion was significantly degraded when the morphing range was between 25-75%. As we previously observed in TE lesioned monkeys, the accuracy score improved over the first few days of exposure to the morphed stimuli, in this case recovering to pre-lesion levels of performance. These results suggest that TEO removal causes a temporary degradation of ability for visual categorization.

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**Poster**

**590. Visual Learning, Memory, and Categorization**

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**Program#/Poster#:** 590.08/GG27

**Topic:** D.07. Vision

**Support:** the New Energy and Industrial Technology Development Organization (NEDO)

**Title:** Comparison between monkeys with bilateral removals of visual area TE and a deep neural network in categorizing feature-ambiguous stimuli

**Authors: *N. MATSUMOTO**[1], M. A. ELDRIDGE[2], B. J. RICHMOND[2]
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**Abstract:** In the canonical view of visual processing, the neural representation of complex objects emerges as visual information is integrated through a set of convergent, hierarchically organized processing stages, ending in the primate inferior temporal lobe (Mishkin et al., 1983). It seems reasonable to infer that visual perceptual categorization requires the integrity of anterior inferior temporal cortex (area TE). Nevertheless, monkeys with bilateral removals of area TE show only mild deficits of perceptual categorization (Matsumoto et al., 2016). Furthermore, the monkeys show greater deficits for feature-ambiguous stimuli of morphed cats and dogs than control monkeys (Eldridge et al., SFN abstract 2012). Here we evaluated the performance of a simulated hierarchical model of vision in discriminating the same categorization problems previously presented to the monkeys. We employed a deep neural network (DNN) consisting of 5 convolution layers (conv1-5) and 3 fully connected layers (fc6-8) (Krizhevsky, et al., 2012). In the behavioral experiments, the monkeys were trained to categorize using an image set of 20

cats and 20 dogs prior to TE removal. After the removals, the monkeys were tested using a trial-unique set of 240 cats and 240 dogs. In the modeling experiments, the set of 20 cats and 20 dogs was used to train the model. Linear support vector machine (SVM) analysis was applied to the output vectors for cat/dog images in each model layer to determine the cat/dog boundary in that layer. We then assessed the classification accuracy of the model layers when presented with the set of trial-unique stimuli. The accuracy increased at successive model layers (from superficial to deep). Even in the conv5 layer the accuracy was above 70%. This result indicates that the convolutional layers are sufficient for supporting a reasonable accuracy of categorization. It has been reported that the information derived from the fully connected layers of this DNN is consistent with the degree of information that can be derived from neuronal activity in area TE (Yamins and DiCarlo, 2016). For the trial-unique cat/dog stimuli, the output of the conv5 layer was similar to the performance of monkeys after area TE removals. However, the fully connected layers were necessary to recognize feature-ambiguous morphed cat/dog images, whereas the monkeys with TE removals retained some ability to perform this task, albeit at a lower level of accuracy than controls. These results indicate that some revision to the DNN may be necessary to increase flexibility across stimulus types if we wish to be able to map the function of modules of the visual processing hierarchy to layers of the network.

**Disclosures:** **N. Matsumoto:** None. **M.A. Eldridge:** None. **B.J. Richmond:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.09/GG28

**Topic:** D.07. Vision

**Title:** Hippocampus is not required for visual perceptual categorization in the rhesus monkey

**Authors: *G. MAMMARELLA**[1], M. A. G. ELDRIDGE[2], R. C. SAUNDERS[3], B. J. RICHMOND[4]
[1]NIMH, [2]NIH, Bethesda, MD; [3]Lab. Neuropsychol, [4]NIMH, Bethesda, MD

**Abstract:** We assign objects into categories based on similarities in their appearance in a process called visual perceptual categorization. The medial temporal lobe (MTL) has been implicated in learning, categorization, and memory. Inferior temporal (IT) cortex is considered as a late stage of visual processing in the ventral visual pathway, and provides input to areas in the MTL. Previously we have shown that rhesus monkeys quickly learn (in less than one testing session) a visual category task discriminating between cat-dog stimuli, and that intact monkeys can learn a more complex task containing computer-generated images that are morphed (blended and warped) between many dog-cat pairs. Three normal monkeys, 3 with bilateral TE ablations, and 3 with bilateral rhinal ablations, reported whether the morphed image was more dog-like or more

cat-like. The normal and rhinal lesion monkeys performed at a high level having difficulty only when the stimuli approached the category border. Monkeys with TE ablations were somewhat impaired when the images contained more than about 10% morphing.
This outcome led us to look elsewhere for candidate areas in the MTL that may play a role in visual categorization, such as the hippocampus. The hippocampus is important in declarative memory, e.g. processing memories that are recalled consciously. It seems reasonable to ask whether declarative memory may play a role in visual perceptual categorization; for example, if the monkeys were constructing 'prototype' representations for each category to which all stimuli are compared to for classification, the hippocampus might be necessary for this process. However, the categorization performance of monkeys with excitotoxic hippocampal lesions was indistinguishable from that of controls. Thus, from these data it seems as if the hippocampus is not necessary for the categorization of feature-ambiguous stimuli.

**Disclosures: G. Mammarella:** None. **M.A.G. Eldridge:** None. **R.C. Saunders:** None. **B.J. Richmond:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

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**Program#/Poster#:** 590.10/GG29

**Topic:** D.07. Vision

**Support:** JSPS KAKENHI Grant Number JP26120535, JP16H01561, JP16H01684

the New Energy and Industrial Technology Development Organization (NEDO)

**Title:** Upright and inverted faces are separately represented in feed-forward processing in the visual cortex

**Authors: *Y. SUGASE-MIYAMOTO**[1], N. MATSUMOTO[1], Y. MOTOTAKE[2], K. KAWANO[1], M. OKADA[2]
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**Abstract:** A picture-plane inversion of a face reduces the ability to recognize both facial identity and expression in humans. To study the effect of face inversion on the neural activities in monkey area TE, we previously applied principal component analysis (PCA) to the responses of 119 face-responsive neurons to upright and inverted pictures of human faces (3 models with 4 expressions) and monkey faces (4 models with 4 expressions), and calculated a population activity vector for each picture consisting of averaged spike counts of the individual neurons in the windows of 115-165 ms and 140-190 ms after stimulus onset. The former and latter windows are known to be where the global (human vs. monkey) and fine categorizations (human

individuals or monkey expressions) take place, respectively. In the 115-165 ms window, separation of the vectors of the upright and inverted faces was observed. In the 140-190 ms window, the separations of human individuals and monkey expressions of the upright faces were larger than those of the inverted faces. The result suggested that the changes in the information represented by the TE neurons might cause the psychological face inversion effect. In the present study, we employed a convolutional neural network in an effort to understand the information processing of the face inversion effect. The model consists of 5 convolution layers (conv1-5) and 3 fully connected layers (fc6-8) (Krizhevsky, et al., 2012). Upright and inverted pictures of a human or monkey face used in the previous work (Sugase-Miyamoto, et al. 2014) were input to the model. We applied PCA to the activity vectors in each layer to visualize the relationship between the vectors of the upright and inverted faces. In conv1, the vectors of the human and monkey faces were separated. The vectors of the upright and inverted pictures of the monkey faces were overlapped, while those of the human faces were separated. In conv5, the vectors of the upright and inverted pictures of the monkey faces were separated, showing consistency with the neuronal data in the 115-165 ms time window. In fc6, the separations of the human individuals and monkey expressions of the upright faces were larger than those of the inverted faces, showing consistency with the neuronal data in the 140-190 ms window. These results indicate that the human and monkey categorizations develop even in the lower visual cortex, and the separation of the upright and inverted faces takes place progressively in feed-forward processing in the visual cortex.

**Disclosures:** **Y. Sugase-Miyamoto:** None. **N. Matsumoto:** None. **Y. Mototake:** None. **K. Kawano:** None. **M. Okada:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.11/GG30

**Topic:** D.07. Vision

**Support:** NSF 1232530

NSF PIRE OISE-0730255

Spanish MINECO PCIN-2015-079

**Title:** Escaping the frontal bottleneck: Extensive practice of a visual categorization task shifts category representations from dorsolateral prefrontal cortex to ventral occiptio-temporal cortex

**Authors: *P. H. COX**[1], C. A. SCHOLL[1], C. A. SPROUSE[1], J. C. RONKIN[1], R. L. KLEIN[1], K. WIMMER[2], K. GLOMB[2], N. E. JAIMES[1], G. DECO[2], X. JIANG[1], M. RIESENHUBER[1]
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**Abstract:** The process of learning to assign a category to a sensory stimulus has been well studied in the visual domain, typically involving several hours of training and up to a few thousand trials, generally showing a sharpening of shape-tuning in visual areas and the formation of task dependent category responses in dorsolateral prefrontal cortex (dlPFC). However, experience with real-world stimuli is far more extensive and categorization of common objects happens independent of task context. Other studies have identified a bottleneck in PFC that limits processing to one task at a time, potentially limiting our ability to categorize objects in multitasking scenarios. Here we test the hypothesis that extensive training leads to a shift in the neural circuitry underlying categorization, from later in prefrontal cortex to earlier in more posterior brain regions, moving the decision out of the frontal bottleneck and increasing the ability to perform the trained task automatically.
We trained 14 subjects to categorize morphed car stimuli (as in our previous studies, Jiang *et al., Neuron,* 2007; Scholl *et al., JOCN,* 2014) using a training app and used fMRI and EEG rapid adaptation (RA) techniques to identify the brain regions and temporal dynamics underlying the categorization process after initial training (~4 hours over one week, as in our previous studies) and extensive practice (~16 more hours over 4 more weeks, ~30,000 trials in total). In agreement with previous studies, after initial training subjects show categorical responses in left dlPFC and shape-selective responses in occipito-temporal cortex (OTC) in fMRI-RA, and, in EEG-RA, a shape-selective signal over posterior channels ~170ms followed by a categorical signal starting just after 200ms over left frontal channels. After extensive training, fMRI-RA showed categorical responses in left OTC, as well as left parietal cortex and right striatum, but no longer showed a categorical response profile in dlPFC. Frontal regions also showed reduced effective connectivity with the rest of cortex. In agreement with our fMRI-RA results, EEG-RA reveals earlier categorical signals starting ~150ms over posterior channels. These data suggest that extensive practice changes the brain networks performing perceptual categorization, with category-selective representations being pushed out of the frontal bottleneck, down into OTC. These category-selective representations then could permit a direct recruitment of parietal or striatal circuitry for response selection, circumventing the frontal bottleneck. Indeed, after extensive single task training subjects' ability to categorize cars in dual task scenarios increased.

**Disclosures: P.H. Cox:** None. **C.A. Scholl:** None. **C.A. Sprouse:** None. **J.C. Ronkin:** None. **R.L. Klein:** None. **K. Wimmer:** None. **K. Glomb:** None. **N.E. Jaimes:** None. **G. Deco:** None. **X. Jiang:** None. **M. Riesenhuber:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

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**Program#/Poster#:** 590.12/GG31

**Topic:** D.07. Vision

**Support:** NEI Intramural

**Title:** A prefrontal long-term memory mechanism for discrimination of objects with stable reward associations

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**Abstract:** We have previously shown that given sufficient reward training, a single valuable object among others can be detected rapidly during visual search (single saccade <150ms). Such efficient detection is sustained in long-term memory for many weeks without seeing the objects or reward exposure (Ghazizadeh et al 2016 JOV). However, the neural mechanism for such long-term memory formation is not fully understood (but see Hikosaka et al 2014 Annu Rev Neurosci). Our recent results with whole brain fMRI revealed that the prefrontal cortex ventral to principal sulcus (vlPFC) is involved in discrimination of objects based on months-long memory of value associations (SfN 2016). To further study the neural mechanism of such long term memory, we trained two macaque monkeys to associate more than one hundred random fractal objects with either high or low reward (‘good’ and ‘ bad’ objects, 10+days, 300+ trials). We then recorded the activity of the visually responsive neurons in vlPFC in a passive fixation task while ‘good’ and ‘bad’ objects were presented inside the neurons receptive field and in the absence of reward. We did this hours, days, weeks or months after this reward training. Results showed that as a population, vlPFC neurons responded to ‘good’ objects more strongly than ‘bad’ objects (GB discrimination) and sustained the value-based response bias up to many months. The onset of GB discrimination in the vlPFC population was rapid (<120ms). This reveals a long-term high capacity memory mechanism in vlPFC for object’s old values. We also examined the interaction of GB discrimination and object eccentricity. Interestingly, the GB discrimination was stronger in the periphery (15-20 deg) than the center (0 deg). Such peripheral value-based discrimination can be the neural basis of efficient visual search reported previously. Together, the high capacity and the longevity of the value memories, as well as the rapid peripheral discriminability of ‘good’ vs ‘bad’ objects, can be critically useful for animals as they encounter many valuable objects (e.g. food) in their lifetime and have to quickly retrieve them any time in future.

**Disclosures: A. Ghazizadeh:** None. **O. Hikosaka:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

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**Topic:** D.07. Vision

**Support:** NIH R01-EY019041

NSF CAREER award 0955640

McKnight Scholar award

**Title:** Visual image familiarity learning at multiple timescales in the inferotemporal cortex

**Authors: *K. MOHAN**, D. J. FREEDMAN
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**Abstract:** Humans and other primates have an exceptional ability to learn to recognize familiar visual items, and to discriminate them from novel ones. This ability is thought to rely on experience dependent changes in neuronal representations in the ventral visual pathway, particularly the inferotemporal cortex (IT). Past work has shown that neurons in primate IT cortex differentiate between novel and familiar stimuli, with stronger activity on average for novel images. To date, experimental studies have primarily sampled from the extremities of the familiarity spectrum, comparing highly familiar to highly novel images. Thus, it is unknown whether IT supports the acquisition of familiarity or whether it merely reflects learned familiarity signals encoded elsewhere? To probe this question, we familiarized the monkeys with multiple sets of 40 novel images for up to 10 days each, while monitoring responses of IT neuronal ensembles in awake, behaving monkeys during the familiarization process. Additionally, on every session, we also presented the monkeys with 40 initially-novel images which were familiarized through repeated presentations (N~=30) in each session, as well as 40 highly familiar images. Neuronal responses and selectivity were compared between highly familiar, intermediately familiar, and novel stimuli. Consistent with previous reports, we observed a decrease in the average neuronal response (N=120) with familiarity, both within and across sessions. While the average transient response (80-150 ms) was comparable for all stimuli, the average sustained response (150-300 ms) was highest for novel, followed by intermediately familiar, and the lowest for highly familiar stimuli. We also investigated how firing rates and visual selectivity evolve across each session with repeated presentations of initially novel stimuli. This revealed a diversity of changes with neurons increasing their firing rates to some stimuli, while they decrease with others. A subset of neurons showed global increases in firing rates for all stimuli, independent of familiarity. For each neuron, we quantified the proportion of stimuli that elicit robust firing rates and in line with previous studies, found that individual

neurons (75%) responded to a smaller fraction of familiar than novel stimuli. Interestingly, within a session, we observed that the proportion of stimuli that evoked robust firing rates decreased more for novel stimuli than for familiar stimuli, suggesting that IT shows rapid changes in activity during learning. Together, these results give insight into how experience affects visual representations in ITC across multiple timescales.

**Disclosures: K. Mohan:** None. **D.J. Freedman:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.14/GG33

**Topic:** D.07. Vision

**Support:** NIH R01EY019041

**Title:** Differential roles of LIP and MST during motion categorization

**Authors: *Y. ZHOU**[1], K. MOHAN[1], D. J. FREEDMAN[2]
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**Abstract:** Categorization is an important cognitive function for recognizing the behavioral significance of sensory stimuli and choosing appropriate behavioral responses. Previous studies have shown that frontal-parietal cortical areas are closely involved in the categorization process. Specifically, studies from our lab have used a delayed match to category (DMC) task to reveal that activity in prefrontal (PFC), lateral intraparietal (LIP) and medial intraparietal (MIP) cortices all encoded learned visual categories, with evidence suggesting that LIP plays a preferential role in motion category encoding. Here, we further examine the roles of multiple cortical areas during motion categorization process by comparing neuronal activity in LIP with that in the medial superior temporal (MST) area, an important motion processing area which is interconnected with both LIP and the middle temporal (MT) area. We recorded from 100 MST neurons and 55 LIP Neurons in one monkey during performance of the motion DMC task. Interestingly, we found that many MST neurons appeared to eencode the learned motion categories, qualitatively similar to that observed in LIP. MST category selectivity peaked in strength during the late sample period, but also persisted during the delay of the DMC task. We further compared category encoding in LIP and MST by using several analyses including a category tuning index , percentage of explained variance, and support vector machine decoding. This gave several lines of evidence suggesting that LIP and MST play different roles during the DMC task. First, category selectivity in LIP and MST arose with a similar latency after sample onset. Second, LIP showed stronger average category selectivity during the early sample and delay periods than

MST. Third, LIP but not MST neurons showed reduced category selectivity in the error trials than in the correct trials during the delay period, indicating that category selectivity in LIP was more closely correlated with task performance than in MST. Fourth, LIP neurons showed relatively stable category encoding during the delay period, while MST neurons showed more dynamical category encoding during the delay. Finally, MST neurons showed stronger direction encoding than LIP neurons during the sample period. In summary, our results support the idea that LIP is closely involved in mediating categorical decisions and holding the category information within the short-term memory during the DMC task. MST neurons might be also involved in the transformation of direction tuning to category encoding during the sample period, but plays a lesser role in retaining category information within working memory.

**Disclosures:** **Y. Zhou:** None. **K. Mohan:** None. **D.J. Freedman:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.15/HH1

**Topic:** D.07. Vision

**Support:** NIMH IRP ZIA-MH002898

**Title:** Tracking response changes due to familiarity and adaptation in AM and AF face patch neurons

**Authors: J. H. HONG**, K. W. KOYANO, B. E. RUSS, *D. A. LEOPOLD
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**Abstract:** The ability to distinguish faces of unknown individuals from those of familiar ones is a crucial aspect of a primate's social life. However, the process of learning familiar faces is still not well understood because it is difficult to track activities of the same neurons across multiple days. Here, we report our preliminary results of longitudinal recording of neurons in AM and AF face patches while monkeys were repeatedly shown the same face stimuli. To track the changes of neuronal responses over weeks, we chronically implanted microwire bundles (McMahon et al., 2014; 2015) in the face patches whose locations were identified using fMRI (Russ et al., 2015). With the microwire bundles, we could longitudinally record more than 30 neurons from both patches for two weeks. More than 60% of neurons were selective for visual face stimuli. We prepared 120 novel face stimuli that the monkeys had never seen before and repeatedly showed them to the monkeys in consecutive days. During the two-week experimental period, neurons in AM face patch gradually decreased their responses to a subset of the novel faces. This response change was obvious in the sustained, late response phase between 200-400 ms after the stimulus onset, but nearly absent in the transient, early response during initial 200 ms. In contrast to the

plastic responses of AM face patch, responses of neurons in AF patch showed no overall change during the two-week experimental period, consistent with a previous study from this area (McMahon et al., 2014). However, neurons in the AF face patch did exhibit repetition suppression, or a decrease in response strength to repeatedly shown stimuli within in each session, whereas neurons in AM face patches did not. These decreased responses recovered by the following day, thus there was no accumulation of repetition suppression across days in AF. These results indicate that AM neurons change their activity to novel faces gradually over weeks, whereas AF neurons change their activity rather quickly within a few trials in a given day. This suggests the different roles across face patches in learning new faces through familiarity and adaptation mechanisms.

**Disclosures:** **J.H. Hong:** None. **K.W. Koyano:** None. **B.E. Russ:** None. **D.A. Leopold:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.16/HH2

**Topic:** D.07. Vision

**Support:** NSF Grant 1545668

**Title:** Using real-time magnetoencephalography neurofeedback training to reduce the time of shifting spatial attention from one visual field to another

**Authors: K. D. RANA**[1], J. VOJTECH[1], S. BROWN[1], *L. M. VAINA[2]
[1]Biomed. Engin., Boston Univ., Boston, MA; [2]Biomed Engineering&Neurology, Boston Univ. & Harvard Med. Sch., Boston, MA

**Abstract:** Adaptive behavior requires rapid shifting of spatial attention among different locations in the environment. Even without any training, covert spatial attention can be shifted in just a few hundred milliseconds (Muller et al, 1998). In this project, our objective was to train subjects to increase their speed of shifting their spatial attention. To do this we developed a real time magnetoencephalography (MEG) neurofeedback training method that decodes spatial attention location (left (LVF) or right visual field (RVF)) over time using MEG sensors activation. Subjects were cued to attend to the direction of motion of one of two random dot kinematograms (RDK) displayed in either the LVF or RVF (“attend” period). After a variable time period (750-1500 ms), the motion direction in the attended RDK changed, cueing the subject to switch attention to the opposite visual field (VF) and report the color of a disk (red/green) superimposed on the RDK display in that VF (“switch” period). Feedback was presented as a thermometer where its level represented the speed of attention switching. To generate the feedback we trained a decoder to discriminate between LVF and RVF from

oscillatory activity across the 306 MEG sensors during the “attend” periods in the trials (n=80) of the previous block. After each trial in the current block, the decoder was applied to the time course of sensors activity in the “switch” period and then it computed the speed of switching attention from one VF to the other. In 5 of the 7 subjects trained on this task (6 days, 320 trials/day) the speed of switching attention significantly decreased (Wilkoxin Ranked Sum $p < 0.05$). Directional functional connectivity between active regions of interest (ROIs) was computed through frequency Granger causality within each stimulus period in the α, β, and γ bands. We calculated Dynamic Granger Causality (DGC), a sliding time-windowed form of Granger causality (Lin et al, 2009; Vaina et al, 2010), to compute the time segments in the stimulus when any two ROIs were significantly connected. During the “attend” period, increased switch speed was correlated with higher β-band Granger scores between bilateral MT+, precuneus, and frontal eye fields. During the “switch” period, increased switch speed was correlated with lower γ-band Granger scores among frontoparietal ROIs. DGC computed between these ROIs revealed shorter time segments after training. Taken together these results suggest that the engagement of preparatory top-down attention connections in the β-band prior to “switch” and the short lived γ-band connectivity among frontoparietal ROIs during the “switch” underlie successful training of switch speed.

**Disclosures:** **K.D. Rana:** None. **J. Vojtech:** None. **S. Brown:** None. **L.M. Vaina:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

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**Program#/Poster#:** 590.17/HH3

**Topic:** D.07. Vision

**Support:** Whitehall 2014-5-18

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**Title:** Maintenance of spatial information enhances visual processing via phase modulation of ongoing brain rhythms

**Authors: *Z. BAHMANI DEHKORDI**[1], Y. MERRIKHI[1], K. L. CLARK[2], M. DALIRI[1,3], B. NOUDOOST[2]
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**Abstract:** We have recently shown that in the absence of firing rate changes, a spatially-specific working memory (WM) signal drives subthreshold modulations in visual areas. Whether and how this subthreshold input results in enhanced visual processing is crucial for understanding how sensory processing is altered by WM. We simultaneously recorded the spiking activity and local field potentials from multiple sites within the middle temporal (MT) cortex during a modified version of the memory guided saccade task. In this task, a set of visual probe stimuli are presented during the fixation and memory periods. We quantified the receptive fields (RFs) in MT and altered the locus of WM relative to each RF to study the effect of WM in isolation and when interacting with incoming visual signals. In the absence of visual stimuli, there was a robust increase in the locking of spikes to the ongoing αβ oscillations when remembering a location near the MT RF, even though the overall firing rate remained unchanged. Interestingly, in the presence of visual stimuli during the memory period, the WM-induced change in the locking between spikes and ongoing αβ oscillations was sufficient to enhance the ability of MT neurons to encode visual stimuli (quantified using the mutual information between the visual stimulus location and the phase of αβ oscillations at which spikes occurred). Importantly, the increased gain and discriminability of the response to incoming visual stimuli occurs mostly during the preferred phase of ongoing αβ oscillations. This finding indicates that the WM-induced enhancement of the visual representation depended upon these oscillatory changes. These results provide a mechanistic understanding of how, by altering the oscillatory activity within visual areas, a spatially specific WM signal is capable of enhancing the efficacy of visual sensory processing, potentially underlying the behavioral benefits of WM for visual perception.

**Disclosures: Z. Bahmani Dehkordi:** None. **Y. Merrikhi:** None. **K.L. Clark:** None. **M. Daliri:** None. **B. Noudoost:** None.

**Poster**

**590. Visual Learning, Memory, and Categorization**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 590.18/HH4

**Topic:** D.07. Vision

**Support:** 3 R01 EY014978-06

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NARSAD Young Investigator Award 2013

**Title:** Neural responses in non-memory tasks predict the likelihood that LIP neurons will exhibit environmental memory

**Authors: *M. SEMEWORK**[1], M. E. GOLDBERG[2]
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**Abstract:** Neurons in the lateral intraparietal area (LIP), demonstrate a form of environmental memory (EM)(Steenrod & Goldberg 2009, Semework et al 2012,), a memory for task-irrelevant objects that may become important in the future. When a monkey makes a saccade that brings the spatial location of a probe flashed in a recent trial (hence attention-grabbing) into the neuron's receptive field, the neurons respond even though the stimulus has not appeared on the current trial. However, we also discovered that classic statistical measures did not classify units precisely. We therefore used other approaches to increase our classification accuracy and create tools to predict precisely memory performance. Our analysis demonstrated that baseline and visual activity of these neurons in non-EM tasks can predict the likelihood of having EM. Using logistic regression models, we found that the strongest influence on memory activity came from pre-saccadic (baseline) mean activity. Moreover, maximum visual response is highly predictive of memory responses. There is a significant relationship between pre-saccadic baseline median and maximum visual response. Linear Discriminant Analysis (LDA) performed at about 72% correct classification rate when using maximum visual responses to predict memory responses (as "good", "none", or "not-defined"). Consistent with non-parametric data distributions, comparison between Principal Component Analysis (PCA) and LDA classification of memory responses into different classes resulted in first LD explaining 88.1% between-group variance, and first PC explaining 98.8% total variability in the data (based on baseline pre-saccadic max and median). Leave-One-Out and 10-fold cross-validation (CV) analysis on prediction of memory activity by baseline pre-saccadic median shows a similar (minimum) CV error for a degree 1 or 2 polynomial. A non-parametric clustering (k-Nearest Neighbors algorithm) using baseline pre-saccadic median and visual responses predicted memory responses with 73% accuracy. Together, these results indicate that, LIP neuron environmental memory dynamics can be predicted reasonably, suggesting this response is not a task-specific epiphenomenon but possibly useful neuronal activity which may help us process retino-spatial signals that this brain region is known to compute.

**Disclosures: M. Semework:** None. **M.E. Goldberg:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.01/HH5

**Topic:** E.02. Cerebellum

**Support:** Merit Review Award

NIH Grant204345

**Title:** Inhibition of polyamine catabolism leads to cerebellar injury and overt ataxia

**Authors: K. ZAHEDI**[1], S. BARONE[2], J. XU[1], R. CASERO[3], *M. SOLEIMANI[2,4]
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Med., Univ. of Cincinnati Med. Ctr., Cincinnati, OH; [3]The Johns Hopkins Sch. of Med., Baltimore, OH; [4]VA Res. Services, Cincinnati, OH

**Abstract:** Polyamines, spermidine and spermine, are aliphatic cations that play multiple indispensible roles in the regulation of DNA structure, protein-nucleic acid interactions, and cell growth and viability. Their cellular content is tightly regulated through the balance of their synthesis, degradation, import and export. Polyamine catabolism increases and exacerbates organ damage and dysfunction in a variety of injuries (e.g. ischemia/reperfusion, traumatic and toxic). However, the role of polyamine catabolism under normal conditions is not known. In order to address this, we developed mice with the complete deletion of polyamine catabolism pathway through the ablation of spermidine/spermine $N^1$-acetyltransferase and spermine oxidase (SSAT/SMOX) genes by generating mice with global double knockout (dKO) of SSAT and SMOX. Examination of the SSAT/SMOX dKO mice did not reveal any overt growth or developmental deficits. SSAT/SMOX dKO mice exhibited a general build-up of polyamines in various organs that were tested. The SSAT/SMO dKO mice developed progressively ataxia as verified by the worsening of neural deficits (average ataxia score of 5.07+/-0.26 at 3 months of age), while age matched WT mice did not show any neural deficits (average ataxia score of 0.51+/-0.07). The comparison of brain histology of WT and SSAT/SMOX-dKO mice revealed an increased presence of intensely stained contracted nerve cell clusters and vacuolization in the granular layer and white matter of the cerebellum. Our results also show that compared to WT mice, the number of Purkinje cells is decreased and staining intensity of calbindin-1 in Purkinje cells and α-synuclein punctates in the white matter of SSAT/SMOX-dKO is significantly increased. Analysis of brain structure by magnetic resonance imaging revealed increased hyperintensity and significant atrophy in the cerebellum of SSAT/SMOX-dKO but not WT animals. Chemical analysis revealed decreases in glutamate and N-acetylaspartate and an increase in the inositol content of the cerebellum. Gene expression analysis by RNAseq also revealed alterations in the expression of mRNAs that account for potential mechanisms that lead to the onset of ataxia in SSAT/SMOX-dKO mice. Our results indicate that polyamine catabolism is important in the maintenance of normal cellular polyamine levels, and that the accumulation of polyamines due to the disruption of their catabolism is cytotoxic, especially in cerebellum and causes overt ataxia in mutant mice with the combined deletion of SSAT and SMOX genes.

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**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.02/HH6

**Topic:** E.02. Cerebellum

**Title:** Comparison of anodal and cathodal tDCS on cerebellum using an *In vivo* approach

**Authors: R. MANCHANDA**[1], A. KEITH[1], A. DE LA CRUZ[1], *H. LU[2]
[1]GA Campus Philadelphia Col. of Osteo. Med., Suwanee, GA; [2]PCOM - Georgia Campus, Suwanee, GA

**Abstract:** According to recent studies, the use of transcranial direct current stimulation (tDCS) has shown promise as a potential therapy for cerebellar movement disorders. Our previous studies explored the effects of anodal tDCS stimulation on the Purkinje cell firing rate and local field potentials (LFPs) in the cerebellar cortex. The aim of our current study is to investigate the effects of cathodal tDCS stimulation on Purkinje cell firing rates and cerebellar cortical LFPs. From 5 Sprague-Dawley rats, 6 Purkinje cells were isolated from the cerebellar cortex along with LFP recordings in the cerebellar and cerebral cortices. The intensity of cathodal stimulation was set at 100 µA and 200 µA. Power spectrum analysis was conducted to study the general cerebellar cortical activity changes by tDCS. The results revealed an increase in the amplitude at approximately 10 Hz in 2 out of 6 cells. The remaining 4 cells did not show any significant changes in amplitude. Analysis of the mean frequency was conducted to study the individual Purkinje cell output. Three cells showed a decrease in firing rate following cathodal stimulation, while other three cells showed an increase in firing rate following the same stimulation. In general, cathodal stimulation resulted in an average decrease of 20% in the Purkinje cell firing rate. Our previous studies using anodal stimulation resulted in a 13% decrease in firing rate of Purkinje cells on average. Cross correlation and coherence analyses were used to assess the relationship between the cerebellar and motor cortices. A cross-correlation level of 0.2-0.5 was observed in all recordings, which is consistent with result from anodal stimulation. Four out of 6 cells showed coherence between the cerebellum and cerebral cortices at 10 Hz. Two of those 4 cells showed additional coherences at 75 and 80 Hz respectively. These changes that occurred at the high frequency range are not consistent with either anodal or cathodal stimulation.

**Disclosures:** **R. Manchanda:** None. **A. Keith:** None. **A. De La Cruz:** None. **H. Lu:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.03/HH7

**Topic:** E.02. Cerebellum

**Title:** Trajectories of myelinated axons that travel within the purkinje cell layer

**Authors: *M. ARIEL**[1], D. T. DALY[2]
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**Abstract:** At SfN ’16, we described a transverse cerebellar commissure within the Purkinje Cell Layer (PCL) of red ear pond turtles, *Trachemys Scripta*. This path was observed by tracer transport in myelinated axons that connect each lateral edge of the cerebellar cortex (Cb) to one another. Those axons also connected with vestibular regions elsewhere, properties that were suggested by previous physiological recordings (Brown et al, J Neurophysiol 2011). However, these axons were only viewed as thick fibers in thin transverse sections near the Cb midline. The present work evaluates axonal orientations within the narrow (20-50 µm) layer of the PCL at the Cb midline, when sectioned tangential to the pia surface. Turtle brains were studied due to their unfoliated cerebellar cortex whose neurons transport tracers when maintained in vitro. One day following fluorescent dextran placement in the lateral Cb in vitro, the Cb was gently flattened between two glass coverslips, fixed (4% para in PBS) and cryoprotected (25% sucrose). 30-µm tangential sections were examined by confocal microscopy. Alternatively, a fresh Cb was immediately flattened, fixed, cryoprotected, sectioned and labeled immunofluorescently for Myelin Basic Protein (MBP). In these tangential sections, as previously observed in transverse sections, dextran filled axons were observed crossing the PCL at the midline. Using 120x magnification, orientations were measured for axons that extended at least 70 µm on either side of midline. Cluster analysis revealed that the orientation of the largest set of fibers (80% of the sample) was not statistically different than the 0° transverse plane (-6.3° ± 0.7; N= 289, $p < 0.05$ V test; Berens 2009). At 20x magnification, labeled axons were also observed with oblique trajectories, indicating a path to the Cb peduncle. For Cb with anti-MBP immunolabeled axons, orientations were also analyzed at the midline with 120x magnification. Surprisingly, two other sets of axons were observed in addition to the commissure. One large set of axons traveled primarily along the sagittal midline (Cb raphe) within the PCL and ventral to it. A second smaller group had transversely oriented axons dorsal to the PCL; a population of thin myelinated parallel fibers with varicosities. In summary, tangential Cb sections reveal myelinated axons with multiple orientations. Unlike white matter tracts, these thick axons traveled tortuously through the PCL, avoiding large Purkinje cell bodies in their path. Whereas the commissural axons that

connect the two vestibulocerebellar regions may enable bilateral vestibular processing, a function for a myelinated tract along the Cb midline remains to be elucidated.

**Disclosures: M. Ariel:** None. **D.T. Daly:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.04/HH8

**Topic:** E.02. Cerebellum

**Support:** Wellcome

ERC

Gatsby

**Title:** Graded sign inversion via chemical and electrical circuits in the cerebellar cortex

**Authors: *B. A. CLARK**[1], S. RIEUBLAND[2], A. ROTH[2], C. ARLT[2], M. HÄUSSER[2]
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**Abstract:** Inhibitory pathways are essential functional elements of the nervous system and the interaction between excitation and inhibition underlies key operations performed by neural circuits. The organisation of networks of inhibitory neurons into overlapping groups connected via direct synaptic connections and gap junction coupling provides a framework for diverse recruitment scenarios that expand on the classical roles of inhibitory influence on principal cells. While action potential-driven, chemical synaptic signalling is unidirectional in sign and necessarily involves a threshold, subthreshold electrical signalling is bidirectional in sign and does not involve a threshold. How these circuits interact to shape the functional output of a circuit is not well understood. In the cerebellar cortex, the sole output is provided by Purkinje cells (PCs), which are regulated by synaptic inhibition provided by molecular layer interneurons (MLIs). MLIs are interconnected by both chemical and electrical synapses, and both PCs and MLIs are spontaneously active. To explore the interactions between INs and PCs, we made paired and triple recordings from MLIs and PCs in cerebellar slices at physiological temperatures. In addition to the expected spike-triggered inhibitory connections between monosynaptically connected pairs of INs and PCs, we observed an unexpected effect of INs on PC firing. In some pairs, a steady state hyperpolarization of the MLI caused a subthreshold depolarization of the PC (0.29±0.26 mV n=36). Remarkably, this effect could be observed in both monosynaptically connected and unconnected pairs (71%, n=51). The effect was reversibly blocked by the $GABA_A$ antagonist gabazine. Recordings from cell triplets (MLI-MLI-PC) revealed that this effect was mediated by electrical coupling within the MLI network, such that

the manipulated MLI suppresses firing in coupled INs which, if directly connected to the PC, reduces inhibitory drive, thus disinhibiting the PC. This disinhibition is powerful enough to change spike output in Purkinje cells both during spontaneous and parallel fiber-evoked firing, increasing firing frequency, reducing ISI variability and enhancing the number of PF-evoked spikes. Importantly, the effect was both graded and bidirectional depending on the polarity of the membrane potential change in the IN, and could thus both enhance or suppress PC firing. This mechanism therefore allows both digital and analog, graded signalling modes, allowing the cerebellar network to implement a wide range of mathematical operations. We are currently exploring how this mechanism could influence sensory processing in the cerebellar cortex in vivo.

**Disclosures: B.A. Clark:** None. **S. Rieubland:** None. **A. Roth:** None. **C. Arlt:** None. **M. Häusser:** None.

**Poster**

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**Program#/Poster#:** 591.05/HH9

**Topic:** E.02. Cerebellum

**Support:** NS084996

**Title:** Morphological constraints on cerebellar granule cell layer recombination

**Authors: *J. GILMER**[1], A. L. PERSON[2]
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**Abstract:** Granule cells are theorized to encode combinations of mossy fiber inputs, but their capacity to recombine mossy fiber inputs is naturally subject to spatial limitations created by the morphological specializations specific to the granule cell layer. Using computer modeling and anatomical measurements, we explored the consequences of the limitations of granule layer architecture on mixing of mossy fiber afferents by granule cells. We show that the number of unique combinations of mossy fiber inputs is rapidly saturated as mossy fiber inputs diversify in space, and that patchy mossy fiber ramification patterns produce unexpectedly robust representations of specific mossy fiber combinations. Several features of the granule layer organization positively enhance combinatorial diversity including sparse rosettes and mossy fiber filopodia. These predictions of the model were then validated in anatomical measurements from mouse cerebellum. Our results complement information theoretic studies of granule layer structure and provide insight into the contributions of granule layer anatomical features that may favor dense information representation useful for temporal expansion recoding.

**Disclosures:** **J. Gilmer:** None. **A.L. Person:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

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**Topic:** E.02. Cerebellum

**Support:** JSPS Overseas Research Fellowships

NIH Grant RO1 NS084996

**Title:** Diverse modes of cerebellar Golgi cell recruitment

**Authors: *S. TABUCHI**, A. L. PERSON
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**Abstract:** Temporal diversity of granule cell (GrC) activity is hypothesized to serve cerebellar associative learning, but the mechanisms of temporal diversification are obscure. Golgi cells (GoCs), the inhibitory neurons in the GrC layer, mediate both feedforward- and feedback-inhibition, and therefore are proposed to participate in the circuit mechanisms producing temporal diversity. We examined how GoCs mediate these different forms of inhibition and the consequences of these modes of recruitment on GrC activity. We focused on the recruitment of GoCs by a single mossy fiber type (nucleocortical MFs) using electrophysiology and anatomy. In some GoCs, optogenetic stimulation of nucleocortical MFs reliably elicited EPSCs which followed the stimuli and drove robust increases in firing rate. By contrast, other GoCs responded with low-probability EPSCs during stimuli despite dense labeling of MFs in the vicinity of the cell. To observe the detailed morphology of GoCs, we labeled GCL neurons sparsely by injecting full titer AAV.CAG.FLEX.eGFP and low titer AAV8.hSyn1.mCherry.Cre to the GCL in wild type mice. We imaged a single GoC and produced a complete 3D reconstruction, revealing sparse basal dendrites and a massive basal axonal arbor, which possessed over 4200 boutons and over 10000 $\mu m^3$ volume. This arrangement of input and output structures of the same GoC indicate that a spatially restricted population of MFs with access to basal dendrites and soma can drive feedforward inhibition compared to larger populations of MFs that must recruit large numbers of GrCs to drive feedback inhibition. In keeping with this idea that GoC-mediated inhibition is widespread relative to its excitation, optogenetic activation of the nucleocortical pathway elicited IPSCs in GrCs without necessarily eliciting EPSCs. We propose that these spatiotemporal recruitment patterns can produce diversification of activity in GrCs.

**Disclosures:** **S. Tabuchi:** None. **A.L. Person:** None.

**Poster**

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**Topic:** E.02. Cerebellum

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CONACyT (ZSHB) 326816

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CA-28-Neurociencias

**Title:** Multiunit recording of the cerebellum after electrolytic lesion of the ventrolateral striatum

**Authors: *L. VASQUEZ CELAYA**[1], J. R. GUTIERREZ[1], Z. S. HERNANDEZ[1], M. E. HERNANDEZ[2], G. A. CORIA-AVILA[2], P. CARRILLO[3], J. MANZO[2], M. A. MIQUEL[4], L. I. GARCIA[2]
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**Abstract:** In patients with Parkinson's disease (PD), as a possible compensatory mechanism for the cortico-basal circuitry deficit, the cerebellar function is altered by basal ganglia. For this, several studies support the idea that both structures function as an integrated system. The present study was designed to analyze the bioelectric activity of cerebellum´s granular neurons of sim B y crus II lobules and the dentate nucleus after ventrolateral striatum (VLS) electrolytic lesion. This lesion provoked a mandibular tremor similar to the patients with PD. Male Wistar rats (250 and 350 g) were assigned to 3 groups: 1) the intact group, were the cerebellar multiunitary activity (MUA) was recorded; 2) the sham group, in which only one electrode was descended in VLS, and 3) the lesion group, in which in addition to the cerebellar registration implant, an electrolyte lesion (3.5 mV/30 sec) were performed on the VLS. In all three groups three subgroups were formed to record single structures (lobes Sim b, Crus II and dentate nucleus) in which the MUA was recorded (steel electrode of 250 µ diameter). In all groups, the basal activity and the maximum/minimum amplitude reached during mandibular tremor provoked by the lesion were analyzed. Following MUA recordings, it was performed the histological verification of the implant. The results show differences in baseline MUA among groups and structures, evidencing a decreased basal activity in the sham and lesion groups compared to the intact group. On the other hand, we unexpectedly observed the presence of mandibular tremor in the sham group, as observed in the lesion group. Mandibular tremor was correlated with an increase in MUA,

especially in Crus II in both sham and lesion groups. These findings suggest a functional relationship of the cerebellum with the VLS.

**Disclosures: L. Vasquez celaya:** None. **J.R. Gutierrez:** None. **Z.S. Hernandez:** None. **M.E. Hernandez:** None. **G.A. Coria-Avila:** None. **P. Carrillo:** None. **J. Manzo:** None. **M.A. Miquel:** None. **L.I. Garcia:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.08/HH12

**Topic:** E.02. Cerebellum

**Title:** Direct current stimulation modifies activity of Purkinje cells: Using *In vitro* and in computo approaches

**Authors: *C. MOORE**, A. WANG, T. PHAN, H. LU
Philadelphia Col. of Osteo. Med., Duluth, GA

**Abstract:** The cerebellum plays essential roles in movement coordination and motor control. Modulation of cerebellar activity using transcranial direct current stimulation (tDCS) has become a potential method of treatment for cerebellar dysfunctions. tDCS has been shown to have an effect on Purkinje cell activity through *in vivo* studies, but the underlying mechanisms are not well understood. In this project, *in vitro* method was used to quantitatively measure the effects of DCS on basic properties of Purkinje cells using patch clamp technique. The frequency changes of Purkinje cell action potentials under anodal and cathodal stimulation were compared using a paired Student's *t*-test ($p=0.27$, $n=5$). No significant changes were observed in relationship with orientation of the Purkinje cell with the intensity of DCS set at 200 μA. We also analyzed the effect of graded intensity on the firing rate of Purkinje cells starting at 200 μA to maximum 800 μA. Additional efforts were made to study the relationship between the orientation of the dendrites of a cell and the electrical field using a multi-compartment model. A model with a single dendrite displays specific polarization along the soma-dendrite axis within an electric field (+0.5 to -0.5 mV) generated by DCS. To simulate the effect of DCS on a cell with branching dendrites, additional dendritic compartments were added to the model. With the same setting of electric field, the model with branches demonstrated less voltage change and a smaller increase in firing rate when anodal stimulation was delivered.

**Disclosures: C. Moore:** None. **A. Wang:** None. **T. Phan:** None. **H. Lu:** None.

**Poster**

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**Topic:** E.02. Cerebellum

**Support:** NIH R37-NS39395

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**Title:** Sensory and motor representations in Purkinje cell firing during spontaneous and sensory-evoked whisking

**Authors: S. BROWN**, *I. M. RAMAN
Neurobio., Northwestern Univ., Evanston, IL

**Abstract:** To explore sensorimotor processing associated with whisking in the cerebellum, we made extracellular recordings from 32 Purkinje cells in crus I of awake, head-fixed mice habituated to sitting in a tube. Simple and complex spikes were measured during non-contact whisking. During spontaneous whisking, 17 cells showed increases in simple spike rate triggered to the initiation of whisking. Cross-correlations indicated that the rate increase coincided with or preceded the onset of movement. Also, 13 cells showed increases in complex spike probability at the initiation of whisking, usually with a latency of 0-100 ms. Purkinje cell firing rates were correlated to slow varying parameters of whisking. To investigate which aspects of Purkinje cell firing relate to sensory input, sensory feedback from whisker movement (reafference), and/or motor commands, we evoked whisking with sensory stimulation and recorded Purkinje activity. Puffs (10-ms) applied to either the ipsilateral or contralateral whisker pad elicited bilateral whisking that lasted >250 ms, and also elicited increases in Purkinje cell simple spike rate that were correlated to whisker movement. The initial, short-latency component of the Purkinje cell response to the ipsilateral puff was altered when the puff was applied contralaterally, consistent with a dependence on the primarily ipsilateral trigeminal (sensory) projections to the cerebellum. Since the duration of the changes in Purkinje cell firing rates often correlated with the duration of evoked whisking, we tested whether sensory feedback from whisker movement was responsible for the prolonged Purkinje cell responses. Lidocaine was infused into the cheek to silence the ipsilateral facial nerve, which abolished ipsilateral whisking while leaving contralateral movement intact. Under these conditions, Purkinje cell firing was indistinguishable from control, suggesting that reafference does not contribute substantially to the response of these Purkinje cells during whisking. Therefore, to test whether the prolonged Purkinje cell activity following puffs contributes to evoked whisker movement, we inhibited Purkinje cell targets in the cerebellar nuclei. Optogenetically stimulating channelrhodopsin-expressing Purkinje cells in the vicinity of the recording sites inhibited even the earliest components of puff-evoked whisker

movements by ~30%. These results suggest that the targets of these Purkinje cells usually modulate reflexive whisking and that Purkinje cell firing sculpts normal puff-evoked whisker movement.

**Disclosures: S. Brown:** None. **I.M. Raman:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

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Johns Hopkins Science of Learning Grant

**Title:** Cerebellar complex spikes encode error direction and magnitude

**Authors: *D. J. HERZFELD**[1], Y. KOJIMA[2], R. SOETEDJO[2], R. SHADMEHR[1]
[1]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Physiol. & Biophysics, Univ. of Washington, Seattle, WA

**Abstract:** The study of motor learning seeks to answer a fundamental question: how do we learn from error? When the outcome of our movement is different than expected, the brain learns from this sensory prediction error by adapting the next movement to partially compensate; a computation that crucially relies on the cerebellum. The hypothesis of cerebellar learning suggests that adaptation is driven by sensory prediction errors communicated via climbing fibers to Purkinje cells (P-cells), resulting in complex spikes (CSs). Here, we ask whether CSs encode both the magnitude and direction of sensory prediction errors experienced in a saccade adaptation task.

We trained monkeys to produce saccades to visual targets while recording P-cell activity from the oculomotor vermis (OMV). In every trial, the monkey made a saccade to a target. However in some trials, we jumped the target during the saccade. In other trials, the target was not displaced but the natural variability of the saccade produced an endpoint error. In each trial, we measured eye position at saccade termination and computed an error vector.

Following completion of the saccade, P-cells produced a complex spike with a probability that depended on the direction of the error vector. That is, following saccade termination, CSs exhibited cosine tuning with respect to the foveal error direction. When the error was in the

preferred direction, the probability of CS was three-fold higher than when it was in the anti-preferred direction. Therefore, the direction of sensory prediction error was encoded in the probability of CS.
The brain, however, learns from both error direction and magnitude. We quantified the trial-to-trial change in the saccade amplitude following an error. The magnitude of error significantly modulated the amount of learning: a large behavioral change occurred following a large error.
How do CSs encode error magnitude?
To answer this question, we considered trials in which a CS occurred during the post-saccadic period. We organized these trials based on the magnitude of the error in each P-cell's preferred direction. We found that when the error was large, the CS tended to occur soon after saccade termination. That is, while the direction of a sensory error was encoded in the probability of CS, the magnitude of the error was encoded in the timing of the CS. We found that trial-to-trial change in eye speed was greatest following an early CS, suggesting that CS timing directly affected the amount of learning from error.
Our results demonstrate that whereas the direction of the error vector is encoded in the probability of the complex spike, the magnitude is encoded in the timing of the spike.

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**Poster**

**591. Cerebellum: Physiology and Circuit Function**

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**Topic:** E.02. Cerebellum

**Support:** MOE2016-T2-1-097

**Title:** Unique circuit organization of novel cerebellar molecular layer interneurons receiving Purkinje cell feedback inhibition

**Authors: *J. KIM**, G. J. AUGUSTINE
Lee Kong Chian Sch. of Med. - NTU, Singapore, Singapore

**Abstract:** A subset of molecular layer interneurons (MLIs) receive inhibitory inputs from Purkinje cells (PCs). These MLIs have distinctive morphological and intrinsic electrical properties, indicating that they are a third type of MLI called PC-MLI. We have used optogenetics and paired whole-cell recordings in cerebellar slices to characterize the synaptic connectivity of PC-MLIs. While basket cells (BC), another type of MLI, form electrical synapses with other MLIs, PC-MLIs do not. Evidence for this is: (1) neurobiotin does not spread between PC-MLIs and BCs, while it does spread between BCs; and (2) although the input resistance of

PC-MLIs is higher than that of BCs, this difference is abolished by treatment with blockers of electrical synapses. In addition to inhibitory input from PCs, PC-MLIs rarely (~4%) receive inhibitory input from other MLIs. Thus, the main inhibitory drive of PC-MLIs comes from PCs. PC-MLIs also receive excitatory input from both parallel fibers and climbing fibers, suggesting that they have the same sources of excitatory drive as other MLIs. The output of PC-MLIs includes inhibition of neighboring basket cells (~33%) and PCs (~10%). No reciprocal connections were detected between PC-MLIs and PCs. Although input from PCs was readily detected in sagittal slices, synaptic connections between PC-MLIs and PCs were detected only in oblique slices. Thus, unlike other MLI circuits, the circuit between PC-MLIs and PCs apparently is not organized in the sagittal plane. This may provide a mechanism for synchronizing PCs in different layers via disinhibition. In summary, PC-MLIs are a third type of MLI with unique circuit organization. These neurons, and their circuits, are likely to play unique and important roles in cerebellar information processing.

**Disclosures: J. Kim:** None. **G.J. Augustine:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

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**Topic:** E.02. Cerebellum

**Support:** NIH Grant EY011027

**Title:** Purkinje cell inhibition charges the neural integrator

**Authors: *T. KODAMA**, S. DU LAC
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**Abstract:** Cerebellar Purkinje cells (PCs) increase and decrease firing rate in specific contexts of behaviors. It is however not fully understood how downstream neurons respond to PC firing rate modulation to adaptively control behaviors. We investigated eye movements evoked by excitation and inhibition of PCs that directly innervate pre-oculomotor neurons in the brainstem, and discovered that PC inhibition preferentially induces persistent changes in eye position. In mouse lines expressing photoactivatable opsins (ChR2 or ArchT) selectively in PCs, both photo-excitation and inhibition of PCs reliably accelerated eye movements in opposing directions (Kodama and du Lac, 2016). Following PC excitation, the eye returns to the initial position in ~1 sec. However, after PC inhibition, changes in eye position are maintained for seconds, although PC firing rate returns to baseline within <0.5 sec. The asymmetric influence of PC activity is evident in extracellular recordings in downstream neurons. These results demonstrate that PC inhibition preferentially charges the neural integrator for gaze holding.

**Disclosures:** **T. Kodama:** None. **S. du Lac:** None.

**Poster**

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**Topic:** E.02. Cerebellum

**Support:** ANR Labex BioPsy

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ANR-13-BSV4-0016

**Title:** Temporal processing in the cereballar cortex enabled by dynamical synapses

**Authors: A. BARRI**[1], M. WIECHERT[2], F. CHABROL[3], *D. A. DIGREGORIO[1]
[1]Neurosci., Inst. Pasteur, Paris, France; [2]Dept. of Physiol., Univ. of Bern, Bern, Switzerland; [3]Biozentrum, Univ. of Basel, Basel, Switzerland

**Abstract:** The cerebellar cortex (CC) is considered to be essential for the learning of precisely timed tasks on the order of several tens of milliseconds to few seconds. Cerebellar adaptive filter models generally consider the CC as a three layered network where mossy fibers (MFs) and Purkinje cells (PCs) form the input and output layer, respectively, and granule cells (GCs) constitute a hidden layer. In this framework, for PCs to learn time-variant signals it is necessary that the GC population responds to MF inputs with sufficiently diverse time-varying responses. It still remains an open question by which mechanisms these time varying GC signals can be produced. Recent findings have established that synaptic transmission between MFs and GCs exhibits various forms of synaptic short-term plasticity (STP). Here we show that these synaptic dynamics can provide a sufficiently rich temporal modulation of GC activity to enable temporal learning by PCs on behaviorally relevant timescales. First, we re-analyzed data from whole-cell recordings of MF-GC synaptic currents from Chabrol *et al.* (2015) and extracted parameters associated with pre-synaptic depression, facilitation and post-synaptic receptor desensitization. This revealed the existence of a rich diversity of synaptic time-constants ranging from a few milliseconds to seconds. In a second step, we used the experimentally obtained synaptic parameter distributions to constrain a firing-rate-based model of the CC. In this model, GCs exhibit transient firing rate modulations in response to rapid changes in MF activity, as observed experimentally (Ishikawa *et al.* 2015). Due to the diversity of the synaptic parameters, GC transients exhibit a broad diversity across cells. Interestingly, during these transients the GC population is densely activated as suggested by recent in vivo recordings (Giovannucci *et al.* 2017). Furthermore, GC transients elicited strong modulations of model PC firing rates, similar

to experimental findings (Bosman *et al.* 2010). We also showed that GC firing transients form a temporal basis that is sufficient to enable PCs to encode stimulus duration and interval length, a critical requirement for rapid time scale auditory perception. We then took advantage of classical supervised learning to demonstrate that GC firing response diversity could also be used to teach PCs to predict delayed stimuli, as in the classical eye-blink conditioning paradigm. Our theoretical study demonstrates that the distribution of STP at the input layer of the CC is sufficient to elicit temporal computations in a purely feed-forward manner.

**Disclosures:** **A. Barri:** None. **M. Wiechert:** None. **F. Chabrol:** None. **D.A. DiGregorio:** None.

**Poster**

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CSC 201306230130

**Title:** Differentiation of cerebellar functioning: The role of TRPC3 in physiology and behavior

**Authors:** ***B. WU**[1], F. BLOT[1], C. OSORIO[1], H.-J. BOELE[1], C. DE ZEEUW[1,2], M. SCHONEWILLE[1]
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**Abstract:** Purkinje cells (PCs) in the mammalian cerebellum are remarkably homogeneous in shape and orientation, yet they exhibit regional differences in spiking activity which correlate with the expression of zebrin. The firing rates of both the simple spikes and complex spikes in zebrin-negative ($Z^-$) PCs are higher than those in the zebrin-positive ($Z^+$) PCs. However, the underlying cause and functional relevance of this physiological difference between zebrin-discriminated PCs remains unknown. We have previously suggested a role for the transient receptor potential cation channel type C3 (TRPC3) protein in the difference in simple spike activity. Here, we recorded the spiking activity of Z- and Z+ PCs in gain-of-function and PC-specific knockout TRPC3 mutants both *in vivo* and *in vitro*. The results indicate that bidirectional manipulation of TRPC3 results in corresponding regulation of PC simple spike activity, particularly in Z- PCs. Next, we performed vestibulo-ocular reflex adaptation and eyeblink conditioning as these tasks are predominantly controlled by Z+ PCs and Z- PCs, respectively.

Our results suggest that TRPC3 contributes to the physiological discrepancy between Z- and Z+ PCs, as it selectively affects the spiking activity of Z- PCs as well as Z- PC-dependent behaviors.

**Disclosures: B. Wu:** None. **F. Blot:** None. **C. Osorio:** None. **H. Boele:** None. **C. De Zeeuw:** None. **M. Schonewille:** None.

**Poster**

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**Support:** NIH Epilepsy Training Grant

Hughes Collaborative Innovation Award

**Title:** Imaging cerebellar granule cells in behaving mice

**Authors: *M. J. WAGNER**[1], T. H. KIM[1], M. J. SCHNITZER[1,2], L. LUO[1,2]
[1]Stanford Univ., Stanford, CA; [2]Howard Hughes Med. Inst., Stanford, CA

**Abstract:** The human brain contains ~60 billion cerebellar granule cells[1], which outnumber all other brain neurons combined. Through extensive closed-loop connectivity with most regions of the cerebral cortex[2,3], the cerebellum is poised to play a role in a wide variety of brain computations. Yet relatively little is known about the types of information that reach the cerebellum via granule cell inputs. To better understand the full range of cerebellar computations, we are studying granule cell signaling during motivated behaviors using two-photon $Ca^{2+}$ imaging. We have found that granule cells convey information about the expectation of reward during multiple types of operant and Pavlovian behaviors. Granule cells selectively encoded reward, reward omission, and reward anticipation in ways that were unexplained from sensorimotor signaling. Such reward representations were found throughout multiple cerebellar lobules. Individual granule cell reward representations emerged over several days of learning[4]. In other complex movement tasks, we have also found high-level representations of planning information in granule cells. Both reward and planning signals are reminiscent of known signaling in various prefrontal and premotor neocortical areas. Thus we are also studying neural representations of our tasks in cortical output ensembles to determine how cortex gives rise to cerebellar input signals, with the goal of understanding coordinated computations in these circuits.
1. Herculano-Houzel, S. Coordinated Scaling of Cortical and Cerebellar Numbers of Neurons. *Frontiers in Neuroanatomy* **4**, 12 (2010). 2. Coffman, K. A., Dum, R. P. & Strick, P. L. Cerebellar vermis is a target of projections from the motor areas in the cerebral cortex.

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**Disclosures: M.J. Wagner:** None. **T.H. Kim:** None. **M.J. Schnitzer:** None. **L. Luo:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.16/HH20

**Topic:** E.02. Cerebellum

**Support:** NIH Grant (092809-01)

**Title:** Presynaptic $GABA_A$ receptors can enhance or inhibit vesicle release depending on $GABA_B$ receptor activity

**Authors: *R. D. HOWELL**[1], Y. YANG[1], S. MELLEY SADANANDAN[1], J. R. PUGH[2]
[1]UT Hlth. San Antonio, San Antonio, TX; [2]Physiol., UTHSCSA, San Antonio, TX

**Abstract:** A wide variety of synapses in the CNS express $GABA_A$Rs at the presynaptic membrane. Many recent studies agree that these receptors depolarize the presynaptic terminal and enhance synaptic vesicle release. However, these studies were conducted with either $GABA_B$Rs pharmacologically blocked or using a $GABA_A$R specific agonist to avoid $GABA_B$R mediated inhibition of vesicle release. It has recently been shown that $GABA_B$R activation enhances currents through extrasynaptic, delta subunit-containing $GABA_A$Rs in the soma or dendrites of several neurons, including cerebellar granule cells (Tao et al., 2013, Connelly et al., 2013). This raises the possibility that presynaptic $GABA_A$Rs, which may also contain delta subunits, are also enhanced by $GABA_B$Rs. To test this possibility, we made whole cell patch clamp recordings from molecular layer interneurons in acute cerebellar slices and evoked parallel fiber EPSCs. On alternating sweeps GABA was applied 50 ms before the stimulus by photolytic uncaging of RuBi-GABA (60 μM). EPSC amplitudes and paired-pulse ratios were compared between control and uncaging sweeps. We found that GABA uncaging increased EPSC amplitudes, and this effect was blocked by the $GABA_A$R antagonist, picrotoxin. However, this increase was also blocked by a $GABA_B$R antagonist CGP55845, suggesting that without $GABA_B$R activity, the $GABA_A$R currents were not sufficient to enhance vesicle release. In fact, when we used high concentrations of RuBi-GABA (1-5 mM), we found that GABA increased EPSC amplitudes even in the presence of CGP55845. These data are consistent with $GABA_B$R

enhancement of presynaptic $GABA_AR$ currents.We then measured presynaptic $GABA_AR$ function over a range of GABA concentrations by systematically changing the uncaging laser intensity. Surprisingly, we found that in control conditions ($GABA_BR$ not blocked) presynaptic $GABA_ARs$ inhibit, rather than enhance, vesicle release at all but the lowest uncaging light intensities. There was only a very narrow range of light intensities over which GABA enhanced vesicle release. In picrotoxin, EPSC amplitudes were not altered at any laser intensity, suggesting both the enhancement and inhibition are mediated by $GABA_ARs$. In the presence of CGP55845, we observed enhancement of EPSCs only at high uncaging intensities and never saw inhibition, consistent with previous studies of presynaptic $GABA_ARs$. These data suggest that presynaptic $GABA_ARs$ are normally enhanced by $GABA_BRs$. Furthermore, when $GABA_BR$ activity is intact, the primary effect of presynaptic $GABA_AR$ activity may be to inhibit, rather than enhance, vesicle release.

**Disclosures:** **R.D. Howell:** None. **Y. Yang:** None. **S. Melley Sadanandan:** None. **J.R. Pugh:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.17/HH21

**Topic:** E.02. Cerebellum

**Support:** EMBO

Wellcome Trust

**Title:** Probing the functional interactions between neural populations in the cerebellar cortex and deep nuclei of awake behaving mice

**Authors: *M. BEAU**[1], D. KOSTADINOV[3], M. BLANCO POZO[1], M. HAUSSER[2]
[1]WIBR, [2]UCL, London, United Kingdom; [3]Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

**Abstract:** The functional interactions between neurons in the cerebellar cortex and the deep cerebellar nuclei (DCN) are crucial for understanding cerebellar computations, and yet are almost completely unexplored in behaving animals. This requires simultaneous recordings from neurons in the two brain areas, which is technically extremely challenging. We have addressed this problem by harnessing a new recording approach - Neuropixels silicon probes, which allow for sampling from 384 densely-spaced recording channels along a linear shank that is capable of spanning both cerebellar cortex and DCN. We have performed Neuropixels recordings in awake head-fixed mice performing a behavioural task. In these recordings, we could harvest up to 100

well-isolated units simultaneously recorded across a recording depth of almost 4 mm. We were able to classify a majority of these units into putative cell types using prior knowledge about the distinctive electrophysiological signatures of cerebellar neurons, as well as by post-hoc histology. In the cerebellar cortex, recordings typically spanned several folia, with the major cell types - Purkinje cells, molecular layer interneurons and granule cells - being readily identifiable. Typical recordings yielded 25 ± 5 Purkinje cells, 15 ± 5 molecular layer interneurons and 10 ± 2 granule cells (n = 3 mice). We also identified units consistent with published firing patterns of mossy fibre bouton terminals and Golgi cells. Recordings from interpositus nucleus revealed units (typically about 15) with diverse firing properties and spike shapes consistent with recordings from principal cells and inhibitory neurons. Thus, we have been able to record simultaneously from all the major cell types in the cerebellar cortex while also sampling from the targets of Purkinje cells in the interpositus DCN. Our preliminary results have identified both inhibitory and excitatory relationships between neurons in close proximity (both Purkinje cell and DCN neuron pairs) as well as between neurons separated by hundreds of micrometres. We are now using this system to probe the dynamics of interactions between these cell types during a sensorimotor integration task in virtual reality, allowing us to determine how sensory and motor variables are encoded in the patterns of activity. This approach highlights the power of this new generation of silicon probes for examining millisecond timescale cellular-resolution interactions between multiple brain regions, and will provide unprecedented insights into how the cerebellar cortico-nuclear interactions govern sensorimotor behaviour.

**Disclosures:** **M. Beau:** None. **D. Kostadinov:** None. **M. Blanco Pozo:** None. **M. Hausser:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.18/HH22

**Topic:** E.02. Cerebellum

**Support:** NIH Grant NS083894

**Title:** Cerebellar-dependent motor memory depends on patterned changes in molecular layer interneuron activity

**Authors:** ***A. BONNAN**[1], M. A. GAFFIELD[1], K. ZHANG[1], J. M. CHRISTIE[2]
[1]MPFI, Jupiter, FL; [2]Synapse Physiol. Group, Max Planck Florida Inst., Jupiter, FL

**Abstract:** During vestibulo-ocular motor learning, Purkinje cells (PCs) acquire phase shifts in their pattern of spiking thus providing a neural correlate for the expression of adaptive movements. Temporal re-patterning of PC spiking has long been attributed to synaptic plasticity

at their parallel fiber inputs. However, inhibitory molecular layer interneurons (MLIs) also form connections onto PCs. Therefore MLIs are poised to influence PC excitability and spike output. To assess for their role in the expression of motor learning, we measured MLI activity in the flocculus during recalibration of the vestibulo-ocular reflex (VOR) using genetically encoded $Ca^{2+}$ indicators. We found that over the course of gain increase learning, MLIs progressively developed a shift in the phase of their activation induced by passive head rotation. Although optogenetic stimulation of MLIs could induce eye movements in quiescent animals, suppressing their activity had no effect on baseline VOR performance indicating that MLIs were dispensable for accurate movement in naïve mice. . In contrast, after gain increase learning, suppressing MLI activity led to the immediate reversal of adapted eye movements. Interestingly, MLI activity remained unchanged following gain decrease learning and its expression was not susceptible to reversal upon MLI suppression pointing to mechanistic distinctions between different types of learning. Together, our results indicate that a change in MLI activity is required for the accurate expression of certain forms of motor learning. This points to their synaptic connections as potential storage sites for motor memory.

**Disclosures:** **A. Bonnan:** None. **M.A. Gaffield:** None. **K. Zhang:** None. **J.M. Christie:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.19/HH23

**Topic:** E.02. Cerebellum

**Support:** MH46904

MH74006

**Title:** Recordings across sessions is the foundation for using eyelid conditioning as a model system to study aging

**Authors: *H. E. HALVERSON**[1], M. D. MAUK[2]
[1]Ctr. for Learning and Memory, Univ. of Texas At Austin, Austin, TX; [2]Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

**Abstract:** Characterizing change in neural responses across time allows for identification of the mechanisms supporting learning in the short term (days) and how age might interact with those mechanisms in the long term (years). Extracellular recording of single unit activity with tetrodes in rabbits provides a method to track the same neuron(s) over the course of a training session (1 hour), possibly across many sessions (multiple days) and even the same population over the entire life span of the animal (years). Tracking the activity of neurons across different training

sessions allows for investigation of neural responses within a given brain area in response to changes in task demands. Comparing recordings from the same neuron types at different points across the life span allows for investigation of how aging interacts with neural mechanisms of learning and memory. Single unit tetrode recordings from the cerebellum of rabbits were done during eyelid conditioning at different inter-stimulus intervals. Recordings focused on the subset of neurons controlling the kinematics of conditioned eyelid responses that are solely driven by the cerebellum. Recordings were done over multiple sessions from eyelid Purkinje cells and eyelid interpositus neurons during eyelid conditioning. Recording protocol focused on tracking individual single units until the recording was lost. Identification of the proper neurons in the cerebellum combined with the ability to record the same neurons across sessions provides a method to investigate how learning mechanisms might change over time in response to changes in task demands. Stable recordings across sessions also allowed for longitudinal investigation of single unit activity of the same population of eyelid cells in the cerebellum over the life span. Tetrode recordings in the cerebellum during eyelid conditioning over the life span of rabbits provides a good model system to investigate how aging interacts with the mechanisms supporting learning and memory.

**Disclosures: H.E. Halverson:** None. **M.D. Mauk:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.20/HH24

**Topic:** E.02. Cerebellum

**Support:** The Wellcome Trust

ERC

Gatsby Charitable Foundation

**Title:** Structured synaptic input to spiny branchlets of cerebellar Purkinje cells

**Authors: *A. ROTH**, M. BOZNAKOVA, S. RIEUBLAND, M. JAKUBOWSKA, M. HAUSSER
Univ. Col. London, London, United Kingdom

**Abstract:** The dendrites of cerebellar Purkinje cells receive up to 200,000 parallel fibre inputs, one climbing fibre, and inhibitory inputs from molecular layer interneurons. Different synaptic inputs to Purkinje cells are uniquely identifiable in EM data, providing the singular opportunity to examine their spatial distribution across an entire neuron with ultrastructural resolution. Here we use 3D reconstructions of rat Purkinje cell spiny branchlets (including all spines and input

synapses) from data obtained with focused ion beam scanning EM to map the spatial distribution of spines and synapses on Purkinje cell dendrites. Synapses were classified as excitatory or inhibitory based on the orientation and branching pattern of the presynaptic axon. On average, spiny branchlets received 7.1 ± 2.6 excitatory and 0.45 ± 0.49 inhibitory synapses per micron of dendritic length (n = 68 branches). The average ratio of the number of excitatory and inhibitory synapses was 14.3. Interestingly, 40% of inhibitory inputs arrive on spines. Of all spines, 98.3% receive excitatory input (10.4% more than one excitatory input), 3.51% receive inhibitory input, and 1.86% receive both types of inputs. We use this data to ask whether the placement of excitatory and inhibitory synapses on Purkinje cell dendrites is random, or whether fluctuations in local synapse density - and in particular the ratio of excitatory and inhibitory synapses - deviate from those expected from random placement. To test these hypotheses, we compare the actual data and a hierarchy of different models of random synapse placement using three different measures: nearest neighbour distance distributions, the output of the clustering algorithms UPGMA and DBSCAN, and network K-functions. We find that excitatory and inhibitory synapses are non-randomly distributed, but most of the deviations from randomness can be explained by the fact that some spines receive multiple synapses. However, even in models accounting for the grouping of synaptic inputs on spines, the distribution of nearest neighbour distances between inhibitory synapses in the data shows an excess at distances between 0.7 and 1.0 µm when compared with most models. Spines themselves are spatially clustered and not randomly distributed. We conclude that the distribution of synaptic inputs on Purkinje cell spiny branchlets is non-random due to non-random placement of spine necks along the dendrite, grouping of synapses on spines, and due to an as yet unexplained association of inhibitory synapses at short distances. This structured input may have direct consequences for the rules of synaptic integration and synaptic plasticity in Purkinje cell dendrites.

**Disclosures:** **A. Roth:** None. **M. Boznakova:** None. **S. Rieubland:** None. **M. Jakubowska:** None. **M. Hausser:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.21/HH25

**Topic:** A.10. Development and Evolution

**Support:** Zhejiang Province Natural Science Foundation (LQ16C060002)

start-up funding (KYQD141109) from Wenzhou Medical University

**Title:** Loss of serine rasemase lead a transient delay in CGC migration

**Authors: *H. ZHANG**[1], S. WU[2]
[1]Sch. of Optometry and Ophthalmology and the Eye Hosp., Wenzhou Med. Univ., Zhejiang, China; [2]Wenzhou Med. Univ., Zhejiang, China

**Abstract:** Inward migration of cerebellar granule cells (CGCs) after birth is critical for lamination of cerebellar cortex. CGCs migrate across Purkinje cell layer, and reach the final destination, the deep strata of inner granule cell layer. N-methyl-D-aspartate subtype of glutamate receptor (NMDAR) tethering CGCs into Bergmann glial fiber mediates this inward movement during the glial-dependent migratory phase. D-Serine, a co-agonist of NMDAR, has been reported to account for CGC migration in cerebellar section. We hypothesized that D-Serine and its synthesizing enzyme serine racemase (SR) may mediate the inward movent of CGCs *in vivo*. Thus, we analyzed cerebellar cortical architecture in wild-type (WT) and *Srr* (the gene for coding SR) mutant mice ($Srr_{null}$) during postnatal stage-- CGC migration is seminal at this time period. The thickness of external germinal cell layer (EGL) in $Srr_{null}$ at postnatal, P7 were37.7±1.7 μm, which were significantly different with WT at P7 29.9±1.8 μm (p=0.00), but the difference disappeared on P12 ($Srr_{null}$ at 10.2±1.7μm *vs*.WT at 10.6±0.6 μm, p=0.484). To elucidate the underlying mechanism, we examined the content of NMDAR ligands and the subunits of NMDAR in cerebellum at this postnatal stage. We found that the level of glycine increased on P7, 10, 12, and the expression of GluN2B on P10 was upregulated in $Srr_{null}$ compared to WT. We are exploring whether additon of D-Serine to cerebella could rescue the temporay postponement before P7. In conclusion, our study revealed that loss of SR lead a transient delay in CGC migration towards the IGL and was compensated by upregulation of Glycine and GluN2B on P10 *in vivo*.

**Disclosures: H. Zhang:** None. **S. Wu:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.22/HH26

**Topic:** E.02. Cerebellum

**Support:** Academic Research Fund Tier2

Academic Research Fund Tier1

National Medical Research Council

**Title:** Conditional deletion of Cadherin13 perturbs inhibitory synaptic function in the cerebellum and disrupts cognitive behaviors

**Authors: *L. GUO**, M. TANTRA, A. CHEN
Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore

**Abstract:** Members of the Cadherin superfamily have been linked to neurological disorders. Because of the diversity in structures, expression pattern and function, the functional significance of Cadherins in mediating neuronal processes and behavior is not clear. Cadherin13 (Cdh13) has recently been implicated in autism spectrum disorder (ASD) and extreme violence in genome-wide association studies (GWAS). We found that *Cdh13* is expressed specifically in a subtype of inhibitory neurons in the cerebellum. To examine the function of *Cdh13* in the cerebellum and other glycinergic neurons in the CNS, we generated *Cdh13* conditional knockout mice through *GlyT2::Cre* mediated deletion, analyzed the synaptic strength in the Golgi cells and examined a range of motor and cognitive functions in mice with *Cdh13* ablation. We found that inhibitory synaptic strength is reduced in Golgi cells lacking *Cdh13*. Interestingly, deletion of *Cdh13* has no impact on general motor function. Instead, we observed impairments in cognitive function using several behavioral paradigms. Together, our findings indicate that *Cdh13* is critical for inhibitory function and that disruption of *Cdh13* in GABAergic/glycinergic neurons in non-executive centers of the brain, such as the cerebellum, may contribute to cognitive behavioral deficits linked to neurological disorders.

**Disclosures: L. Guo:** None. **M. Tantra:** None. **A. Chen:** None.

**Poster**

**591. Cerebellum: Physiology and Circuit Function**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 591.23/HH27

**Topic:** E.02. Cerebellum

**Support:** R01NS079750

F31NS089716

**Title:** Cerebellar modulation of substantia nigra

**Authors: *S. G. KEE**, R. BHUVANASUNDARAM, K. KHODAKHAH
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

**Abstract:** The basal ganglia and cerebellum are two components of a complex system of interconnected brain regions that coordinate body movement. The long-held view that these structures interact primarily at the level of the cerebral cortex through closed-loop circuits has been challenged by a growing body of evidence suggesting the presence of a number of cortex-independent pathways that mediate direct communication between these two structures. Understanding how the cerebellum communicates with the nuclei of the basal ganglia may

provide insight into motor disorders caused by dysfunction of these brain regions, including Parkinson's disease and dystonia. The substantia nigra pars compacta (SNc) is a nucleus in the basal ganglia that gives rise to the nigrostriatal tract, which provides dopaminergic input to the striatum and degenerates in Parkinson's disease, leading to motor deficits. Multiple studies using lesions of the cerebellar nuclei and viral tracing have suggested that the cerebellar nuclei send monosynaptic projections to dopaminergic neurons of the SNc. The strength of this connection and its relevance to motor activity is unknown. It has also been shown that trains of electrical stimulation to the cerebellar nuclei can alter dopamine levels in both the substantia nigra and the striatum. Whether this is mediated directly, through the monosynaptic cerebellar projection to the SNc, or indirectly, via other brain regions, remains to be established. Here, we test the hypothesis that the cerebellum can drive the activity of neurons in the SNc through a direct, monosynaptic connection. To test this hypothesis, Channelrhodopsin (ChR2) was expressed in cerebellar nuclei neurons. Immunohistochemistry confirmed that cerebellar fibers expressing ChR2 were present in SNc. Cerebellar fibers in substantia nigra were activated by light, and the responses of neurons in substantia nigra were recorded. We found *in vivo* that short light pulses increased the firing of neurons in substantia nigra with a short latency. We performed complementary whole-cell voltage clamp recordings from neurons in SNc in slices from mice injected with ChR2 into the cerebellum and found that stimulation of ChR2-positive fibers evoked inward currents in recorded neurons. Both tyrosine hydroxlase (TH)-positive and TH-negative cells respond to stimulation of cerebellar fibers. Taken together, these data provide support for a monosynaptic, cortex-independent pathway capable of relaying information directly from the cerebellum to the substantia nigra. Future studies aim to characterize this pathway in more detail and scrutinize its role in motor control.

**Disclosures: S.G. Kee:** None. **R. Bhuvanasundaram:** None. **K. Khodakhah:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.01/HH28

**Topic:** E.03. Basal Ganglia

**Support:** Data was collected in the lab of Dieter Jaeger

**Title:** Cortico-subthalamic projection stimulation increases maximum running speed in 6-OHDA lesioned mice

**Authors: L. HOGEWOOD**, *T. H. SANDERS
Pharmacol., Vanderbilt Univ., Nashville, TN

**Abstract:** Open field locomotion has been shown to increase in 6-OHDA lesioned mice during optogenetic stimulation of motor-cortico-subthalamic projections (Sanders et al., 2016). Here I report that the maximum running speed of these mice when tested on a variable speed treadmill is increased during stimulation as well. Surprisingly, the maximum running speed of the unlesioned control was decreased when tested using the same stimulation protocol and treadmill. Electrophysiological measures showed increased power during stimulation for both lesioned and unlesioned animals. However, cortico-subthalamic (M1-STN) theta coherence was decreased in the lesioned mice while M1-STN coherence was increased in the control. The results suggest that while the local neural population response may be similar between lesioned and unlesioned mice, circuit effects lead to different behavioral and synchronization responses.

**Disclosures: L. Hogewood:** None. **T.H. Sanders:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.02/HH29

**Topic:** E.03. Basal Ganglia

**Support:** NIH Grant DA038208

VA Grant BX002525

Portland VA PADRECC

**Title:** Enhancement of K-ATP currents by AMP kinase activation is modified by phosphoinositol metabolism in substantia nigra dopamine neurons

**Authors: *S. W. JOHNSON**[1], A. C. MUNHALL[1], K.-Z. SHEN[2]
[1]Dept Neurol, Portland VA Med. Ctr., Portland, OR; [2]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

**Abstract:** Our lab reported recently that ligand-gated ATP-sensitive K+ (K-ATP) current is potentiated by AMP-activated protein kinase (AMPK) that is activated during whole-cell patch-clamp recordings of substantia nigra compacta (SNC) dopamine neurons (Neurosci 330:219-228, 2016). Although it is not yet known how whole-cell recordings activate AMPK, it is well known that K-ATP channel function is enhanced by the presence of phosphatidylinositol 4,5-biphosphate (PIP-2) in cell membranes. Therefore, we used whole-cell patch-clamp recordings to characterize effects of phospholipase C (PLC) activators and inhibitors on AMPK-dependent changes in K-ATP currents in SNC dopamine neurons in slices of rat midbrain. As we reported previously, recording in whole-cell configuration caused a time-dependent 220% increase in diazoxide (200 μM)-evoked outward current, from an initial current of 38 ± 5 pA to 122 ± 24 pA

as measured 60 min after starting recording. This enhancement of diazoxide current was AMPK dependent because diazoxide current remained at baseline when slices were superfused with the AMPK inhibitor Compound C (20 μM). Because PLC activity cleaves PIP-2, we reasoned that an inhibitor of PLC might increase diazoxide current by reducing PIP-2 degradation. Consistent with this hypothesis, we found that the PLC inhibitor U73122 (5 μM) significantly enhanced the diazoxide current to 177 ± 18 pA at 60 min (P less than 0.001) Moreover, this increase in current was blocked by Compound C. Enhancement of diazoxide current was also completely prevented by superfusing either the PLC activator m-3M3FBS (25 μM) or the metabotropic glutamate agonist DHPG (3 μM). Results of these studies suggest that AMPK activation, which occurs during the course of whole-cell recording, enhances K-ATP channel function by a process that requires PIP-2. Experiments are in progress that will characterize effects of phosphoinositol metabolism on firing properties of dopamine neurons. Results should further characterize how the excitability of dopamine neurons are regulated by the combined influences of K-ATP channels, AMPK, and lipid membrane constituents.

**Disclosures: S.W. Johnson:** None. **A.C. Munhall:** None. **K. Shen:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.03/HH30

**Topic:** E.03. Basal Ganglia

**Support:** MoST105 2321-B-001-018

**Title:** ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3 (ST8SIA3) mediates sialylation of striatal proteins and functions as a signal coordinator in the striatum

**Authors: *C.-Y. LIN**, H.-L. LAI, H.-M. CHEN, J.-J. SIEW, C.-P. CHANG, Y. CHERN
Academia Sinica/Institute of Biomed. Sci., Taipei, Taiwan

**Abstract:** ST8 alpha-*N*-acetyl-neuraminide alpha-2,8-sialyltransferase 3 (ST8SIA3) exists in the Golgi apparatus and belongs to the type II membrane protein. It is responsible for transferring one or multiple sialic acids to the terminal galactose of the *N*-linked or *O*-linked glycan chains of glycoproteins. ST8SIA3 is highly expressed in the striatum, the brain area that controls motor coordination *in vivo*. To investigate the pathophysiological function of ST8SIA3, we generated a *St8sia3*-knockout (KO) mouse model using the CRISPR/Cas9-mediated genome engineering technology by disrupting the translation start site to ablate RNA expression. No apparent abnormality was observed in the embryonic viability, gross development and fertility of *St8sia3*-KO mice. Interestingly, *St8sia3*-KO mice revealed reduced performance on the accelerating rotarod test, designed to evaluate maximal motor performance. Magnetic resonance imaging

(MRI)-based *in vivo* morphometric and volumetric assessment revealed that *St8sia3*-KO mice had a smaller striatum, but not other brain areas tested, than their littermate controls. To identify the protein substrates of ST8SIA3, we evaluated the expression of several striatum-enriched proteins in *St8sia3*-KO mice. Western blot analyses suggested that the $A_{2A}$ adenosine receptor ($A_{2A}R$), the type V adenylyl cyclase (AC5) and the $D_2$ dopamine receptor ($D_2R$) might be potential substrates of ST8SIA3, because these proteins harvested from the striatum of *St8sia3*-KO mice traveled faster in SDS-PAGE gels than those in their littermate controls. Treatment with sialidase, which released all sialic residues from sialylated glycans, eliminated the difference in protein sizes of $A_{2A}R$, AC5, and $D_2R$ between WT and *St8sia3*-KO mice, further suggesting that these three proteins might be modified by the ST8SIA3-mediated sialylation. Consistently, locomotor activity analyses suggested that the motor responses to an $A_{2A}R$ agonist (CGS-26180, intraperitoneally injection (i.p.)), an $A_{2A}R$ antagonist (SCH-58261, i.p.) and a $D_2R$ antagonist (L-741626, i.p.) were altered in *St8sia3*-KO mice. Collectively, ST8SIA3 appears to play a critical role in the striatum by mediating sialylation of several important striatal proteins and subsequently modulate striatal functions.

**Disclosures: C. Lin:** None. **H. Lai:** None. **H. Chen:** None. **J. Siew:** None. **C. Chang:** None. **Y. Chern:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.04/HH31

**Topic:** E.03. Basal Ganglia

**Support:** NIH Grant RO1-NS076772

**Title:** Real-time striatal measurements of oxidative stress and dopamine in the dyskinetic rat during chronic l-dopa treatment for parkinson's disease

**Authors: *C. MASON**, L. R. WILSON, C. A. LEE, L. A. SOMBERS
Chem., North Carolina State Univ., Raleigh, NC

**Abstract:** Parkinson’s disease (PD) is a neurodegenerative disease characterized by the slow degeneration of dopaminergic neurons found in a region of the midbrain called the substantia nigra. Dopamine (DA) plays a key role in regulating motor function. Thus, the destruction of these neurons and the consequential decrease in DA concentrations in the striatum leads to the deterioration of motor control. The drug Levodopa (L-DOPA) has been used to treat PD by helping to increase the concentration of DA in the brain. This drug has been proven to alleviate the motor symptoms of PD; however, after a short period of time, dyskinetic symptoms can develop. It is thought that oxidative stress is a principal contributor to the destruction of

dopaminergic neurons, and possibly to the development of dyskinesias, in PD and its treatment. To date, oxidative stress has been difficult to measure due to the high reactivity of oxygen radicals, however the generation of hydrogen peroxide (H2O2) can serve as an indicator of the presence of oxidative stress. This experiment uses fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes to simultaneously monitor rapid, real-time, fluctuations of DA and H2O2 in the dorsal striatum. These neurochemical dynamics can be time-locked to dyskinetic episodes. Overall, these studies will aid in our understanding of how oxidative stress modulates nigrostriatal DA signaling, as well as the behavioral consequences of this interaction. The results will inform improved therapeutic strategies for the treatment of PD.

**Disclosures: C. Mason:** None. **L.R. Wilson:** None. **C.A. Lee:** None. **L.A. Sombers:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Title:** Untangle glycinergic immunoreactivity in the basal ganglia

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**Abstract:** The basal ganglia circuitry involves the modulation of GABAergic medium-sized spiny neurons within the striatum by dopaminergic (DA) projections from the substantia nigra pars compacta (SNc), via the direct and indirect pathways. Although the involvement of glycinergic circuitry has not been considered to be part of the basal ganglia, the data to be presented suggests the presence of glycinergic immunoreactivity within several basal ganglia structures. Staining of rat coronal and saggital sections with the microtubule associated protein MAP2 showed co-localization with glycine transporter 1 (GlyT1), a transporter believed to be expressed in glia cells. Moreover, GFAP staining clearly demonstrate that the majority of GlyT1 immunoreactivity is absent from astrocytes. Further staining with glycine receptor antibodies

showed immunoreactivity in the globus pallidus (GP) and the striatum, suggesting the presence of pre- and post-synaptic glycinergic fibers. In addition, Injection of retrograde tracers and adeno- associated viral particles into the GP of rodents suggests that the cell bodies of these putative neurons are located within areas of the midbrain. Additional characterization of these neurons and the postsynaptic connections are currently under development. These data together suggest that glycinergic neurons may contribute to the regulation of the indirect pathway of the basal ganglia.

**Disclosures: R.A. Perez:** None. **V. Garcia:** None. **R. Ortega:** None. **E. Castañeda:** None. **M. Miranda-Arango:** None.

**Poster**

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**Topic:** E.03. Basal Ganglia

**Support:** SfN-IBRO Travel Award

**Title:** Angiotensin II type 1/adenosine $A_{2A}$ receptor heteromerization in mice striatum: a novel potential target for tardive dyskinesia

**Authors: *P. A. DE OLIVEIRA**[1,2], J. A. R. DALTON[3], J. GIRALDO[3], M. LÓPEZ-CANO[4], X. MORATÓ[4], V. FERNÁNDEZ-DUEÑAS[4], C. E. MÜLLER[5], A. S. CUNHA[2], F. C. MATHEUS[2], R. N. TAKAHASHI[2], R. D. S. PREDIGER[2], F. CIRUELA[4]
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**Abstract:** Tardive dyskinesia (TD) is a serious motor side effect that may appear after long-term treatment with neuroleptics and mostly mediated by dopamine $D_2$ receptors ($D_2$Rs). Striatal $D_2$R functioning may be finely regulated by either adenosine $A_{2A}$ receptor ($A_{2A}$R) or angiotensin receptor type 1 ($AT_1$R) through putative receptor heteromers. Here, we examined whether $A_{2A}$R and $AT_1$R may oligomerize in the mice striatum to synergistically modulate dopaminergic transmission. Initially, we observed high overlapping in the distribution of $AT_1$R and $A_{2A}$R in transfected cells that transiently expressing the receptors, through the fluorescence detection of CFP/YFP tagged receptors. Then, we demonstrated a physical $AT_1$R-$A_{2A}$R interaction in cultured cells by using bioluminescence resonance energy transfer (BRET), indicating a heteromers formation in living HEK-293T cells. Interestingly, by protein-protein docking and molecular dynamics simulations by computacional modeling, we described that a stable

heterotetrameric interaction may exist between $AT_1R$ and $A_{2A}R$ bound to antagonists (i.e. losartan and istradefylline, respectively). In addition, angiotensin II ($AT_1R$ - agonist) significantly increased intracellular $Ca^{2+}$ mobilization from co-transfected cells ($AT_1R$-$A_{2A}R$) by Fluo4 determinations, when compared with cells expressing only $A_{2A}R$ or $AT_1R$, suggesting a functional interplay between $AT_1R$ and $A_{2A}R$ might exist, upon expression in these cells. Once demonstrated these functional interaction complexes in living cells, we determined the existence and functionality of these heteromers in CD-1 mice. We initially demonstrated the distribution of both $AT_1R$ and $A_{2A}R$ in mice striatum by immunofluorescence and interestingly, both receptors showed a high degree of co-distribution throughout the striatal neuropil. Subsequently, by proximity binding in the *in situ* assay, a positive signal was observed in the striatum of wild-type mice that strongly support the existence of $AT_1R/A_{2A}R$ heterodimer in the striatum. Finally, we took advantage of a TD animal model, namely the reserpine-induced vacuous chewing movement (VCM), to evaluate a novel multimodal pharmacological TD treatment approach based on targeting the $AT_1R/A_{2A}R$ complex (all procedures aforementioned were approved by local Ethical Committee in Animal Research). Thus, reserpinized mice were co-treated with sub-effective losartan and istradefylline doses, which prompted a synergistic reduction in VCM. Overall, our results demonstrated the existence of striatal $AT_1R/A_{2A}R$ oligomers with potential target for pharmacological intervention in TD.

**Disclosures: P.A. De Oliveira:** None. **J.A.R. Dalton:** None. **J. Giraldo:** None. **M. López-Cano:** None. **X. Morató:** None. **V. Fernández-Dueñas:** None. **C.E. Müller:** None. **A.S. Cunha:** None. **F.C. Matheus:** None. **R.N. Takahashi:** None. **R.D.S. Prediger:** None. **F. Ciruela:** None.

**Poster**

**592. Transmitters and Modulators**

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**Topic:** E.03. Basal Ganglia

**Support:** DPBM/University of Helsinki

**Title:** Histamine deficient mice display altered exploratory activity and striatal monoamine neurotransmitter metabolism

**Authors: *S. ABDURAKHMANOVA**, S. SEMENOVA, P. PANULA
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**Abstract:** Histamine is a biogenic amine synthesized by histidine decarboxylase (HDC) from L-histidine. It is stored and released in both periphery and CNS. In concert with other neurotransmitters such as dopamine, acetylcholine, serotonin and noradrenaline, hypothalamic

histamine regulates homeostatic and cognitive functions in the brain. Decreased locomotor activity and increased striatal dopamine turnover have been shown in histamine deficient HDC knock-out (HDC KO) mice. Furthermore, a functional mutation in *HDC* gene has been found in a family diagnosed with Tourette syndrome. We investigated novelty-induced exploratory activity, monoamine turnover and mRNA levels of monoamine neurotransmitter degrading enzymes in HDC KO mice. Näive HDC WT and KO male mice without prior handling and habituation were tested in an open field. While both genotypes showed similar distance travelled and velocity, detailed analysis of the recorded behavior revealed distinct behavioral patterns in HDC KO mice. Locomotor activity of HDC KO mice was interrupted by seated rearing to a greater extent than in control group. Moreover, mutant mice displayed a higher number of stereotypic behaviors such as jumping, head and paw twitching and wall licking. The level of blood plasma corticosterone was similar in both groups. The measurement of monoamine content in striatal homogenates showed decreased levels of dopamine and serotonin in HDC KO mice. The level of dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and DOPAC/dopamine ratio was increased in histamine deficient mice. Also, the ratio of serotonin metabolite 5-hydroxyindoleacetic acid /serotonin was increased in the striatum of HDC KO mice. Quantitative PCR showed a decrease of striatal monoamine oxidase B and catechol-O-methyltransferase mRNA levels in HDC KO mice. Our data suggest that lack of brain histamine leads to impaired novelty-induced exploratory activity and alterations in dopamine and serotonin metabolism accompany this behavioral phenotype. These changes could be due to lack of striatal histamine receptors activation; alternatively, histamine neurotransmission could modulate the activity and transcriptional level of monoamine neurotransmitter degrading enzymes.

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**Poster**

**592. Transmitters and Modulators**

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**Title:** The role of Gas2-like 2 (Gas2l2) in the $A_{2A}R$ adenosine receptor-mediated signaling

**Authors: *M.-S. LIN**[1,2], H. PAI[1,3], C.-P. CHANG[1], H.-L. LAI[1], H.-M. CHEN[1], Y.-C. WU[1], Y. CHERN[1,2,3]
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**Abstract:** The $A_{2A}$ adenosine receptor ($A_{2A}R$) is a G protein coupled receptor, which is widely expressed in the brain with the highest level in the striatum. Cross-regulation between $A_{2A}R$ and the dopamine D2 receptor ($D_2R$) plays a critical role in the control of basal motor function. Several laboratories including ours had demonstrated that the c-terminus of $A_{2A}R$ acts as a scaffold domain to recruit various functional partners (such as TRAX, α-actinin, and calmodulin), and contributes to the dimer formation of $A_{2A}R$ and $D_2R$. We have previously reported that Gas2-like 2 (Gas2l2) is a novel interacting protein of the c-terminus of $A_{2A}R$, and functions in the fine-tuning of Gsα-mediated cAMP signaling during $A_{2A}R$ activation. Results of reverse transcription polymerase chain reaction (RT-qPCR) demonstrated that Gas2l2 is expressed in most brain areas with moderate levels. Through the direct interaction with Gas2l2, the $A_{2A}R$-mediated adenylyl cyclase (AC) activity is enhanced. To study the physiopathological role of Gas2l2 in the $A_{2A}R$-mediated signaling *in vivo*, we developed a conditional knockout mouse model of Gas2l2. The profile of gene expression measured by RT-qPCR revealed that the deletion of Gas2l2 slightly increased the transcript levels of $D_2R$ and α-actinin in both the striatum and hippocampus. Interestingly, the level of $A_{2A}R$ in the striatum was also enhanced. The effect of lacking Gas2l2 on the signaling and functions of $A_{2A}R$ *in vivo* will be discussed.

**Disclosures: M. Lin:** None. **H. Pai:** None. **C. Chang:** None. **H. Lai:** None. **H. Chen:** None. **Y. Wu:** None. **Y. Chern:** None.

**Poster**

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**Topic:** E.03. Basal Ganglia

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**Title:** Stereological estimations and neurochemical characterization of neurons expressing $GABA_A$ receptor gamma 2 subunit in the rat pedunculopontine and laterodorsal tegmental nuclei

**Authors: B. PATERNAIN**, E. LUQUIN, *E. MENGUAL
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**Abstract:** Both the pedunculopontine (PPTg) and laterodorsal tegmental (LDTg) nuclei receive abundant GABAergic projections from basal ganglia components. Thus far, the presence of $GABA_A$ receptors ($GABA_ARs$) in the PPTg and LDTg has been reported both electrophysiologically and pharmacologically; however, their anatomical localization has not been investigated yet. Furthermore, both nuclei comprise three different neurochemical phenotypes - cholinergic, GABAergic and glutamatergic - which play different roles in locomotion and sleep/wake behavior. To better understand GABAergic transmission in these

nuclei we aimed 1) to estimate the total number of neurons in PPTg and LDTg expressing $GABA_AR$ gamma 2 subunit ($GABA_AR$ γ2), and 2) to identify which of the three neurochemical subpopulations expresses this $GABA_AR$ subunit. Unbiased stereological methods were used in sections dually labeled for $GABA_AR$ γ2-immunoreactivity ($GABA_AR$ γ2-ir) and NADPH-diaphorase staining, a marker of cholinergic neurons. Cell counts were carried out within the nuclear boundaries of the PPTg and LDTg, demarcated around the most peripherally located cholinergic neurons. The mean number of cells expressing only $GABA_AR$ γ2 -ir was 9,848 ± 1,856 in the PPTg and 8,285 ± 962 in the LDTg, whereas those of cholinergic cells were 3,850 ± 580 and 3,235 ± 510, respectively. Dually labeled sections immunoreacted against choline acetyltransferase (ChAT) and $GABA_AR$ γ2 revealed a ≈ 100% expression of the γ2 subunit in cholinergic cells of both nuclei. Subsequently $GABA_AR$ γ2-ir was combined with *in situ* hybridization against either GAD67 or Vglut2 mRNA, and also with ChAT immunoreactivity for delineation purposes. Quantification of dually labeled cells revealed that 76% of GABAergic cells in the PPTg and 45% in the LDTg also expressed $GABA_AR$ γ2, indicating that there are at least two different subsets of GABAergic neurons in the PPTg and LDTg, represented in different proportions in the two nuclei. Finally, 90% of glutamatergic cells in the PPTg and 65% in the LDTg also expressed $GABA_AR$ γ2. These differences in the presence and/or subunit composition of $GABA_A$Rs in the glutamatergic and GABAergic cell subpopulations of PPTg and LDTg may account for some of the reported functional differences between the two nuclei.

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**Poster**

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**Title:** Mapping projections of molecularly-defined dopamine neurons using intersectional genetic strategies

**Authors: *J.-F. POULIN**[1], G. CARONIA-BROWN[2], Q. CUI[3], S. CHAN[5], D. A. DOMBECK[4], R. AWATRAMANI[3]
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**Abstract:** Recently, the molecular diversity of midbrain dopamine (DA) neurons has been under intense scrutiny and several distinct subtypes of DA neurons have been identified. How molecular profiles are related to other properties like axonal projections or neuronal functions, remains to be determined. Progress in this regard has been hindered by the lack of genetic tools to study genetically-defined DA neuron subtypes. Here, we present a genetic toolbox and three distinct intersectional strategies to target these subtypes based on combinatorial gene expressions. The genetic toolbox we generated includes a mouse expressing Flp recombinase under the control of tyrosine hydroxylase promoter, as well as two novel Cre drivers, to gain genetic access to key DA neuron subtypes in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Using our intersectional genetic labeling strategies, we mapped the projections of molecularly defined DA neuron subtypes of the SNc and VTA. Our approach revealed the underestimated diversity of the DAergic innervation of the striatum, nucleus accumbens and amygdala. Together, the genetic toolbox and DA neuron subtype projections presented here constitute a resource that will accelerate the investigation and understanding of the functions of this clinically significant neurotransmitter system.

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**Poster**

**592. Transmitters and Modulators**

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**Title:** Pramipexole promotes dopamine transporter internalization and lysosomal degradation through dopamine $D_3$ receptor

**Authors: *T. GONZÁLEZ-HERNÁNDEZ**, D. LUIS-RAVELO, F. FUMAGALLO-READING, D. AFONSO-ORAMAS, I. CRUZ-MUROS, J. SALAS-HERNÁNDEZ, J. RODRÍGUEZ-NÚÑEZ
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**Abstract:** The dopamine transporter (DAT) is a membrane glycoprotein expressed in dopaminergic neurons and is responsible for the uptake of dopamine (DA) into presynaptic neurons after its release into the extracellular space. DAT dysfunction has been involved in different neurological and psychiatric disorders, including Parkinson's disease, depression and attention deficit hyperactivity disorder. DAT activity is regulated by different factors, including DA $D_2$ and $D_3$ autoreceptors. Current data indicate that $D_2$ receptor ($D_2R$) promotes DAT recruitment to the plasma membrane and an increase of DA uptake, but $D_3$ receptor ($D_3R$) seems to have a dual effect on DAT. Its acute stimulation induces an increase in DAT activity, but after prolonged stimulation, DA uptake becomes lower than in basal conditions. The mechanisms underlying this phenomenon are still poorly known. To shed light on this issue, DAT-$D_3R$ transfected HEK cells, midbrain dopaminergic neurons and rats were treated with the $D_3R$ preferential agonist pramipexole (PPX). The results show that prolonged treatment with PPX (10µM for 90 minutes in cell cultures; 0.15mg/kg/d/6d in rats) causes: 1. A decrease of [$^3$H]DA uptake and glycosylated DAT levels in the plasma membrane (an effect that was blocked by the protein kinase C inhibitor bisindolylmaleimide-IV), 2. A decrease in total DAT levels together with an increase in DAT phosphorylation, ubiquitination and binding to p62, and 3. DAT colocalization with LC3 and lysotracker (two markers of the autophagy-lysosome pathway). These findings indicate that PPX induces internalization and degradation of DAT through the autophagy pathway. The modulatory effect of PPX on DAT can contribute to strengthening dopaminergic transmission in the mesostriatal system, suggesting new therapeutic possibilities of $D_3R$ agonists in neurological and psychiatric conditions.

**Disclosures: T. González-Hernández:** None. **D. Luis-Ravelo:** None. **F. Fumagallo-Reading:** None. **D. Afonso-Oramas:** None. **I. Cruz-Muros:** None. **J. Salas-Hernández:** None. **J. Rodríguez-Núñez:** None.

**Poster**

**592. Transmitters and Modulators**

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**Title:** Ontogenetic effects of DA receptor inactivation on the efficacy of D2 receptors and the cAMP and Akt signaling pathways

**Authors: V. REAL**, A. TERAN, C. A. CRAWFORD, *S. A. MCDOUGALL
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**Abstract:** DA receptor inactivation causes dramatic age-dependent behavioral effects across ontogeny. In adolescent and adult rats, D2 receptor inactivation fully attenuates the locomotion caused by DA agonist treatment. In preweanling rats, on the other hand, agonist-induced locomotion is paradoxically enhanced after IP or IC injection of the irreversible DA antagonist EEDQ. Although EEDQ inactivates a similar percentage of D2 receptors in young and adult rats, the affinity of the remaining receptors varies according to age. Specifically, EEDQ-treated preweanling rats, relative to older rats, have a disproportionate number of high affinity D2 receptors. This finding led us to propose that an excess of $D2^{High}$ receptors is responsible for the potentiated locomotor response in preweanling rats. If this explanation is accurate: (a) the efficacy of D2 receptors should be enhanced in EEDQ-treated preweanling rats, and (b) age-dependent changes in $D2^{High}$ receptors should differentially affect down-stream signaling. To test these hypotheses, we examined the efficacy of D2 receptors by measuring stimulated [$^{35}$S]GTPγS binding in EEDQ-treated rats. We also measured the effects of EEDQ on the cAMP and Akt signaling pathways. In both experiments, rats were given an IP injection of vehicle or EEDQ (2.5 or 7.5 mg/kg) on PD 17, PD 39 or PD 84. After 24 h, CPu sections were dissected and stored at -70 °C. Agonist-effect curves of [$^{35}$S]GTPγS binding were performed in assay buffer containing 10 µM GDP and NPA. Nonspecific binding was determined using 10 µM cold GTPγS. Western blots were used to quantify the functional status of the cAMP and Akt systems. Namely, the PKA catalytic subunits α and β (PKA-Cα and PKA-Cβ) were measured, as were phosphorylated PKA-Cα (pPKA-Cα) and the PKA regulatory subunit (PKA-RII). Phosphorylated and nonphosphorylated forms of Akt and GSK-3β were also measured using immunoblotting. We found that EEDQ increased the efficacy ($E_{max}$) of NPA-stimulated [$^{35}$S]GTPγS specific binding to a greater extent in preweanling rats than adults. Age-dependent differences in second messenger system functioning were apparent, as striatal Akt levels were greater in preweanling rats than adults, while adolescent rats had reduced pGSK-3β relative to the other age groups. Receptor inactivation had minimal effects on these signaling pathways, with the exception that EEDQ reduced pAkt in all age groups. In sum, the GTPγS data are consistent with the hypothesis that EEDQ's age-dependent behavioral effects are caused by changes in the affinity and efficacy of D2 binding sites, but there is no evidence that alterations in the cAMP and Akt signaling pathways mediate these behavioral effects.

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**Poster**

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**Topic:** E.03. Basal Ganglia

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American Parkinson's Disease Foundation

**Title:** Burst firing of dopamine neurons co-releases dopamine and sonic hedgehog which have opposing effects on cholinergic neurons

**Authors: D. ZUELKE**[1,3], A. W. STUCKY[4], *A. H. KOTTMANN[5,3,2,6]
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**Abstract:** DA neurons play critical roles in procedural learning, action selection and motivation, and perturbations of dopaminergic influences on basal ganglia circuitry contribute to neurological and psychiatric disorders. It is assumed that the main modulator of these processes is DA. However, DA neurons communicate with their targets by a host of secreted cell signaling factors in addition to DA. The physiological functions of these auxiliary modes of dopaminergic communication are only beginning to emerge.
We found previously that all DA neurons release the secreted cell signaling factor sonic hedgehog (Shh) in the striatum. Only cholinergic- and fast spiking (FS), gabaergic- interneurons among all dopaminergic targets express the receptor for Shh. We further revealed that Shh signaling, in a dose dependent manner, inhibits the transcription of the dopaminotrophic factor GDNF and the muscarinic autoreceptor M2 leading to a graded modulation of extracellular acetylcholine (ACh) (Gonzalez-Reyes et al., Neuron 75, 306).
Here we exploit unilateral optogenetic stimulation of DA neurons in conjunction with cholinergic and Shh signaling specific pharmacology, to show that Shh signaling to ACh neurons specifically from DA neurons plays a critical role in the formation of locomotion. The effect of Shh signaling is seen within 2 minutes and reveals an antagonistic relationship of DA and Shh when co-released from DA neuron terminals in that DA inhibits while Shh stimulates ACh signaling. Continuous, forced burst stimulation of DA neurons reveals a rapid onset and linear exhaustion of Shh signaling over the course of 40 minutes while DA signaling remains constant. These results indicate that the physical identity of the reward prediction error signal, which is critical for reinforcement learning and is encoded by trains of dopaminergic bursts, changes over the course of several bursts. Consistent, we find that mice with selective ablation of Shh from DA neurons reveal a learning deficit but increased motor habit formation. Our results are likely

of clinical relevance since the co-administration of L-Dopa together with Shh agonists inhibits the formation of L-Dopa induced dyskinesia (LID) in animal models of Parkinson's Disease (SFN poster Lauren Malave & Andreas H. Kottmann).

**Disclosures: D. Zuelke:** None. **A.W. Stucky:** None. **A.H. Kottmann:** None.

**Poster**

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**Topic:** E.03. Basal Ganglia

**Title:** D3 receptor blockade increases dopamine, glutamate and GABA levels in the substantia nigra reticulata and increases locomotor activity

**Authors: *M. RODRÍGUEZ**[1], S. LOYA[2], E. ESCARTÍN[4], V. AYALA[2], F. PAZ[3], D. ERLIJ[5], B. FLORÁN[3]
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**Abstract:** Substantia nigra reticulata (SNr) is the output nucleus of the basal ganglia whose modulation of their neural firing rate by GABA and Glutamate allows to explain the modulation of movement through basal ganglia circuit. In the SNr, GABA, Glutamate and Dopamine release is controlled by presynaptic dopamine receptors. D1 receptors modulate GABA and Glutamate release from striatal and subthalamic afferents whereas D4 receptors modulate GABA release from pallidal terminals. D3 dopamine receptor has been located at striatal and subthalamic afferents and in dopaminergic dendrites as well; however, the participation of D3 receptor in the modulation of motor behavior at this level is uncertain.
Here we performed in vivo microdialysis experiments in the SNr of naive rats to measure dopamine, glutamate and GABA levels and correlate them with motor behavior. The blockade of nigral D3R with the highly selective antagonist GR-103691 (100 nM i.c.) produced an increase of Dopamine, Glutamate and GABA levels correlating with an increase in the ambulatory distance and non-ambulatory behavior. Systemic blockade of D3 receptors with U99194 (25 mgrs/kg i.p.) mimicked the effects of local blockade in SNr. Co-perfusion of SCH 23390 (1µM i.c.) with GR-103691 prevented the effects of D3 receptor blockade in GABA but not in Dopamine and Glutamate increases. Co-perfusion of Kinurenic acid (3 µM i.c.) prevented the effects of D3 receptor blockade in Dopamine and GABA but not in Glutamate.
These data suggest that D3 receptors tonically modulate (decrease) Glutamate release from subthalamo-nigral terminals. Glutamate in turn, as previously suggested (Rosales et al., 1977), increases Dopamine through NMDA receptors, and Dopamine stimulates GABA through D1

receptors. These increases in GABA levels decreases firing rate of output neurons and increases motor behavior, in consequence local or systemic blockade of D3 receptors increases locomotor activity by increasing Glutamate release at SNr.

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**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.15/II6

**Topic:** E.03. Basal Ganglia

**Support:** NIDA Intramural Funds

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**Title:** Dissecting the biochemical properties of dopamine D1-D3 receptor heterotetramers and their role in the modulation of locomotor activity

**Authors: *X. GUITART**[1], E. MORENO[2], W. REA[1], C. QUIROZ[1], M. SANCHEZ-SOTO[1], V. KUMAR[1], A. CORTES[2], E. I. CANELA[2], C. BISHOP[3], A. H. NEWMAN[1], V. CASADO[2], S. FERRE[1]
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**Abstract:** A quaternary structure of the dopamine $D_1$ receptor (D1R)-$D_3$ receptor (D3R) heteromer has been proposed as constituted by D1R and D3R homodimers coupled to their respective cognate Gs and Gi proteins. The use of biophysical techniques and specific disruptive peptides in mammalian transfected cells demonstrated a D1R-D3R heteromer-dependent positive synergistic interaction of D1R and D3R agonists on MAPK signaling. In addition, D1R and D3R agonists interacted antagonistically at the level of adenylyl cyclase (AC) signaling, with D3R activation counteracting D1R agonist-induced cAMP accumulation. For adenosine $A_{2A}$-dopamine $D_2$ receptor heteromers, this canonical Gs-Gi antagonistic interaction at the level of AC has been demonstrated to depend on receptor heteromerization. We now show that synthetic peptides that disrupt D1R-D3R heteromers also disrupt the canonical Gs-Gi-AC interaction in transfected cells, demonstrating that it is also a biochemical property of the D1R-D3R receptor heteromer. This would support the hypothesis that the synergistic effect of D1R and D3R

agonists on MAPK signaling should involve G protein-independent D1R signaling. However, also in transfected cells, D1R-agonist induced activation of MAPK was found to be dependent on Gs-AC signaling, since it was counteracted by a PKA inhibitor. The results support a model where, in the D1R-D3R heteromer, D1R activation induces MAPK activation by a Gs-AC-mediated mechanism, but not when D3R is co-activated. In this case, a D1R agonist cannot signal though MAPK, because of the canonical Gs-Gi-AC interaction, but can allosterically potentiate D3R-mediated MAPK activation. This would imply that, if dependent on D1R-D3R heteromerization, the main mechanism behind the strong locomotor activity produced by co-administration of D1R and D3R agonists in reserpinized mice, should be mostly dependent on D3R-mediated MAPK activation. The biased antagonism of a novel and highly selective D3R antagonist, VK4-116, provided strong support to our hypotheses. In transfected cells, when compared with the non-selective $D_2$-like receptor antagonist eticlopride, VK4-116 was equipotent at counteracting D3R agonist-mediated forskolin-induced cAMP accumulation, but 300 times less potent at counteracting D3R agonist-mediated MAPK activation and locomotor activation in reserpinized mice induced by co-administration of D1R and D3R agonists. Ongoing *ex vivo* and *in vivo* experiments with infusion of disruptive peptides should unequivocally demonstrate that the effects of co-administration of D1R and D3R agonists on MAPK activation and on locomotor activity depend on D1R-D3R heteromerization.

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**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

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**Topic:** E.03. Basal Ganglia

**Support:** DFG, Grant EXC 1086

**Title:** Beyond "high" and "low" dopamine: complex spatio-temporal concentration maps and receptor activation in the striatum

**Authors: *L. HUNGER**[1], A. KUMAR[2], R. SCHMIDT[3]
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**Abstract:** Dopamine (DA) plays an important role for reward-related learning, motor control and action-selection. While single DA neuron firing has been linked to reward prediction error

responses, direct measurements of DA concentration in the striatum have indicated a more complex picture of the DA signal. For example, the spatio-temporal DA concentration in the striatum does not seem to be a straightforward result of the DA cell firing. Furthermore, the activation of D1 and D2 DA receptors of striatal neurons does not follow the time course of the DA concentration directly, but is altered by the slow binding kinetics of DA receptors.
To better understand how DA cell firing determines changes in DA concentration and receptor activation, we created a restricted diffusion model that takes into account the reduced rate of diffusion in the brain due to obstacles like neurons and glia cells (Nicholson 2000; Dreyer et al. 2010). In our model we incorporate non-homogenous DA uptake, DA axonal tree morphologies, detailed DA receptor kinetics, and spike trains based on rat DA cell recordings (Hyland et al. 2002). Thereby, we obtain three-dimensional DA maps describing how DA concentration varies over time in a striatal cube. We find that the DA maps have complex spatio-temporal dynamics that cannot simply be classified as "high" or "low" DA concentration. For example, for tonic DA cell firing the time course of the DA concentration at a given location in the striatum strongly depends on the distance to the nearest DA synapses.
Furthermore, we calculate the activation of D1 and D2 receptors in the striatum. During tonic DA cell firing the average DA concentration is constant long enough so that the DA receptor activation equilibrates. In this case the receptor affinity describes the fraction of activated receptors accurately. However, the timescale of the phasic DA cell firing is too short for the receptors to reach equilibrium. In this non-equilibrium case the affinity of the DA receptors does not accurately describe their activation. In particular, even though D2 receptors have a high affinity for DA, not all D2 receptors are activated during phasic DA signals due to the slow kinetics of DA receptors. Therefore, during phasic DA cell firing the DA receptor activation does not simply follow the time course of the DA concentration, but is also determined by the interplay with receptor kinetics. Moreover, we find that the time course of the DA receptor activation is very similar for D1 and D2 receptors. We conclude that the kinetics of the DA receptors might be more important for disentangling the effects of phasic and tonic DA cell firing than the differences in D1 and D2 receptor affinity alone.

**Disclosures: L. Hunger:** None. **A. Kumar:** None. **R. Schmidt:** None.

**Poster**

**592. Transmitters and Modulators**

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**Program#/Poster#:** 592.17/II8

**Topic:** E.03. Basal Ganglia

**Support:** MH101207

**Title:** Phasic dopamine response in the dorsal striatum correlates with specific movements

**Authors: *H. R. KIM**, N. UCHIDA
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**Abstract:** Dopamine (DA) in the striatum is critical for the generation of movements as indicated by motor impairments in Parkinson's disease. However, the exact mechanism by which DA regulates movements remains elusive. Previous studies have long failed to find a tight relationship between specific movements with phasic DA activities, but recent studies in rodents have begun to show the presence of phasic DA responses at the time of motor initiations (Jin and Costa, 2010; Barter et al., 2015; Howe and Dombeck, 2016). It was argued, however, that movements studied in these studies (e.g. locomotion) involve the activity of many muscles as well as various sensory experiences (Schultz et al., 2017). Whether striatal dopamine correlates with specific movements remain to be clarified.
To examine whether dopamine signals in specific striatal regions correlate with specific movements, we monitored calcium signal in dopamine axons in the striatum while head-fixed mice voluntarily ran on a treadmil in the absence of appetitive reward. Viruses expressing GCaMP6m in a Cre-dependent manner were injected in VTA and SNc of mice expressing Cre recombinase under the control of the DAT gene. DA axonal activity in the dorsomedial and dorsolateral striatum (DMS and DLS, respectively) was measured using fiber fluorometry (photometry). In addition to locomotor speed, we recorded whisking and whole-body movement using high-speed videography.
Whisking preceded locomotion with a variable interval ranging from zero to two seconds, which allowed us to examine the behavioral correlate of phasic DA activity in DLS. We found that phasic DA activity in DLS was more tightly correlated with the onset of whisking rather than locomotion (median d' = 0.20 for locomotion, d' = 0.54 for whisking, p = 0.003, n = 6 mice). Furthermore, whisking also occurred without initiating locomotion. The magnitude of DA activity at the onset of spontaneous whisking was comparable to that of whisking preceding locomotion (d' = 0.60 for spontaneous whisking and d' = 0.54 for whisking with locomotion). In contrast, we did not observe clear spontaneous whisking-related activity in DMS (median d' = -0.02, n = 5 mice), as opposed to locomotion onset-related activity (median d' = 0.29). These results indicate that DA signals in different parts of the dorsal striatum are correlated with specific types of movements. These results also suggest that movement-related DA signals are wide-spread in the dorsal striatum.

**Disclosures: H.R. Kim:** None. **N. Uchida:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

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**Topic:** E.03. Basal Ganglia

**Support:** Beckman Young Investigator Award

William and Bernice E. Bumpus Young Innovator Award

Rita Allen Foundation Scholar Award

NARSAD Young Investigator and P&S Fund Grant

National Institutes of Health (T32 AG20506)

Arnold O. Beckman Postdoctoral Fellowship

**Title:** Regulation of midbrain dopamine systems by oxytocinergic projections

**Authors: *L. XIAO**[1], M. F. PRIEST[1], J. NASENBENY[1], T. LU[2], Y. KOZOROVITSKIY[1]
[1]Dept. of Neurobio., [2]Roxelyn and Richard Pepper Dept. of Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

**Abstract:** The hormone and neuromodulator oxytocin has been linked to the function of dopaminergic reward systems through its effects on social behavior including pair bond formation, parenting, and mating. The possibility of oxytocinergic modulation of midbrain dopaminergic systems has been suggested by behavioral and anatomical studies, but the neural circuits and physiological consequences of this potential modulation remain elusive. Here, we anatomically and functionally characterize hypothalamic oxytocinergic projections that regulate the activity of midbrain dopaminergic neurons. This oxytocin source derives from parvocellular neurons in the hypothalamic paraventricular nucleus (PVN). Electrophysiological recordings of dopaminergic neurons reveal a functional oxytocinergic pathway that modulates neuronal activity of VTA and SNc neurons in a location-specific manner. Since hypothalamic oxytocinergic projections also target the striatum, oxytocin is poised to bias the balance of dopamine tone through multiple sites in vertebrate reward circuits.

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**Poster**

**592. Transmitters and Modulators**

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**Topic:** E.03. Basal Ganglia

**Support:** Natural Sciences and Engineering Research Council of Canada Grant 171354-2011

**Title:** Effect of membrane cholesterol removal and replenishment on rat and monkey brain monoamine transporters

**Authors: *T. P. DIPAOLO**[1,2], M. MORISSETTE[1], N. MORIN[1], C. ROUILLARD[1]
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**Abstract:** Neurotransmitters dopamine (DA) and serotonin (5-HT) homeostasis in presynaptic neurons is regulated by the DA transporter (DAT) and 5-HT transporter (SERT) respectively and by the vesicular monoamine transporter (VMAT2). Dysregulated release of DA as a false neurotransmitter from 5-HT neurons following L-DOPA therapy may lead to L-DOPA-induced dyskinesias as a consequence of DAT loss. Changes in membrane cholesterol levels were shown to modulate ligand binding activity and function of DAT and SERT. This study investigated the effect of cholesterol removal and replenishment *in vitro* on DAT in rat and monkey brain homogenates as compared to VMAT2 and SERT. Incubation of rat striatum with methyl-β-cyclodextrin (MCD) to deplete membrane cholesterol or with cholesterol-MCD complex to increase membrane cholesterol content showed no change of DAT protein levels measured by Western blot. By contrast, striatal DAT specific binding labelled with [$^{125}$I]-RTI-121 decreased in a dose-dependent manner after incubation with MCD (-82% in rat striatum; -56%, -54%, and -62% in monkey caudate nucleus, putamen and nucleus accumbens respectively with 10 mM MCD) while the opposite was measured with cholesterol loading (+34% in rat striatum; +12%, +17% and +31% in monkey caudate nucleus, putamen and nucleus accumbens respectively with 0.1 mM cholesterol). Moreover, [$^{125}$I]-RTI-121 specific binding of striatal membranes depleted of cholesterol with MCD was restored to their initial DAT content with addition of cholesterol showing a rapid and reversible effect of cholesterol. By contrast, no change of rat striatal SERT specific binding labelled with [$^{3}$H]Citalopram was observed following cholesterol manipulations. In monkey brain, incubation with MCD left unchanged SERT specific binding in the caudate nucleus and putamen whereas it increased in the nucleus accumbens (+25%). Incubation with cholesterol decreased SERT specific binding in the caudate nucleus and putamen (-49% and -34% respectively with 10 mM cholesterol) while no effect was observed in the nucleus accumbens. Incubation of rat striatal membrane with MCD decreased VMAT2 specific binding labelled with [$^{3}$H]Dihydrotretrabenazine (-26% with 10 mM MCD) whereas no change was observed in monkey brain. Incubation with cholesterol decreased VMAT2 specific binding in the caudate nucleus and putamen only at higher cholesterol doses (5 and 10 mM). These results show an important modulatory effect of cholesterol on monoamine transporters mainly DAT suggesting a close relationship between the membrane lipid environment and DAT function.

**Disclosures:** **T.P. DiPaolo:** None. **M. Morissette:** None. **N. Morin:** None. **C. Rouillard:** None.

**Poster**

**592. Transmitters and Modulators**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 592.20/II11

**Topic:** E.03. Basal Ganglia

**Title:** Dynamic monitoring of phase-amplitude coupling for phase-dependent stimulation

**Authors: Y. SALIMPOUR**[1], *K. A. MILLS[3], W. S. ANDERSON[2]
[2]Dept. of Neurosurg., [1]Johns Hopkins Sch. of Med., Baltimore, MD; [3]Neurol., Johns Hopkins Univ., Baltimore, MD

**Abstract:** Background: Synchronous, rhythmic changes in the membrane polarization of neurons form oscillations in local field potentials. High-frequency brain activity reflects local cortical information processing and a low-frequency brain rhythm projects information flow across larger cortical networks. This provides for a complex form of information transmission based on interactions between oscillations at different frequency bands, which can be displayed by cross-frequency coupling (CFC) metrics such as phase-amplitude coupling (PAC). PAC reflects the coupling of the phase of oscillations in a specific frequency band to the amplitude of oscillations in another frequency band. Methods: In awake patients undergoing functional neurosurgery for Parkinson's disease or essential tremor, we used a parametric spectral estimation method for modelling band limited oscillations. We used a state space-based Bayesian algorithm for estimation and optimization of the model parameters with time-varying coefficients that adapt according to a band limited oscillation of the brain. The optimal parametric model allows temporal prediction of the brain signal and increases the accuracy of Hilbert-transform-based instantaneous frequency and phase estimation. Results: Using electocorticography data recorded intraoperatively on patients with Parkinson's disease or essential tremor during deep brain stimulation surgery, we were able to detect PAC (Figure 1) and used auto-regressive modeling to predict the future phase of slow oscillations on which high frequency gamma was entrained. Conclusions: We established a system for PAC monitoring and then predicted future phase of the slower rhythm oscillations. This is a first step toward phase-dependent cortical stimulation and may allow cortical neuromodulation to enhance intraoperative physiology recordings to guide functional neurosurgery and potentially, as a therapeutic intervention. Figure 1. PAC between slow cortical oscillations and gamma activity in a PD (A) and ET (B) patient.

**Disclosures:** **Y. Salimpour:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **K.A. Mills:** A. Employment/Salary (full or part-time):; Johns Hopkins University. B.

Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH KL2 Award through NCATS. **W.S. Anderson:** A. Employment/Salary (full or part-time):; Johns Hopkins University.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.01/II12

**Topic:** E.04. Voluntary Movements

**Title:** Variability and motor learning: Neural processing noise supports visuomotor adaptation

**Authors: *J. N. VAN DER GEEST**[1], R. VAN DER VLIET[1,2], L. DE VREEDE[1], Z. JONKER[1,2], G. RIBBERS[2], R. SELLES[2], M. A. FRENS[1,3], O. DONCHIN[4,1]
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**Abstract:** The nervous system seems to exploit variability (noise) to support behavioural learning. However, this claim has not been consistently observed in experiments on human motor learning. This may be because the noise that underlies the motor variability in human movements is comprised of two components that contribute to motor adaptation in different ways: neural processing noise and output noise. If people learn from errors, optimal control theory using a Kalman filter suggests that learning rate will correlate positively with neural processing noise (state noise) and negatively with output noise. We tested this hypothesis in a visuomotor reaching adaptation experiment in 69 participants. They made rapid pointing movements in 450 baseline trials with and without visual feedback, and in 450 adaptation trials in which the feedback was perturbed. We extracted state noise, output noise and adaptation rate using a state-space model of trial-to-trial behaviour. We found that adaptation rate during perturbations correlates positively with baseline state noise but negatively with baseline output noise. This suggests that motor noise can be decomposed into a supporting factor for motor adaptation and a factor that hampers learning.

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**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

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**Program#/Poster#:** 593.02/II13

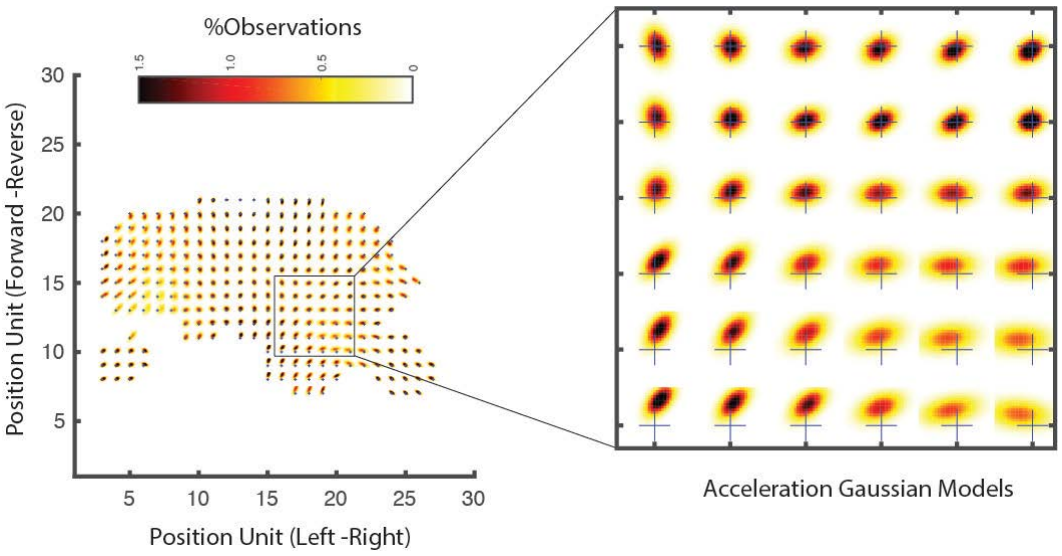
**Topic:** E.04. Voluntary Movements

**Support:** NIH GRANT R01NS053606 – 05A1

**Title:** Simulation of inertial augmentation to counteract abnormal synergies in arm movement

**Authors: *F. C. HUANG**
Arms + Hands Lab., Shirley Ryan Abilitylab, Chicago, IL

**Abstract:** This study examined how a simple model of arm impedance can reveal configuration dependent changes in abnormal synergies in stroke survivors. We also present methods for designing a custom training intervention, by relating the desired change in acceleration covariance in planar motion with a corresponding change in inertia matrix. Using data from a previous study in which stroke survivors (n=10) performed self-directed motor exploration, we first characterized movement in terms of overall covariance in acceleration, and secondly as covariance in acceleration as a function of position in the workspace. Using a forward dynamics simulation of the hand endpoint impedance, we found that the variable change in endpoint inertia resulted in greater reduction in acceleration covariance compared to the fixed change in inertia method. These findings provide support for the use of custom augmentation of limb inertia for upper extremity rehabilitation.




**Disclosures: F.C. Huang:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

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**Program#/Poster#:** 593.03/II14

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant 1F31NS100520-01

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**Title:** Error-augmented feedback accelerates adaptation to nonlinear visuomotor distortions but leads to unstable learning

**Authors: *P. N. PARMAR**[1,3], J. L. PATTON[2,3]
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**Abstract:** Although error-based mechanisms play an important role when acquiring novel motor skills and adapting to perturbations, the dynamic effects of error feedback are not well understood. Recently, Patton et al. (2013) demonstrated how the size of error feedback results in faster learning rates by testing only 3 selected gain values. In this study, we comprehensively tested error feedback gain values from 0 to 3. In addition, we tested interaction effect of these gain values with error bias that was proportional to initial exposure error. We recruited 5 control and 10 test subjects, whose task was to adapt to 8 nonlinear visuomotor distortions by making target-to-target reaching movements. All control subjects perceived normal error feedback (gain 1 and bias 0), while all test subjects perceived augmented error feedback (gain and bias combinations randomly selected for all 8 tasks per every 2 subjects). Results reveal fastest inter-trial change in the feedforward component of movement trajectory error (error calculated from the first 150ms of movement in reference to the straight-line reach) with gain 2 and bias 0. However, this condition led to continue learning beyond zero error with steady-state error lower than the control (unstable learning). As expected, no error-feedback (gain 0, bias 0) led to the slowest inter-trial change in the feedforward component with steady-state error higher than the control (incomplete learning). We also tested the linear, quadratic, and cubic polynomial models to predict the inter-trial change in error. Here, the linear model performed the best, and adding higher order polynomial terms did not improve the model performance.

**Disclosures: P.N. Parmar:** None. **J.L. Patton:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.04/II15

**Topic:** E.04. Voluntary Movements

**Title:** Influence of haptic feedback on exploration of movement variability during motor learning

**Authors: *R. LOKESH**[1,2], R. RANGANATHAN[3,3]
[2]Mechanical Engin., [3]Kinesiology, [1]Michigan State Univ., East Lansing, MI

**Abstract:** Several studies have established that haptic assistance facilitates motor learning in tasks that require minimizing movement errors. However, in tasks with motor redundancy, haptic feedback can be used to modulate two aspects of movement variability - task space variability, which affects task performance; and null space variability, which has no direct effect on task performance. The purpose of this study was to examine the influence of haptic feedback on task and null space variability during motor learning. Participants performed a bimanual task which required them to control a cursor on the screen and navigate a 12mm wide “W-shaped track” as fast as possible. Critically, the cursor on the screen was placed at the mean position of the two hands, which meant that the same trajectory could be achieved with different motions of the left and right hands. We examined two groups - (a) a no-haptic feedback group that learned the task only using visual feedback, and (b) a haptic feedback group that received error-correcting haptic force feedback along a small 6mm wide inner “channel” within the track. The haptic feedback was provided using a spring-like force acting perpendicular to the track when they reached the edge of the channel. Participants practiced for 288 trials distributed equally over two days, and we measured movement time and movement variability during learning. Results showed that movement time decreased with practice for all participants, with haptic group having lower movement times throughout practice. Analysis of movement variability showed greater task space variability for the haptic feedback group, but there were no differences in the null space variability. These results suggest that haptic feedback has differential effects on movement variability in the task and null space, which may be used to facilitate motor learning.

**Disclosures: R. Lokesh:** None. **R. Ranganathan:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.05/II16

**Topic:** E.04. Voluntary Movements

**Title:** Modifying coordination pattersn in throwing by reinforcement

**Authors: *T.-H. LIN**[1], R. RANGANATHAN[2]
[1]Dept. of Kinesiology, [2]Dept of Kinesiology, Michigan State Univ., East Lansing, MI

**Abstract:** The redundancy in the musculoskeletal system allows humans to perform same movement with multiple coordination patterns. This feature of the motor system means that motor learning is not only about improving task performance, but can also involve in shifting between different coordination patterns that all yield the same performance. However, how participants learn to use an alternative coordination pattern to perform a task is still poorly understood. Here, we examined the use of reinforcement feedback to shift participants from one coordination pattern to another. We used a virtual throwing task that required coordination between the trunk and the hand. Specifically, participants threw a virtual ball to a target on the screen, but the motion of the ball was determined by a combination of the participants' trunk and hand velocity (indicating there was redundancy). Typically, participants perform the task with high hand velocities, and low trunk velocities. We examined whether we could shift this pattern to a strategy with lower hand velocities and higher trunk velocity. We provided reinforcement feedback by deviating the trajectory of the ball out of the screen if their trunk velocity did not go above a specific threshold value. We compared two reinforcement schedules - an abrupt change, where this threshold value was introduced immediately at the beginning of practice; and a gradual change, where the threshold was increased gradually with practice. Results showed that although trunk velocities increased in both groups, indicating that the reinforcement was successful in altering their movement strategy, there was not a significant difference between the abrupt and gradual groups. Further analysis revealed that the rate of increase in the threshold in the gradual group have been too high, which resulted in effectively acting like an abrupt group. These results suggest that reinforcement feedback has the potential to alter movement strategies, but that reinforcement may be more effective if it is based on the actual performance of the individual, rather than on a pre-determined schedule.

**Disclosures: T. Lin:** None. **R. Ranganathan:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

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**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant R01NS053606-05A1

**Title:** Detecting state-dependency of patient involvement during force field training

**Authors: *Z. A. WRIGHT**[1,2], J. L. PATTON[1,2], F. C. HUANG[2]
[1]Bioengineering, Univ. of Illinois At Chicago, Chicago, IL; [2]Arms + Hands Lab., Shirley Ryan AbilityLab, Chicago, IL

**Abstract:** This study investigates a method to measure patient involvement during robot-assisted force training. An important lesson in therapy is that stroke survivors fail to improve when they allow the robot to passively move their affected limb. We assert that successful approaches require patients to attempt to actively contribute to movement. Current theories suggest that such active involvement promotes the reorganization of neural pathways - a critical component of motor recovery and learning. While the specific force algorithm likely affects involvement we wish to investigate other factors that could also contribute. We evaluated patient involvement during our recent study in which we investigated our design of a customized force intervention. Patients were allowed to move any way they wanted while forces were pre-programmed to push their hand towards their *neglected* velocities. We adopted a simple approach of using inverse dynamics to estimate elbow and shoulder joint torques and then quantified involvement in terms of power exertion at each joint. In our preliminary analysis, we examined how joint power varied across different movement states (position, velocity). We found that some patients often engaged in repetitive cyclic motions or were only active when their hand reached workspace boundaries while other patients were active throughout their state-space ranges. These observations suggest that some patients altered between modes of active and passive involvement. Further analysis will provide insights on whether degree of severity between patients correlates with involvement. This work provides a tool that can be used to develop and evaluate effective strategies for robot-assisted intervention. It can also be used to fine-tune forces during robotic therapy in order to optimize patient involvement.

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**Poster**

**593. Human Motor Learning: Behavior and Models**

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**Topic:** E.04. Voluntary Movements

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NIDRR Grant H133E120010

**Title:** Sensorimotor learning and co-adaptation in body-machine interfaces

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**Abstract:** Studies have shown that the brain is able to seamlessly incorporate external tools into a motor plan by learning their inherent properties (or structure). This process generally involves remapping of postures between our body and the position of the tool. One particular example is offered by Body-Machine Interfaces (BMIs), in which the user operates external assistive devices using body postures/movements as control signals.
In a BMI, a linear map that performs dimensionality reduction describes the transformation from a high-dimensional space of body movement signals to a lower-dimensional space of device movements. In order to skillfully operate the BMI, the user must effectively learn a suitable inverse of this map.
One of the greatest challenges is facilitating learning through the adaptation of the BMI without interrupting the continuous operation of the device. This feature is relevant not only for providing stable assistance through time, but also to use the system for tracking or for driving functional improvements after neurological injury.
Here we present a novel approach that focuses on the characteristics of the body motions the subject performs while trying to control the BMI. Our hypothesis is that it is possible to modulate learning by adapting the map so as to minimize the variance associated with motions in the map's null-space. This approach draws from the finding that exploration in redundant tasks tends to favor strategies of coordination that lie in the task space (i.e. that are orthogonal to the null space).
To test this hypothesis we trained subjects over multiple days controlling a cursor on a screen using shoulder motions. They practiced a reaching task using either a constant map or a co-adaptive BMI that modified the initial map over time. The co-adaptive mapping algorithm implemented an online PCA for incrementally shifting the task space towards movements that express greater variability. We compared the groups according to their performance in trained locations and non-trained locations of the cursor space as well as in a tracking task.

Subjects who trained with the adaptive BMI learned faster than the control group and performed on average better when tested on reaching to untrained locations.

**Disclosures: D. De Santis:** None. **L. Feeney:** None. **F.A. Mussa-Ivaldi:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.08/II19

**Topic:** E.04. Voluntary Movements

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**Title:** The effect of discrete versus continuous training in hand movement remapping

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**Abstract:** Previous research has demonstrated that participants are able to learn to make novel directed hand movements to control a cursor. However, the effect of discrete versus continuous training in hand remapping tasks has yet to be explored, though it has been shown that continuous training impedes in the proficiency of discrete facial remapping tasks. Here, we aim to evaluate which training protocol - continuous or discrete -participants learn more quickly with and then determine which training protocol leads to a more proficient completion of the respective tasks. We hypothesize that discrete training will be more beneficial as a training system because there is less cognitive overload and it requires the participants to be less precise. Participants wore the Cyberglove that recorded hand motions. Hand motions produced signals, which controlled the cursor position on the computer monitor. The study consisted of two phases, training and testing. Participants were trained on one system for three sessions and then tested on the untrained system. The continuous training task consisted of a screen with a cursor providing visual feedback and one target box. Participants were instructed to move the cursor to the target. Once the cursor entered the target box there was no visual feedback of the cursor and the box turned red. The discrete training task consisted of a four by four grid with the controlled cursor being invisible. As the cursor entered each tile, the tile turned red. Once the cursor entered the target tile the tile turned green.
Our performance metrics were smoothness, path length, variance in the task space in relation to variance in the null space, and time. Our preliminary findings support the hypothesis of a greater efficiency of the discrete training protocol.

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**Poster**

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Undergraduate Research Opportunity Program Grant

**Title:** Gradual practice is not superior to random practice for leg motor skill learning during gait

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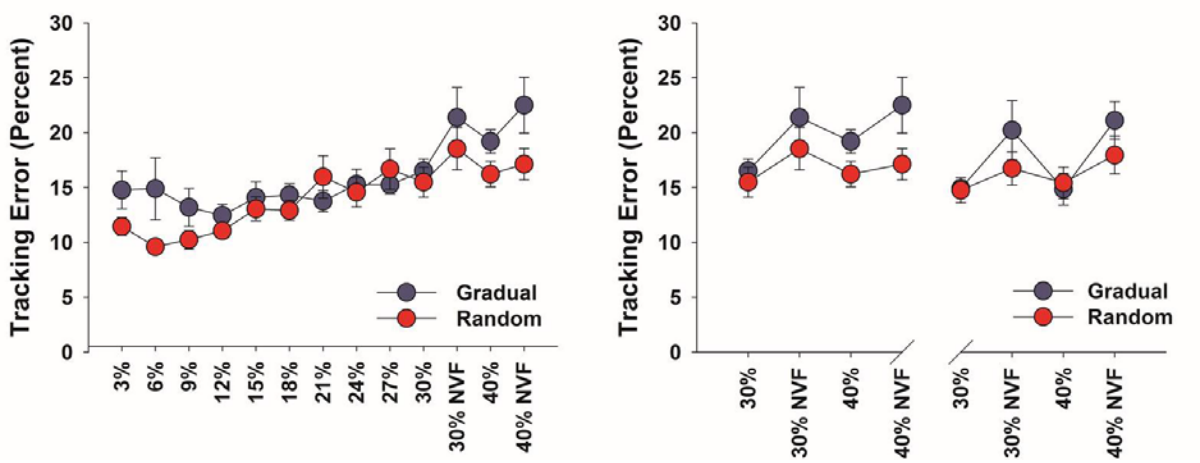
**Abstract:** Motor learning is an important part of rehabilitation; therefore, it is critical to understand the role of different practice structures when learning a motor skill. Here, we compared the benefits of gradual practice with random practice on motor skill learning using a target-tracking paradigm that has been specifically used for gait rehabilitation. Twenty-two right-foot dominant adults (12 male and 10 female) between the ages of 18-26 participated in this study. The participants were asked to walk on a treadmill while performing a motor learning task that required participants to modify their gait pattern to match a template projected on a monitor. Eleven participants practiced matching templates that gradually increased by 3% in size until they were 30% larger than their baseline walking template (gradual group). The remaining participants received the same templates used for the gradual trials, but in a pseudo-random order (random group). Both groups were then required to match a 40% template to evaluate transfer effects, as well as match the 30% and 40% templates without visual feedback. We then compared the tracking-error observed at 30% and 40% templates between groups for both the target-matching with visual feedback and no visual feedback conditions. There were no significant differences between the gradual and random groups when learning to match the template. This finding was consistent between the two groups regardless of whether they were tested with or without visual feedback. Further, there was no difference in retention when subjects returned on the second day for testing. Results indicate both gradual and random practice yielded the same level of tracking-error, and there is no significant difference between the two methods of training. Future studies comparing these paradigms to conventional learning paradigms will further expose the potential benefits of these learning structures.

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**Poster**

**593. Human Motor Learning: Behavior and Models**

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**Topic:** E.04. Voluntary Movements

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**Title:** Force production during holding suggests the presence of a neural integrator in reach adaptation

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**Abstract:** In a point to point movement, the brain first moves the body to a desired position, and then produces commands to maintain that position over time. Is it possible that these moving and holding processes are mediated by different populations of neurons? In the oculomotor system, burst neurons that drive saccades are silent when the eye is held in eccentric gaze. Gaze-holding activity is instead directed by a separate neural integrator that receives an efference copy of velocity-related motor commands from the saccadic burst generators, integrates these commands in time, and communicates a position-related signal to motor neurons of the eye. Is this serial relationship between moving and holding a fundamental property of other parts of the body? In

search of an answer, we noted that during force field adaptation of reaching movements, compensatory forces produced by subjects can continue long after the movement ends, while the arm is held still, despite the absence of external force. We wondered if these “force tails” provide evidence about the arrangement of moving and holding circuits of the arm. Subjects (n=10) held the handle of a robotic arm and made reaches to a peripheral target. We gradually imposed a force field to perturb their outward movements. At the end of every movement, we applied an error-clamp to hold the hand in place and measure force tails. After ramping the force-field magnitude in the CCW or CW direction, we then gradually removed the field, and ramped it in the opposite direction. In each of the four conditions (i.e. adaptation and de-adaptation to CCW and CW force fields) force tails were aligned with the direction of force compensation, with a magnitude that was linearly proportional to the level of adaptation of the movement (linear regression of force tails onto adaptation index; $R^2 = 0.889$). We hypothesize that, like the oculomotor system, motor commands that move the arm may serve as input to an integrator that helps to hold the arm in place. During force field adaptation, integration of additional forces required to counteract the field may lead to a disparity between the true position of the hand at the end of the movement, and the integrated position, which in turn gives rise to the force tail observed during the holding period.

**Disclosures: S.T. Albert:** None. **R. Shadmehr:** None.

**Poster**

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**Topic:** E.04. Voluntary Movements

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17H00874

JSPS #26242062

**Title:** Motor learning rate is influenced by prior motor learning through reconfiguration of directional preference of motor primitives

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**Abstract:** The central nervous system (CNS) corrects motor commands to decrease prediction error. We have previously suggested that the way the CNS corrects motor commands is flexible rather than rigid (Hayashi et al., eNeuro, 2016). Specifically, while reaching towards 30°

clockwise and counter-clockwise targets from straight-ahead (indicates 0°), imposing clockwise or counter-clockwise visual rotation to the cursor, respectively (i.e., outward rotation), led the direction of hand movements to shift inward. This visuomotor learning procedure did not apparently alter the hand movement towards 0° target. However, when a visual rotation was further applied, we found that the induced aftereffect (i.e., motor-learning rate) was significantly reduced. The result suggests that visuomotor learning could change not only how we generate motor commands but also how we learn to generate the commands. This novel finding, which can be considered as evidence of "meta-learning" (Harlow, Psychol Rev, 1949) in motor learning, cannot be reproduced by conventional motor primitive frameworks (e.g., Thoroughman & Shadmehr, Nature, 2000) since they assume that the motor-learning rate is independent of experience of the visuomotor learning. Here, we aimed to account for the novel phenomenon by incorporating a new factor into the framework. A previous neurophysiological study has demonstrated that the preferred directions (PDs) of neurons in motor-related areas rotated towards the direction of an error (Li et al., Neuron, 2001). Inspired by this finding, we assumed that visuomotor learning induces PD rotation towards the directional error. According to this new model, after the procedure of outward rotation described above, the number of motor primitives recruited for the movement towards 0° target should be reduced because the PDs rotate outward. Theoretically, the reduced number of recruited primitives should decrease the motor learning rate. A simulation confirmed that the proposed model could successfully reproduce our experimental results. The success of this new model suggests that visuomotor learning influences how we learn in the subsequent visutomotor learning through the reorganization of the PD distribution of motor primitives.

**Disclosures: T. Hayashi:** None. **K. Takiyama:** None. **D. Nozaki:** None.

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**Program#/Poster#:** 593.12/II23

**Topic:** E.04. Voluntary Movements

**Title:** The effect of energy-matched exercise intensity on brain-derived neurotrophic factor and motor learning

**Authors: *J. BAER**, M. GAUGHAN, H. SAFFER, M. SARZYNSKI, T. HERTER, S. FRITZ, D. DEN OUDEN, J. C. STEWART
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**Abstract:** High-intensity exercise induces an increase in brain-derived neurotrophic factor (BDNF), a protein that facilitates synaptic plasticity, suggesting that an exercise-induced rise in BDNF prior to practice may enhance motor learning. Previous studies comparing high and low-

intensity exercise have observed larger increases in BDNF and better motor learning after high-intensity exercise. However, these studies failed to control for total energy expenditure, thus it is unclear if these results were related to the intensity of exercise or the overall amount of work. The purpose of the current study was to examine the effect of different exercise intensities on BDNF levels and motor learning while controlling for exercise-related energy expenditure. Thirty non-disabled, young participants were randomized into three groups: High-intensity (80% maximal resistance determined from an exercise test), Low-intensity (40% maximal resistance), and Rest (sit quietly for 20 minutes). Durations of the High and Low exercise bouts were individually adjusted so that each participant expended 200 calories. Blood samples were collected immediately before and after each intervention. After the intervention period, all participants completed 144 trials of a 3-dimensional (3D) implicit motor learning reach task. Stimuli were presented in 8-target sequences, and alternated between random and repeated sequences that were matched for difficulty. Retention was assessed 24 hours after initial task practice. Results indicated that BDNF levels increased 36.72% in the High group, while changes were less than 16% in both the Low and Rest groups. All three groups exhibited improvements in task performance, as measured by time to complete a sequence ($p < .001$), and all groups completed the repeated sequence faster than the random sequence ($p < 0.001$). While initially the slowest group at the start of task practice, the Low group showed the greatest change in performance for both the random (High = -22.52% ± 13.28; Low = -25.92% ± 13.80; Rest = -20.49% ± 9.48) and repeated (High = -21.62% ± 11.38; Low = -25.83% ± 12.34; Rest = -21.10% ± 8.80) sequences, although differences between groups were not significant. All groups maintained performance improvements at retention. Although these findings should be interpreted with caution, they suggest that other exercise-related mechanisms that are independent of both intensity and increases in BDNF may be involved in facilitating motor learning. Future work will include investigation of the rate of change of performance and the effects of a BDNF polymorphism.

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**Program#/Poster#:** 593.13/II24

**Topic:** E.04. Voluntary Movements

**Title:** Adaptation to visuomotor rotation along a three-dimensional axis has two components

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**Abstract: Objective:** Previous studies on adaptation to visuomotor rotation primarily focused on a two-dimensional setup: when hand movement is constrained in the horizontal plane and the axis of the visuomotor rotation is always kept vertical. In this study, we tested how the adaptation behaviors observed in two-dimensional (2D) studies generalize to the three-dimensional (3D) setup: subjects adapting horizontal reaching movements in 3D while visuomotor rotation is applied along an arbitrary axis in 3D. Additionally, we test if the observed adaptation behaviors are affected by a positional incongruency between the visual and the physical hand position. **Methods:** Sixteen healthy right-handed subjects participated in the experiment. Subjects grasped a robotic manipulandum and made quick out-and-back reaching movements from a central home position to one of eight peripheral targets on the horizontal plane in a 3D virtual reality (VR) environment using an Oculus Rift. Subject's physical hand position, tracked by the manupulandum, and the home and target positions were presented as spherical objects in VR. After a brief familiarization, a visuomotor rotation along a 3D axis was applied around the home position, and subjects were asked to adapt to the environment to make a visually horizontal reaching movement toward the target. In this environment, we tested two hypotheses. First, is there is a difference in the patterns of the "to-the-plane" adaptation (TP: when the axis of rotation is on the horizontal plane) and the "on-the-plane" adaptation (OP: when the axis of rotation is perpendicular to the horizontal plane - this corresponds to the traditional 2D visuomotor adaptation)? Second, are the observed adaptation speeds affected by the incongruency (a constant positional offset) between the visual and the physical hand positions? Both OP and TP were tested in separate and mixed conditions, during which trial-by-trial adaptation was assessed by maximum perpendicular error to the plane (MPE-TP) and on the plane (MPE-OP) respectively. **Results:** When TP and OP were tested separately, there were no differences between the speed of TP and OP in the congruent condition. In the incongruent condition, however, OP was significantly slowed down ($P < 0.05$) while TP remained at a similar level to the congruent condition. Same results were observed in the combined condition, showing only a significant effect of congruency on OP. Importantly, there were no significant differences in both MPE-TP and MPE-OP between the separated and the combined condition. **Conclusions:** The result suggests that there are two components in 3D visuomotor adaptation.

**Disclosures: S. Yeo:** None.

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**Program#/Poster#:** 593.14/II25

**Topic:** E.04. Voluntary Movements

**Support:** JSPS KAKENHI

**Title:** Low-dimentional modification of musculoskeletal variables in motor adaptation

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**Abstract: INTRODUCTION** Humans can modify motor commands to learn to move in novel environments. The central nervous system (CNS) faces a fundamental problem of how it controls high-dimensional variables in joint and muscle coordination space. To reduce the huge number of variables, motor commands would be constrained in low dimension by organizing the neuromuscular control (Hagio and Kouzaki, 2014; Sadtler et al., 2014). However, how the musculoskeletal variables were modified in motor adaptation is still unclear. In the present study, therefore, we examined the regulation of high-dimensional variables during the adaptation to a novel dynamical environment.
**METHODS** The participants performed horizontal reaching movements holding robotic handles by their right hand. They sat in an adjustable chair to which their trunk was strapped. They were instructed to move cursors representing the hand positions from a start position located at approximately 30 cm in front of their body to one of the 8 equally placed targets by 45° on a horizontal display, as straight as possible. For the training, the participants performed reaching movements under the presence of a velocity-dependent curl force field (Shadmehr et al., 1994). To equalize the endpoint kinematics, we randomly interleaved the error-clamp (EC) trials, with which the movement trajectory of the handle was constrained to a straight path from the start position to the target by a virtual force-channel (Scheidt et al., 2000). The experimental tasks were composed of null field, 384 force field (FF) and 96 washout trials across targets. During the tasks, surface electromyograms (EMGs) were recorded from 14 muscles spanning wrist, elbow and shoulder joints. In addition, the joint torques were estimated from the kinematic data obtained using a motion capture system. The EMGs and estimated joint torques in EC trials were compressed into low dimension using statistical calculation.
**RESULTS AND DISCUSSION** The lateral deviations due to the velocity-dependent force field exponentially decreased to a plateau by approximately half in the FF trials. Both EMGs and joint torques in high-dimensional space were largely explained by computed 3 dimensional variables, the structures of which were maintained throughout the trials across each subject. Low-dimensional variables organizing joint torques and muscle activations were modified with different adaptation rates. The results indicate that the CNS would reduce the high-dimensional musculoskeletal variables modified in the motor adaptation by modularly regulating the motor commands.

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**Topic:** E.04. Voluntary Movements

**Support:** NIH NS092079

**Title:** Target size effects on sensorimotor adaptation

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**Abstract:** Task performance and sensory prediction errors are two feedback signals thought to drive motor learning. In attempts to isolate learning from sensory prediction error, the difference between expected and actual sensory consequences of a motor command, we have used clamped visual feedback (Morehead et al. 2017). Here the angular trajectory of a feedback cursor is invariant with respect to the target location and thus spatially independent of hand position. Participants are fully informed of the manipulation, and are instructed to ignore the cursor and aim directly to the target. This task was designed to make task performance error irrelevant. To test this hypothesis, we conducted several experiments in which we manipulated the size of the target, while holding the clamp size constant. By doing so, we altered the relationship between the cursor and target such that the same clamp offset could result in either persistent target hits or misses. Experiment 1 examined responses to a 1.75° clamp over 220 movement cycles (one movement to each of 4 targets). In a between-subjects design, participants (n=14/group) made fast center-out reaches on a digitizing tablet, with the terminal position of the clamped cursor either fully embedded within a large target (IN) or partially overlapping a small target (IN/OUT group). All participants adapted, evidenced by a shift in heading direction opposite that of the clamp. There was a significant effect of target size: Asymptotic adaptation averaged ~12° for the IN group, compared to ~20° for the IN/OUT group ($p=.02$). In Experiment 2, we increased the clamp to 3.5°. For large, medium, and small target groups (n=12/group), the terminal position of the clamped cursor was fully embedded within the target (IN), halfway in (IN/OUT), or outside (OUT), respectively. The IN group's asymptote was again reduced, ~8° lower than that of both the IN/OUT and OUT groups. In Experiment 3, using a within-subjects manipulation, we tested two groups (n=10/group) on a 1.75° clamp. Following 120 movement cycles with a large (IN) or small target (IN/OUT), the target size switched to the other size for a final 80 cycles. Consistent with Experiments 1 and 2, the initial IN group produced a lower asymptote than the initial IN/OUT group by the end of the first block. As predicted, the IN group's heading angle increased when the target decreased in size, whereas the IN/OUT group's heading angle decreased when their target became larger ($p<.001$). Combined, these results show that target

"hits", even when task irrelevant, attenuates adaptation. Ongoing work is focused on testing whether this effect is due to an interaction between reward and error based learning systems.

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**Topic:** E.04. Voluntary Movements

**Title:** Practicing a de novo skill alters the adaptive response to subsequent perturbations

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**Abstract:** The motor system uses a variety of approaches to compensate for perturbations to movement - e.g. adapting to a 30° rotation of visual feedback about the origin of movement. Foremost, the motor system uses a recalibration mechanism that temporarily and reversibly modifies an existing controller. Alternatively, however, it may maintain the original controller but change the movement aims - e.g. aim to a different target to compensate for a visuomotor rotation (VMR). Another potential solution is to permanently reconfigure the original controller in a way specific to the perturbed environment. Here we examined which of these modes of compensation the motor system employs when compensating for perturbations to a newly acquired skill. We hypothesized that sufficiently practiced de novo controllers could, like baseline controllers, be adapted using recalibration and/or re-aiming, whereas less practiced ones would tend to be permanently reconfigured.
Participants controlled a single on-screen 2-D cursor using both hands. Each hand controlled one dimension of cursor movement so that hand and cursor motion were orthogonal. We compared two groups who practiced this challenging task for either 1 or 4 sessions, during which they primarily moved the remapped cursor to a series of random target locations.
To evaluate how the newly learned skill would adapt to a perturbation, we subsequently exposed them to a 45° VMR of the cursor in a center-out, target-shooting paradigm. We taught them a simple strategy of aiming to a neighboring target, enabling them to counter the perturbation while still experiencing the sensory prediction errors that can drive recalibration (Mazzoni & Krakauer, 2006). We further tested participants for aftereffects by withholding cursor feedback and instructing them to aim directly to each target.
We found that, although both groups adapted similarly to the VMR, neither one exhibited the usual hallmarks of sensory-prediction-error-based recalibration; there was no drift in performance when participants used a provided strategy to counter the VMR, and aftereffects, though present, did not decay during subsequent trials without visual feedback. The sustained

aftereffects, indicative of a permanent reconfiguration of the previously learned controller, were weaker in the 4-session group, suggesting a controller more stable against permanent reconfiguration, and on which re-aiming could be more easily applied. We speculate that this reconfiguration may occur even in conventional adaptation paradigms which apply similar perturbations to more strongly established baseline controllers.

**Disclosures: A.M. Hadjiosif:** None. **A.M. Haith:** None.

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**Topic:** E.04. Voluntary Movements

**Title:** Trial-by-trial updates of internal models during interactive motor learning

**Authors: *S. GAKU**[1], S. KASUGA[1], J. USHIBA[2]
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**Abstract:** We empirically know that in some situations motor skills can improve greater when multiple persons practice together, compared to when they practice alone. A previous study experimentally showed such a synergistic effect during motor learning (Sacheli *et al*, 2012); however, behavioral changes were only investigated in a pre-post design. Therefore, it is unclear how behaviors are interacted in a trial-by-trial manner. In the current study, we aimed at investigating the mechanism in which the visual error information resulting from one person's motor action implicitly updated another person's motor adaptation process. In order to quantify a trial-by-trial interaction of adaptation, we applied Granger causality analysis for the data of an arm-reaching task with visiomotor rotation. Nine pairs of healthy adults participated in this study. They performed 600 trials of a planner center-out reaching task on a computer monitor, using a custom-made manipulandum. Participants were able to see their own and the partner's cursor at the same time on the personal monitor. The cursor of either participant was rotated in ±30 degrees at about 20% of random trials, in the same amount of trials for each participant. As a control experiment, the same participants performed the same task individually in a separate day. To investigate the effect of a partner's movement on one's motor adaptation process, firstly we analyzed the amount of error correction in the next trial of rotation for both perturbed and unperturbed participants. Error correction was significantly decreased compared with the individual control condition ($p$=0.011 ) implying that adaptation of the perturbed participant was interfered by the unperturbed partner's movement that exhibited little error. To further examine the variability of this effect, we computed Granger causality by using trial-by-trial time series of a pair of participants' initial movement direction. We found a significant effect in 6 participants,

indicating the influence from one participant's movement on the other's movement. Furthermore, we found a positive correlation between the difference in the maximum speed of reaching between 2 participants, and a measure of goodness-of-fit for Granger causality model (i.e., F-value; $r = 0.507$, $p = 0.002$), suggesting that the more the speed is different within a pair, the more the slower one is influenced by the faster partner. Overall, the current findings suggest that the brain has the implicit motor learning process which is updated by both own and another person's visually fed-back errors, and that knowledge of the difference on kinematic properties may be related to the strength of the interaction.

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**Topic:** E.04. Voluntary Movements

**Support:** NSF 1553895

**Title:** Subject-specific biomechanical modeling of motor learning

**Authors: *Q. WEI**[1], Q. XING[2], S.-H. YEO[3], W. M. JOINER[1]
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**Abstract:** The human motor system is capable of a range of actions that are modified through experience. Significant work has tested various hypotheses on the underlying learning mechanisms by measuring human behavior in simple behavioral paradigms. This has provided the basis for different theoretical models on how the central nervous system interprets errors and subsequently modifies motor commands. Although these studies have provided significant insight, the changes in biomechanics during learning have been given little attention. In this study, we developed an integrated framework to concurrently probe the biomechanical and muscle activation patterns involved in a simple and highly studied motor learning paradigm. Here, subjects interacted with a robotic manipuladum, and adjust reaching movements in response to a physical perturbation to the limb motion. We provided no mechanical support to the arm so that we could examine the motion of the upper extremity (upper arm, forearm and hand) about the elbow during the compensation. These motions were measured by a custom designed motion capture device, and muscle activities were measured by a wireless EMG system, both synchronized with the robotic manipuladum. The data were used to develop subject specific OpenSim models of the upper extremity dynamics in order to analyze the unique joint kinematics and muscle activation patterns during the motor learning trials. We observed a

significant variation between subjects on how the compensatory response utilized the redundant degree of freedom (the elbow joint in this setting). Together, our subject specific results demonstrate that a thorough understanding of the changes in the biomechanics and muscle activation patterns that accompany motor learning may provide additional insight into the concurrent mechanisms that drive motor learning.

**Disclosures: Q. Wei:** None. **Q. Xing:** None. **S. Yeo:** None. **W.M. Joiner:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.19/JJ3

**Topic:** E.04. Voluntary Movements

**Support:** School of Public Health Student Research Grant

**Title:** The influence of sequence learning on force adaptation

**Authors: *Y. LIU**, H. BLOCK
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**Abstract:** Sequence learning and sensorimotor learning occur together in nature. For example, to play the violin, a student must learn not only the sequence of notes, but also how to move each hand and arm to the correct position with the correct timing, how much force to exert with the bow on the string, and so on. Sensorimotor and sequence learning have much in common, including dependence on working memory and developmental stage, and similar activation of the motor cortex, left dorsal prefrontal cortex, and cerebellum. However, these processes are often studied in isolation. It is unclear whether an implicit sequence would enhance sensorimotor learning, perhaps by making the movements easier to predict, or whether it would interfere with sensorimotor learning, for example by increasing working memory demands. Here we combined a serial reaction time task (SRTT) and a force field adaptation task to investigate this question. Participants performed 40 trials in total: 5 baseline, 25 adaptation, and 10 washout. Each trial consisted of 12 sequenced or randomized reaches among four targets arranged in a square. Subjects grasped a robotic manipulandum (KINARM) throughout. In the block of adaptation trials, an external force was applied perpendicular to the ongoing reaching direction for the sequence/force (N = 3) and random/force (N = 2) groups, while the sequence/null group (N=1) performed sequenced reaches with null force throughout. Participants were asked to reach as fast and straight as possible. The change in reaction time was quantified as the difference in seconds between the last washout trial and the first adaptation trial. This was -0.059 ± 0.016s vs 0.009 ± 0.011s vs -0.059s for the sequence/force, random/force, and sequence/null groups. The decrease in reaction time for the sequence groups suggests that sequence learning occurred. Movement

error was quantified as max perpendicular deviation from the straight line path connecting start and end target. Force adaptation magnitude was quantified as the difference between max perpendicular error on the first and last trials of the adaptation block. This was 3.93 ± 0.11cm in the sequence/force group and 3.69 ± 0.45cm in the random/force group. Negative aftereffect of force adaptation was quantified as the difference between the last baseline trial and the first trial of the washout block. This was -2.94 ± 0.05cm vs -4.17 ± 0.26cm for the sequence/force and random/force groups, respectively. Preliminary data thus suggests that negative aftereffect, reflecting learning and storage of the adaptation, may be reduced when force field learning is accompanied by sequence learning.

**Disclosures: Y. Liu:** None. **H. Block:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.20/JJ4

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant HD40289

**Title:** Increasing motor noise impairs reinforcement learning in healthy individuals

**Authors: *A. S. THERRIEN**[1,2], D. M. WOLPERT[3], A. J. BASTIAN[1,2]
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**Abstract:** Motor variability is crucial to the process of reinforcement learning. It allows the system to explore possible solutions to a task and select those that yield rewarding outcomes. However, in addition to exploration, motor variability also reflects signal noise from stochastic and/or faulty neural processing. While the motor system may possess an internal estimate of exploration variability, it cannot estimate (and thus cannot learn from) variability from noise. Thus, a high proportion of motor variability from noise may impair reinforcement learning by disrupting the mapping between an action performed and the outcome feedback received. We have previously shown that increased motor variability from noise may underlie reduced reinforcement learning performance in individuals with cerebellar damage. Here we examine whether increasing noise in neurologically healthy individuals impairs reinforcement learning in a similar manner to that seen in cerebellar patients. Our experimental task requires participants to use binary feedback signaling to learn to rotate their reach angle in a series of directions. By comparing task performance between conditions with different levels of added noise, we show that adding a small level of noise does not impair learning, but adding a larger level – matched to a group of cerebellar patients – does. Using a mechanistic model, we find that adding a low level

of noise leaves a sufficient proportion of motor variability that can be estimated by the motor system allowing subjects to learn. However, adding a high level of noise leaves participants with an insufficient proportion of estimable variability and this reduces learning performance. Importantly, in a second experiment, we show that clamping the reinforcement rate to that observed in the high noise condition does not impair learning. Finally, by comparing performance between healthy individuals with noise added to their movement and a group of cerebellar patients, we find that the added noise does not impair the control group's learning to the same degree observed in the patient group. We suggest that this may be attributed to a discrepancy between the nature of the added noise in the present study and the source of noise in the cerebellar patients. Overall, our results suggest that motor variability from noise interferes with reinforcement learning. Additionally, we show that a threshold may exist for motor noise, above which the remaining proportion of variability that can be estimated is too small for the motor system to learn from effectively.

**Disclosures:** **A.S. Therrien:** None. **D.M. Wolpert:** None. **A.J. Bastian:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.21/JJ5

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant K01AG047926

NIH grant R01HD065438

**Title:** Dissociating learning- and non-learning-related performance changes during motor skill training in older adults

**Authors: *S. Y. SCHAEFER**[1], N. SCHWEIGHOFER[2]
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**Abstract:** Motor learning has long been thought to decline with age, given that older adults tend to show less improvement from before to after a training session compared to younger adults. Recent data has challenged this idea, however, showing that 'old old' adults (over age 75) do not necessarily learn a novel motor skill less or slower than 'young old' adults (ages 65-74) (Schaefer et al. 2015). A nonlinear mixed-effects model of motor learning may give new insights into the true effects of aging by dissociating learning from other factors unrelated to learning, like fatigue (Park and Schweighofer, 2017). This approach models non-learning-related changes as an increasing linear term that captures performance decrements later in training, in addition to the more traditional 'learning curve' that shows exponential (nonlinear) improvements in

performance early on in training. In other words, older adults' final performance at the end of a training session may not necessarily reflect their motor learning ability. Currently this nonlinear mixed-effects model has been developed to quantify learning of only a reaching task. Thus, the purpose of this study was to test whether this model generalizes to the learning of a more functional, real world motor skill that involves reaching, grasping, and object manipulation. Twenty-eight adults (ages 39-89; median 73 years) completed 150 trials of a functional upper extremity motor task over three days (50 trials/day). Each trial equaled 15 repetitions, where one repetition required participants to use a plastic spoon to transport two raw kidney beans at a time to one of three distal targets using their nondominant hand. Most improvement occurred by the $30^{th}$ trial within the first day. Participants did vary, however, on how much they retained one month later (-9.5% to 46.29% of initial performance). Model fits from the first 30 trials showed a significant relationship between the amount of learning parameter, A, and the observed amount of skill retention one month later ($r=0.44$; $p=0.02$). Normalizing the learning parameter to each participant's initial performance improved the prediction of one month retention ($r=0.50$; $p=0.007$). Interestingly, the non-learning-related parameter, C, was significantly related to age ($rho=0.48$; $p=0.009$). These findings not only demonstrate the robustness of this model in predicting how responsive older adults will be to training on more complex motor skills, but also provides new insights into how fatigue or other age-related effects may have been misinterpreted as impaired motor learning in previous studies in older adults.

**Disclosures: S.Y. Schaefer:** None. **N. Schweighofer:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.22/JJ6

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant K01AG047926

**Title:** Using clinical neuropsychological assessments to predict motor learning in non-demented older adults

**Authors: *J. LINGO VANGILDER**[1], C. R. HENGGE[3], K. DUFF[4], S. Y. SCHAEFER[2]
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**Abstract:** Motor learning diminishes with aging, such that older adults tend to learn less and at a slower rate than younger adults. Poorer motor learning may, in part, be linked to more general age-related cognitive impairments. For example, in patients diagnosed with amnestic Mild

Cognitive Impairment, their ability to learn a functional motor task is related to their performance on a battery of visuospatial tests. The purpose of this study was to test whether these findings generalized to a sample of 24 age-matched older adults with no memory impairment. All participants were assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), from which age-adjusted scores for five cognitive domains were extracted. Participants' baseline performance on a motor task designed to improve upper extremity motor function was also measured prior to a short (9-trial) practice session; motor performance was re-assessed one week later. Motor performance was quantified as the time to complete a given trial of the task, with faster times indicating better performance. Stepwise regression indicated that the visuospatial/constructional index of the RBANS was the only cognitive domain that significantly predicted one-week follow-up motor performance ($R^2$=0.24; p=0.04) once baseline performance was accounted for. As the visuospatial/constructional index is comprised of a line orientation subtest and a complex figure copying subtest, further regression analysis indicated that the line orientation scores were most related to the amount of improvement at one week (p=0.02). These findings are consistent with prior work showing that visuospatial tests may be used prognostically to identify motor learning deficits in older adults, irrespective of any other memory impairments. Future directions will explore the underlying neural mechanism of why visuospatial tests appear to uniquely capture the aging brain's motor learning capacity.

**Disclosures: J. Lingo Vangilder:** None. **C.R. Hengge:** None. **K. Duff:** None. **S.Y. Schaefer:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.23/JJ7

**Topic:** E.04. Voluntary Movements

**Support:** NIH Grant K01AG047926

**Title:** Predicting motor skill retention in older adults with the visuospatial/executive subtest of the Montreal cognitive assessment

**Authors: *P. WANG**, S. Y. SCHAEFER
Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

**Abstract:** Older adults tend to learn novel motor skills at a slower rate and to a lesser extent than younger adults, yet cognitive decline may in part explain the effect of age. In fact, older adults'motor learning capacity may depend on their visuospatial function, rather than on their global cognitive status or their age itself. In adults over 65 with and without significant memory

deficits (i.e., Mild Cognitive Impairment), lower scores on standardized visuospatial tests like line orientation and complex figure copy are associated with less retention of a motor skill. These tests, however, can take tens of minutes to complete, and are typically administered as part of a much longer and comprehensive neuropsychological battery. Thus, the purpose of this study was to determine whether a briefer cognitive screening tool could predict motor skill retention as well. To do so, data from 36 adults over age 65 with no diagnosis of Mild Cognitive Impairment were retrospectively analyzed. Cognitive status was tested with the Montreal Cognitive Assessment (MoCA), which is very brief (under 5 mins) and yields individual subtest scores (Visuospatial/Executive, Naming, Language, Attention, and Delayed Recall) that are summed for a total score of 0-30. Participants also completed fifty trials of a functional novel upper extremity motor task involving reaching, grasping, and object manipulation with their nondominant hand. Skill retention was measured as the amount of improvement retained on the motor task at a 24-hour follow-up, relative to their first trial. Some participants improved by +47%, while others were worse 24 hours later (-19% change). Stepwise regression revealed that the Visuospatial/Executive score alone significantly predicted skill retention ($R^2$=0.25; p=0.003). For reference, the Visuospatial/Executive subtest includes drawing the face of a clock from memory and copying a simple drawing of a cube. Moreover, the effect of age on skill retention was accounted for by Visuospatial/Executive score, such that age was not a significant predictor (p=0.14), nor were any other MoCA subtest scores. Thus, older adults may have less motor learning capacity than younger adults due to age-related declines specifically in visuospatial function, rather than due to just their chronological age alone or other cognitive deficits. While clinical visuospatial tests may be used to predict the extent of motor skill learning in older adults, future research is needed to determine which aspect of visuospatial function (e.g., visual perception, visual attention, mental rotation) is the most critical.

**Disclosures: P. Wang:** None. **S.Y. Schaefer:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.24/JJ8

**Topic:** E.04. Voluntary Movements

**Support:** NSERC

**Title:** Using a real-world chopping task to study motor memory of competent and expert choppers

**Authors: *A. H. NEPOTIUK**, L. E. BROWN
Trent Univ., Peterborough, ON, Canada

**Abstract:** As we learn as specific skill, we develop a motor memory for that task. As people practice to become an expert at a particular task, they may practice specific strategies to achieve a larger skill goal. For example, when practicing to throw a ball, someone might focus on different aspects of the movement in order to achieve the goal of an accurate throw. This may lead to the development of separate motor memories for different task-relevant variations in experts that are not present in people who are competent, but not experts, at performing the task. Task interference is a tool used to study the representation of task performance in motor memory. This paradigm has been used with 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 performance and memory for reaching perturbations, specifically, and motor performance in general. These studies have revealed that memory for the perturbation is formed, and that it is fairly general (it generalizes somewhat to other locations in the workspace or to other hands, for example). We will pair this interference paradigm with an ecologically valid vegetable-chopping task, where we can easily recreate natural performance settings using a task for which we can easily identify experts and competent performers, to compare the representations of knife-skill memory of these two groups.
Participants' performance will be recorded using a motion tracker. 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 performance, participants are exposed to an interference condition where the frequency of chopping is increased or decreased. Participants then perform trials of the original task again. Measures of movement time, slice width and variability are taken. Interference is inferred if the second performance of the original task is impaired, compared to initial performance and that of controls. We believe that competent performers will experience more interference than experts, and that the type and direction of error experienced will illustrate the nature of the differences in motor memory between competent and expert performers

**Disclosures: A.H. Nepotiuk:** None. **L.E. Brown:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.25/JJ9

**Topic:** E.04. Voluntary Movements

**Support:** NSF Grant 1553895

NIH Grant EY021252

**Title:** Dissociating the influence of postural and visual shifts on the transfer of motor adaptation to novel workspace locations

**Authors: *W. ZHOU**[1], K. COLUCCI-CHANG[1], S. M. CHASE[2,3], W. M. JOINER[1]
[1]Bioengineering, George Mason Univ., Fairfax, VA; [2]Dept. of Biomed. Engin., [3]Ctr. for Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA

**Abstract:** Adaptation paradigms that alter the relationship between movement and their sensory consequences provide a method to study the properties of short-term motor learning. Here, we examined how the transfer of such error-driven motor learning to a novel workspace is influenced by dissociated changes in posture and vision. We trained two groups of right handed subjects (N=32 in both groups) to move a robotic manipulandum and perform point-to-point reaching arm movements with their right hands. One group adapted arm movements in response to novel dynamics (force-field perturbations, FF). The other group adapted to alterations in visual feedback (visuomotor rotation perturbations, VMR). There were two workspace locations: A (9 cm right of midline) and B (9 cm left of midline). After reaching asymptotic performance in one workspace, subjects were either tested for retention in the same workspace or transfer to the other workspace under three conditions. (1) Visual transfer: movements made in the trained workspace, but visual feedback provided at the other. (2) Postural transfer: visual feedback at the trained workspace, but movements made in the other. (3) Combined transfer: both visual feedback and movements provided/made in the untrained workspace. The perturbation direction (toward vs. away from midline) and transfer direction (A to B vs. B to A) were counterbalanced across subjects. For both FF and VMR perturbations, we found that the combined transfer condition resulted in the lowest amount of generalization ($P < 0.001$), with intermediate generalization for postural and visual transfer. There was no significant difference between the postural and visual transfer when all results were combined regardless of perturbation or transfer directions ($P > 0.3$). However, by specifically comparing the transfer by perturbation directions, we found that for FF perturbations towards the body, the postural transfer was significantly greater than the visual transfer ($P < 0.001$). Further, visual transfer was significantly greater than postural transfer ($P < 0.001$) when perturbations were away from body. For VMR, we found that the reverse relationships were significant (e.g., the visual transfer was significantly greater than the postural transfer for perturbations towards the body). Together, these results suggest that movement planning and perturbation modality collectively influence the extent of motor adaptation generalization, implicating an important role of higher level cortical processing.

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**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.26/JJ10

**Topic:** E.04. Voluntary Movements

**Title:** Change to Dissecting the relationship between motor variability and motor learning ability

**Authors: *M. A. SMITH**
Sch. Engin., Harvard Univ., Cambridge, MA

**Abstract:** .

**Disclosures: M.A. Smith:** None.

**Poster**

**593. Human Motor Learning: Behavior and Models**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 593.27/JJ11

**Topic:** E.04. Voluntary Movements

**Support:** NSF Graduate Research Fellowship

**Title:** Fast-forwarding feedback to improve performance in motor skill learning

**Authors: *R. B. SINGH**[1], L. ALHUSSEIN[2], M. A. SMITH[3]
[1]Sch. of Engin. and Applied Sci., [2]SEAS, [3]Sch. Engin., Harvard Univ., Cambridge, MA

**Abstract:** Motor skills that require high spatio-temporal precision often develop over years of practice. Studies of simple motor skills, which can be mastered over a few hours of practice, regularly show an initial period of rapid improvement with practice, but diminishing gains with additional practice as performance approaches ideal levels. In realistic every-day movements however, asymptotic performance is often less than ideal. Basketball provides a poignant example: despite improved training methods and years of dedicated player practice, the mean free-throw shooting percentage of professional basketball players in the NBA has remained essentially unchanged over the past 50 years, with the league-wide shooting percentage at 73.2% in the 1966-67 season and 77.2% in the 2016-17 season. Seeing such stark constancy, we wondered what may be limiting free-throw shooting performance. As temporal delays in

feedback have been shown to impair both reinforcement learning and neuronal plasticity, we hypothesized that the temporal delay between movement execution and the resulting success/failure in free throw shooting may be limiting performance, and that substantially reducing this delay will increase performance. To test this hypothesis, we can provide shooters with feedback indicating shot outcome well before they would normally see it themselves (~1 second after ball release). However, providing such early feedback is a challenge in that it requires an accurate prediction of shot outcome based on information available early in the free throw. While the biomechanics of postural changes during the act of shooting should, in theory, predict shot outcome, the relationship between biomechanics and shot outcome is idiosyncratic. In contrast, the physics of ball motion after release is universal, depending on the physics of projectile motion rather than the biomechanics of shooting. Thus, we focus on predicting shot-outcome based on the initial position and velocity of the ball immediately after release. To estimate the initial state, we have developed a high accuracy stereo computer-vision system to detect ball position in 3D. In particular, we developed novel methods for detecting ball position with subpixel accuracy from video frames, and for improving the 3D stereo camera calibration via better localization of calibration features. With this system, we are currently able to detect a basketball's position within 1 mm on average within a scan volume of approximately 10 cubic meters. The current accuracy would allow for 90% accuracy in predicting shot-outcome.

**Disclosures: R.B. Singh:** None. **L. Alhussein:** None. **M.A. Smith:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.01/JJ12

**Topic:** E.05. Brain-Machine Interface

**Support:** NSF EEC-1028725

**Title:** Evaluating electrocorticography signals during sustained grasping and upper-limb kinectic output

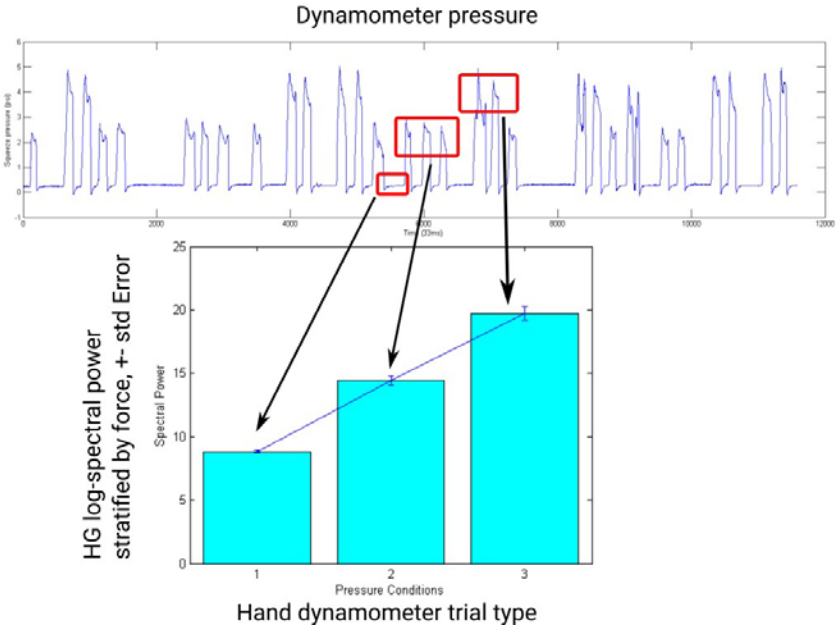
**Authors: *K. LY**[1], J. WU[2], R. P. RAO[3], J. G. OJEMANN[4]
[1]Ctr. of Sensorimotor Neural Engin., Seattle, WA; [2]Ctr. for Sensorimotor Neural Engin., [3]Dept. of Computer Sci. and Engin., [4]Dept Neurosurg., Univ. of Washington, Seattle, WA

**Abstract:** How the human brain controls arm movement is important in refining brain-computer interfaces (BCIs) for the development of upper-limb neuroprostheses. Many earlier decoding methods used recorded sensorimotor signals, as well as the parietal and premotor cortices. However, it is unknown whether movement-related signals in these regions can predict force

magnitudes generated by the upper-limb, or how these regional cortical activation occurs as a function of upper-extremity kinetic output. We investigated electrocorticographic (ECoG) data collected from 3 patients, undergoing epilepsy monitoring with normal pre-operatively motor function, during directed isometric arm force and power grasp tasks.

In one task, one subject was prompted via visual cues to apply isometric force in one of 6 directions (up, down, left, right, forward, backward) to an affixed AMTI force transducer handle with approximately 15lbs (67N) of force; there were 3 seconds of rest after each hold. In a separate task, two subjects prompted to grasp a pneumatic dynamometer and applied one of 3 force conditions (0kPa, 20kPa, 40kPa) which was displayed on a pressure gauge. γ- and β-band spectral power were extracted across implanted ECoG electrodes and compared to captured force and pressure in the two tasks respectively.

We found a time-averaged linear relationship between γ-band log-spectral power and sustained grasping force output with visual feedback. However, the same linear relationship between spectral power and cued isometric force generation, without concurrent visual feedback, in arm force application was not found. We find that a combined feature of γ- and β-band spectral power can be successfully used to predict sustained kinetic outputs that are generalized in both grasping and arm force applications.




**Disclosures:** **K. Ly:** None. **J. Wu:** None. **R.P. Rao:** None. **J.G. Ojemann:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.02/JJ13

**Topic:** E.05. Brain-Machine Interface

**Support:** NSF Grant EEC-1028725

NDSEG Fellowship

**Title:** Transient paresthesias experienced during closed-loop deep brain stimulation

**Authors: *M. C. THOMPSON**[1], B. C. HOUSTON[2], T. E. BROWN[3], J. G. OJEMANN[4], A. L. KO[4], H. J. CHIZECK[1]
[1]Electrical Engin., [2]Neurosci., [3]Philosophy, [4]Neurosurg., Univ. of Washington, Seattle, WA

**Abstract:** Introduction: Paresthesias, defined as unnatural tactile sensations such as “pins-and-needles,” are a known side effect of deep brain stimulation (DBS) for neurological movement disorders. In traditional DBS therapy, paresthesias are experienced only briefly (less than 1 minute) after stimulation is initiated. It has not previously been shown how paresthesias may manifest during closed-loop DBS (CLDBS), where stimulation parameters are modified in real time based on feedback from worn or implanted sensors. We investigated subject-reported paresthesias as a function of stimulation parameters for CLDBS.
Methods: Perceptions of paresthesias were tested in 2 subjects with a DBS system (Medtronic Activa PC+S) for essential tremor implanted in the ventral intermediate nucleus (VIM) of the thalamus contralateral to the affected limb. Paresthesias were intentionally evoked during a pseudorandom series of stimulation updates (bipolar stimulation of clinically effective contacts, 0-4 V, 0-2.5 V/s) while subjects were asked if they experienced physical sensations. Subjects were at rest and blinded to the stimulation parameters. Queries were made to subjects during both ramp and hold periods. The location, strength, and quality of sensations were recorded at each query (ex: “In my right arm I feel weak tingling”). After the experiment, paresthesias for each test were coded on a scale from 0 (no paresthesias experienced) to 3 (strong paresthesias experienced).
Results: Paresthesias were experienced in the upper and lower limbs contralateral to the implanted electrode (Patient 1) and in the scalp (Patient 2). Patients described paresthesias as a radiating tingling or “electric” feeling ranging from very mild to very strong. Paresthesias were only experienced during and immediately following increasing ramp periods, and were not experienced during decreasing ramp or hold periods. We found that slew rate, absolute voltage, and total change in voltage all contributed to the strength of paresthesias. Particularly, slew rates above 1 V/s often caused noticeable paresthesias at therapeutic voltage levels.

Conclusion: Our results indicate that paresthesias could be more prevalent during CLDBS than during traditional DBS. Furthermore, prevention of paresthesias may be at odds with development of algorithms that respond quickly via high slew rates. In addition to precautions that are typically taken during patient programming to avoid high voltages that may elicit side effects, CLDBS requires consideration of additional parameters—such as slew rate—to avoid undesirable side effects.

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**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.03/JJ14

**Topic:** E.05. Brain-Machine Interface

**Support:** NSF 1630178

**Title:** Neural prediction of motor activity in natural data with multimodal techniques

**Authors: *X. WANG**, A. FARHADI, J. OJEMANN, R. RAO, B. BRUNTON
Computer Sci. and Engin., Univ. of Washington, Seattle, WA

**Abstract:** There have been many successes in using experimental ECoG (Electrocorticography) and EEG (Electroencephalography) studies to decode brain activity; however, natural data has been rarely analyzed. Signals collected from humans “in the wild” are affected by a noisy environment and modulated by a complex range of behaviors. The goal of home Brain-Computer Interface (BCI) use and long-term neural monitoring will need to be robust to these factors. As well, with the great advancements in machine learning and computer vision in recent years, recordings of multiple modalities can provide information about a patient’s behavior without the need of strict experimental protocols. In this project, we collected thousands of hours of simultaneous video (including a depth channel), audio and neurophysiological data from patients undergoing clinical long term monitoring for more than 10 ECoG patients. Previously, we were able to show that the latest state-of-the art techniques based on deep learning can track human upper arm joint coordinates accurately from the video. Our current results show and compare the brain mapping and decoding as well as prediction of motor movement from the natural ECoG signals using various neural network models. We also begin to elicit some insight into neural functions using this novel data and technique. Our approaches overall show the value and

opportunity of natural data, especially when combined with fully automated video and audio analyses.

**Disclosures: X. Wang:** None. **A. Farhadi:** None. **J. Ojemann:** None. **R. Rao:** None. **B. Brunton:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** E.05. Brain-Machine Interface

**Support:** NSF EEC-1028725

Washington Research Foundation Fund for Innovation in Neuroengineering

**Title:** Human parahippocampal dynamics during visuomotor rotation tasks

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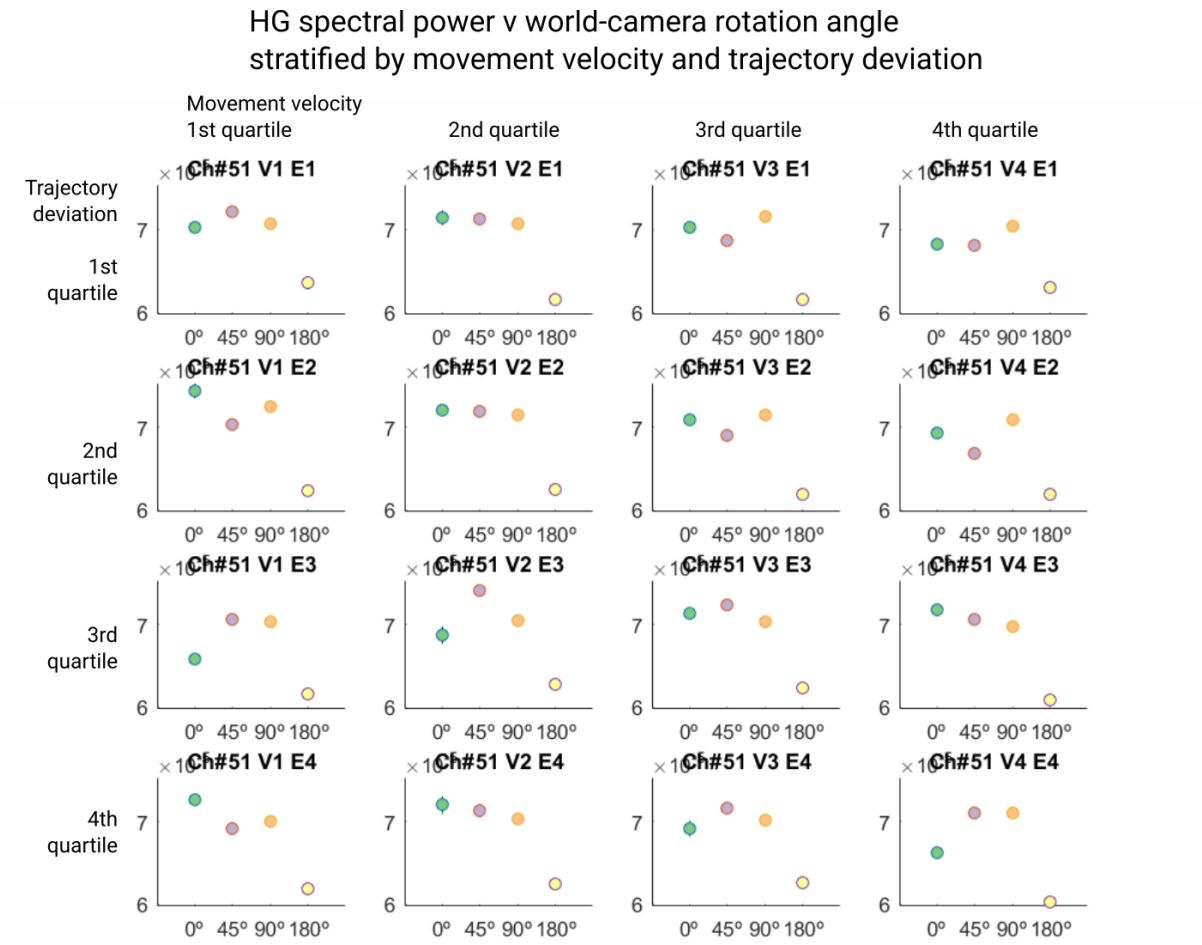
**Abstract:** Cortical decoding of dexterous upper-body movements, especially during dexterous manipulation, relies on the consistent understanding of the representation of spatial coordinates in visual and motor systems. However, the mechanisms and cortical regions that underlie our ability to rapidly adapt to visuomotor rotation is unclear. A generalized model of how visual processing of spatial cues combine with existing motor skills to effect future motor planning behavior is required for the development for future decoding of volitional control of assistive prosthetics.

We investigated spatial-time-frequency models of cortical signals during a touchpad-navigated virtual maze task, recorded in epileptic patients with implanted clinical stereotactic EEG (sEEG) electrodes and clinical electrocorticography (10mm spacing). Patients were instructed to repeatedly navigate an avatar through a virtual maze, following a mandated path through a series of markers. Maze navigation were performed while observing the same maze in world-centered reference frame, from one of 4 possible camera angles, panned at 0° (unrotated), 45°, 90°, and 180° around the maze.

We observed viewing-angle-specific tuning of γ-band spectral power in sEEG recordings of dorsal parahippocampal regions. The tuning depth of the spectral power are task-performance dependent to the degree of visuomotor adaptation, controlled for instantaneous finger movement velocity and movement direction. We further observe two behavioral correlates of the strength of

visuomotor adaptation as a characteristic tradeoff between task performance error and performance speed, and examine the relationships between viewing angle and the relative success rate of transferring adaptation between viewing angles. The temporal dynamics and connectivity between primary motor cortex and dorsal parahippocampal regions may offer a high degree of information about visuomotor adaptation learning.




**Disclosures:** **J. Wu:** None. **L. Levinson:** None. **K. Casimo:** None. **R.P. Rao:** None. **J.G. Ojemann:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.05/JJ16

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH R44NS083183

Robert Plonsey Fellowship from the Duke University Department of Biomedical Engineering

**Title:** Electrodeposited platinum-iridium coating (EPIC) improves in-vivo chronic recording performance of microwire electrode arrays (MEA)

**Authors: *I. R. CASSAR**[1], C. YU[1], A. PETROSSIANS[5], J. J. WHALEN[5], C. D. LEE[5], J. SHARKEY[6], W. M. GRILL[1,2,3,4]
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**Abstract:** Chronic recording of single unit neural activity is an essential tool of neuroscience and useful for neuroprosthetic applications, i.e., brain-machine interfaces. Although single unit recordings are highly informative, unit yield and signal quality degrades over time. One potential cause of this degradation is biofouling of the electrode tip, which can increase electrode impedance, contribute to loss of neurons, and ultimately reduce unit yield and signal quality. We quantified the performance of chronically implanted microwire electrode arrays (MEAs) coated with electrodeposited platinum-iridium coating (EPIC) and compared the performance to uncoated electrodes on the same arrays in the same animals. We implanted seven rats with 16 channel, 0.5 MOhm platinum-iridium MEAs in the substantia nigra pars reticulata. The individual electrodes within each MEA were randomized between a coated and uncoated group. Every week to two weeks we measured electrode impedance at 1 kHz and conducted five-minute unit recordings between 0 and 12 weeks after implant. Electrode impedance, unit yield, and signal to noise ratio (SNR, calculated as the peak amplitude of the unit over the RMS of the noise) were compared via repeated measures ANCOVA, with results given as mean ± SE. The initial impedance of the EPIC microwires (365 ± 88 kOhm) was significantly lower than the uncoated group (991 ± 77 kOhm, $p < 0.01$). The impedance of the uncoated microwires significantly increased over time (+45 kOhm/week, $p < 0.01$), as is typical for implanted arrays. However, the impedance of the EPIC microwires did not change significantly over the same period ($p = 0.73$). The EPIC microwires also had a higher average number of discernable units per electrode in each array (0.80 ± 0.06) than the uncoated microwires (0.39 ± 0.06, $p < 0.01$). In addition, the number of units per electrode increased over time in the EPIC group (+0.045

units/week, $p < 0.01$), but did not change in the uncoated group. There was no significant difference in the SNR between the coated and uncoated microelectrodes. Immunohistochemistry to visualize gliosis (ED1, GFAP) in select animals did not reveal any discernable difference between the response to coated vs uncoated electrodes. This suggests that the increases in recording performance are due to a reduction in biofouling and not prevention of gliosis. The EPIC coating appeared to maintain low electrode impedance over time, and resulted in more discernable single units. With this improved performance, chronically implanted microwires should provide higher quality units for longer durations, thus increasing the feasibility of future brain-machine interfaces.

**Disclosures: I.R. Cassar:** None. **C. Yu:** None. **A. Petrossians:** A. Employment/Salary (full or part-time):; Platinum Group Coatings LLC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Platinum Group Coatings LLC. **J.J. Whalen:** A. Employment/Salary (full or part-time):; Platinum Group Coatings LLC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Platinum Group Coatings LLC. **C.D. Lee:** A. Employment/Salary (full or part-time):; Platinum Group Coatings LLC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Platinum Group Coatings LLC. **J. Sharkey:** A. Employment/Salary (full or part-time):; Platinum Group Coatings LLC.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Platinum Group Coatings LLC.. **W.M. Grill:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.06/JJ17

**Topic:** E.05. Brain-Machine Interface

**Support:** NSF EEC-1028725

NINDS 5R01NS065186

NIH 5T90DA032436

Washington Research Foundation Fund for Innovation in Neuroengineering

Achievement Rewards for College Scientists, Seattle Chapter

**Title:** The “Oops” Detector: Spontaneous vs in-task error-related potentials in long-term humanelectrocorticography

**Authors: *N. R. WILSON**[1], X. WANG[2], R. C. SHEAN[3], J. G. OJEMANN[4], R. P. RAO[2], B. BRUNTON[5]
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**Abstract:** In interaction with our surroundings, we constantly receive and interpret feedback regarding our actions, allowing our brain to assess discrepancies between expected and actual outcomes. These error-related signals are vital to how we adjust our behavior. Reward and error have been the subjects of extensive study over the past decades in multiple modalities and timescales; however, these studies have primarily focused on in-task error in well controlled experiments.
Here, for the first time, we compare in-task errors that occur in an instructed context with spontaneous errors committed by the same subjects in a naturalistic, task-free setting using a long-term human electrocorticography (ECoG) dataset. For the in-task errors, the subjects performed a word and picture association task. Spontaneous errors were extracted from 4 days of recordings per patient using automatic speech recognition of error-related keywords (e.g., “oops,” “sorry,” and various expletives). These keywords indicated the patient had realized their mistakes; we sorted each instance based on if they had been uttered in an error or non-error (control) context.
In preliminary results (n=3 patients), we compared high-gamma (70-100Hz) power across spontaneous and in-task errors. We found increased activation in the ventrolateral prefrontal cortex in both conditions; in addition, we found activation in Brodmann Area 8 (BA8) only in the natural condition (Figure 1). As activation in BA8 is thought to be associated with uncertainty estimation, this increased high-gamma power implies the subjects may be more uncertain of mistakes made in the natural context. These results suggest there may be important differences between error-related potentials for mistakes made inside and outside of strictly controlled experiments; further, they motivate further investigation of error and reward in spontaneous behavior, an analysis enabled by automated algorithms for extracting features from long-term monitoring.

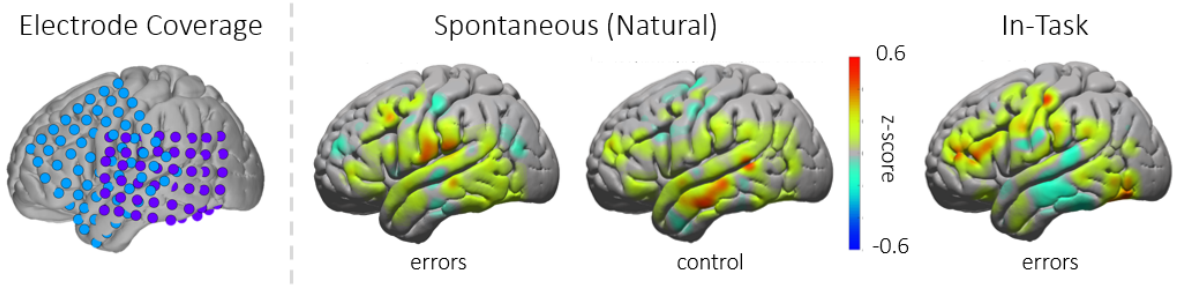


**Figure 1. Differences in high-gamma activation between errors committed in the naturalistic vs in-task contexts.** Mean high-gamma power (normalized to baseline) from two subjects were plotted on a standard MNI brain. A third subject was analyzed with electrode coverage on the right hemisphere (results not shown). Error-related potentials were computed for in-task errors, spontaneous errors, and also compared to a spontaneous control condition.

**Disclosures:** **N.R. Wilson:** None. **X. Wang:** None. **R.C. Shean:** None. **J.G. Ojemann:** None. **R.P. Rao:** None. **B. Brunton:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.07/JJ18

**Topic:** E.05. Brain-Machine Interface

**Support:** NRF Grant 2016M3C7A1904988

**Title:** Changes in network properties of a neuronal ensemble with acceleration and deceleration of point-to-point arm movements

**Authors: *M.-K. KIM**[1], J.-W. SOHN[2], S.-P. KIM[3]
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**Abstract:** A point-to-point arm movement generates a bell-shaped speed profile. It implies that the acceleration and deceleration at two end-point always sequentially occurs with almost equal distribution. Whether and how such coordination of acceleration and deceleration is directed by motor cortical neurons has been of great interest but it is still not fully understood. One of the reason is that speed information is poorly encoded in single neuronal level. However, there is a possibility that an ensemble of neurons, rather than individual ones, may represent speed modulation more clearly. Particularly, dynamics of networking among neurons may be related to the coordination of acceleration and deceleration in point-to-point arm movements. Hence, we aimed to study network properties of primary motor cortical (M1) neurons of non-human primate performing a two-dimensional center-out task. In the analysis, we first divided spiking activities into subsets for the acceleration and deceleration periods during each movement. Then, we fitted a linear regression model to individual firing rates against the hand speed for every subset and utilized the fitted model parameters to build correlation matrices between neurons. In doing so, the correlation matrices were attained for acceleration and deceleration periods, respectively. From the correlation matrices, an adjacency matrix among M1 neurons was constructed by selecting significantly correlated pairs. The network analysis based on the graph theory was conducted to evaluate a number of network metrics, including a small-world-ness index (SWI). The analysis results revealed that SWI was higher for acceleration than deceleration during arm-reaching movements. On the contrary, SWI was higher for deceleration than acceleration during arm-pulling movements. It indicates that M1 neuronal network became more efficient when accelerating arm movement from the body toward a distal location while it became more efficient when decelerating arm movement from a distal location toward the body. Our results suggest that inter-neuronal networking dynamics in M1 may be related to the coordination of

acceleration and deceleration of point-to-point arm movements, which can depend on movement directions pivoting on the body.

**Disclosures:** **M. Kim:** None. **J. Sohn:** None. **S. Kim:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.08/JJ19

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA REPAIR N66001-10-C-2008

NIH 1DP2EB022357-01

**Title:** Chronic intracortical recordings from microelectrode arrays implanted in common marmosets (Callithrix jacchus)

**Authors: *S. DEBNATH**[1], N. W. PRINS[2], R. MYLAVARAPU[1], E. A. POHLMEYER[4], S. GENG[1], J. C. SANCHEZ[5], A. PRASAD[3]
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**Abstract: Background:** Current neuroprosthetics rely on stable, high quality recordings from chronically implanted microelectrode arrays in neural tissue. Microelectrodes implanted in brain tissue can be affected by multiple abiotic and biotic factors that lead to degradation in recording quality, ultimately resulting in electrode failure. While chronic electrophysiological recordings and electrode failure modes have been reported from larger non-human primates, chronic recordings in primary motor cortex from the marmoset model have not been previously described. This study tracks the recording stability and signal quality of microelectrode arrays chronically implanted in behaving marmosets.
**Methods:** Six adult male marmosets were trained to complete reaches for two or four targets and cortical activity was recorded while the animal performed the reaching tasks. After the animals were trained, one 16-channels microelectrode array (MEA) was implanted in the hand and arm region of the primary motor cortex (M1) and another MEA in deep brain structures (targeting the nucleus accumbens). Spike sorting was used to isolate single unit activity from background noise. Signal stability and quality was quantified as a function of single-to-noise ratio, array yield (defined as the fraction of active electrodes that recorded neuronal activity), and neuronal yield (defined as the number of isolated units during a recording session).
**Results:** Neuronal yield and array yield were calculated weekly for the implant duration for each

animal. Out of 11 implanted electrode arrays, 9 arrays provided functional recordings for at least 3 months, with 2 arrays functional for 10 months. In general, all implants had high array and neuronal yield after implant, which remained stable for up to several months. Exceptions resulted from mechanical failure of the MEA itself. In the longest implants, signal degradation occurred, characterized by gradual decline in signal-to-noise ratio, reduced number of isolated single units, and changes in the waveform shape of action potentials.
**Conclusions:** This study shows the feasibility of long-term recordings from marmosets using microelectrode arrays implanted in cortical and deep brain structures. Good quality signals, used to control a brain-machine interface, were recorded up to several months in this animal model. Electrode failure modes could be identified, with mechanical failures related to the MEA occurring most often. The ability to chronically record cortical signals for neural prosthetic applications in the common marmoset suggests the potential of this model in neural interface research.

**Disclosures: S. Debnath:** None. **N.W. Prins:** None. **R. Mylavarapu:** None. **E.A. Pohlmeyer:** None. **S. Geng:** None. **J.C. Sanchez:** None. **A. Prasad:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.09/JJ20

**Topic:** E.05. Brain-Machine Interface

**Support:** Wallace H Coulter Center for Translational Research Neural Engineering SEED Grant

**Title:** Blood-brain barrier (BBB) disruption following implantation of intracortical silicon microelectrodes

**Authors: *C. BENNETT**[1], M. SAMMIKKANNU[1], F. MOHAMMED[2], D. W. DIETRICH[3], S. RAJGURU[1], A. PRASAD[1]
[1]Biomed. Engin., [2]Biol., Univ. of Miami, Miami, FL; [3]The Miami Project to Cure Paralysis, Miami, FL

**Abstract:** Chronically implanted microelectrodes in the neural tissue elicit inflammatory responses that are time varying and have been shown to depend on multiple factors. Among these factors, BBB-disruption has been hypothesized as one of the most dominant biotic factor resulting in electrode failure. Studies have shown the chronic sequelae of electrode-implant related cortical injury in slow-inserted microelectrode arrays (MEA). Typically, microwire arrays and silicon probes are inserted slowly into the neural tissue whereas the silicon Utah MEAs (UMEA) are inserted at a high speed using a pneumatic inserter. Here, we report the sequelae of

electrode-implant induced injury at various acute time-points in fast-inserted UMEA in the brain tissue. Adult, male Sprague Dawley rats (n=50) were used in the study. Animals were divided into implant and stab control groups. All stab animals underwent similar surgical and implantation procedure, however, the UMEA was taken out of the tissue slowly after implantation for stab control animals. We used quantitative polymerase chain reaction (qPCR) to quantify the expression of genes directly involved in the inflammatory and BBB-disruption. For qPCR, we evaluated animals (n=5 for each time-point) implanted for 6-hr, 24-hr, 48-hr, and 72-hr time points, respectively with matched stab controls (n=5) for each time points. We used n=10 rats as naïve controls with no implant or surgery to obtain a baseline of the expression in healthy unimplanted animals. Gene expression was quantified using the $RT^2$ PCR primers for rat inflammatory cytokines for key genes mediating the inflammatory response and tight junction (TJ) and adherens junction (AJ) proteins that form the BBB and are critical to the functioning of the BBB. Our results indicate significant inflammation as suggested by >10-fold expression relative to naïve controls for most pro-inflammatory genes in both groups across all time points. Moreover, there was no significant difference between the stab and implant groups at these time-points suggesting the effect of insertion on both groups. Expression levels for the genes that form the TJ and AJ of the BBB were downregulated suggestive of extensive vascular disruption and BBB-damage. The results of this study provide an insight into the physiological events following BBB-disruption that can lead to neuroinflammation and neuronal degeneration. The gained mechanistic insights into BBB-disruption following fast insertion of UMEAs may provide new opportunities for targeted therapeutics to prevent or reduce neurodegeneration normally observed following UMEA insertion and improve electrode performance.

**Disclosures: C. Bennett:** None. **M. Sammikkannu:** A. Employment/Salary (full or part-time):; University of Miami. **F. Mohammed:** None. **D.W. Dietrich:** A. Employment/Salary (full or part-time):; University of Miami. **S. Rajguru:** A. Employment/Salary (full or part-time):; University of Miami. **A. Prasad:** A. Employment/Salary (full or part-time):; University of Miami.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.10/JJ21

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA

HHMI

NIH

The Bio-X Program, Stanford University

Simons Foundation

**Title:** The effect of task dimensionality on BMI performance

**Authors: *N. EVEN-CHEN**[1], S. VYAS[2], S. RYU[3], K. V. SHENOY[4,5,6]
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**Abstract:** In recent years, non-human primate brain-machine interface (BMI) studies have demonstrated 2D cursor control (e.g., using the ReFIT-KF algorithm) that is approaching native arm control. This approach has been successfully translated into human clinical trials. There have also been promising demonstrations of high degree-of-freedom (DOF) robotic arm control using different algorithms. However, the performance of these studies is not yet close to native arm control. Most high DOF studies focus on functional tasks (e.g., object manipulation) and assess performance with clinical metrics (e.g., ARAT). These metrics are quite important but nevertheless are an indirect measure of underlying control quality. This makes it challenging to directly gleen the reasons for performance limitations. To better understand the sources of performance limits, we compared 2D and 3D BMI control using alternative task designs and metrics.

We designed a virtual reality environment for 3D BMI control that dissociates the effects of 3D control from the confounding effects of studying 3D control with a robotic arm (e.g, dynamics, latency). We trained a rhesus macaque to perform 2D and 3D center-out-and-back tasks using an intracortical BMI system (two 96-channel Utah arrays in M1 and PMd) and the ReFIT-KF control algorithm. To develop an appropriate task to evaluate the BMI control quality (e.g., speed and stability), we manipulated target radius (1, 2, 3 cm) and required hold time (0.1 - 1 sec). We found that a 2 cm sphere with a 500 ms hold time is a good regime to assess the control quality in 3D, and compare it to both a 2D task, and native hand control.

When comparing hand control to BMI control, the average trial length increased by 50% during a 3D task, while only by 20% during a 2D task ($p<0.001$, t-test). To understand this performance difference, which can be a result of slow and/or unstable cursor control, we computed time-target-first-acquired (tFA, as a speed metric) and time required to hold the target once acquired (tDwell, as a stability metric). When comparing 2D vs. 3D native hand control, we found that tFA increased by 10% while tDwell did not change ($p<0.001$, t-test). Surprisingly, when comparing 2D vs. 3D during BMI control, we found that tFA increased by 14% while tDwell increased by 25% ($p<0.001$, t-test). These results suggest that when increasing BMI control dimensionality, both speed and stability differ from native arm control with the latter differing more. This might be explained by the number of task-related dimensions in each phase of the reach. Such underlying control analysis can guide, and likely improve, future BMI designs.

**Disclosures:** **N. Even-Chen:** None. **S. Vyas:** None. **S. Ryu:** None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neuralink Inc., consultant Cognescent, Scientific Advisory Board Heal, Scientific Advisory Board.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.11/JJ22

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA RE-NET IAA

FDA Medical Countermeasures

FDA Office of Chief Scientist

FDA Critical Path Initiative

**Title:** Long-term performance of intracranial electrodes and their effects on brain tissue

**Authors: *Y.-R. GAO**, M. YE, C. M. ALTIMUS, H. RAFI, D. X. HAMMER
US Food and Drug Admin., Silver Spring, MD

**Abstract:** Microelectrode arrays that directly interface with neurons in the nervous system provide high fidelity control signals for advanced neuroprosthetic applications. Those devices have the potential to restore movement capabilities and provide sensory feedback to patients with paralysis or amputation. Although implanted electrode arrays perform well short after implantation, the devices often fail when implanted for durations exceeding one year; and their long-term viability and reliability are unpredictable. We are investigating the dynamics of brain morphological changes with a multimodal approach to correlate the recording signal degradation and brain tissue response. We performed weekly optical coherence tomography (OCT)-guided two-photon microscopy (TPM) through a cranial window. A Thy1-YFP transgenic mouse model was used to image cerebral blood vessels and neurons after implantation. Single-shank, 16-channel, Michigan-style microelectrodes were inserted under the window at a 15-20° angle with an insertion depth up to cortical layer 5. Electrophysiological recordings were conducted for 15 minutes while the animals moved freely in their home cages. Cellular and vascular morphology were monitored using TPM and OCT at time-points matched to the recordings. We found that layer 2/3 dendrite length gradually decreased over time following implantation while layer 1 dendritic tufts density remained relatively stable. There was vascular remapping of the superficial layers of the cortex with an increase in vascular total length, branch number and tortuosity. The deeper capillary network remained relatively stable. We observed a decay of

neural firing rates in most of the channels within two weeks after implantation, while the gamma band power of the LFP signals gradually increased in the first month after implantation and then stabilized. These findings indicate that LFP may have better long-term performance for neural decoding for prosthetic device control. Future studies of the microglia and astrocyte changes after implantation will be conducted to further pinpoint the causes of recording degradation. Our multimodal approach combining electrophysiology and optical imaging provides a broader picture of brain tissue chronic changes to implanted electrodes. It may also provide insight into the long-term performance of implanted electrodes and identifying potential biomarkers for device failure.

**Disclosures:** **Y. Gao:** None. **M. Ye:** None. **C.M. Altimus:** None. **H. Rafi:** None. **D.X. Hammer:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.12/JJ23

**Topic:** E.05. Brain-Machine Interface

**Support:** HHMI

NIH

DARPA

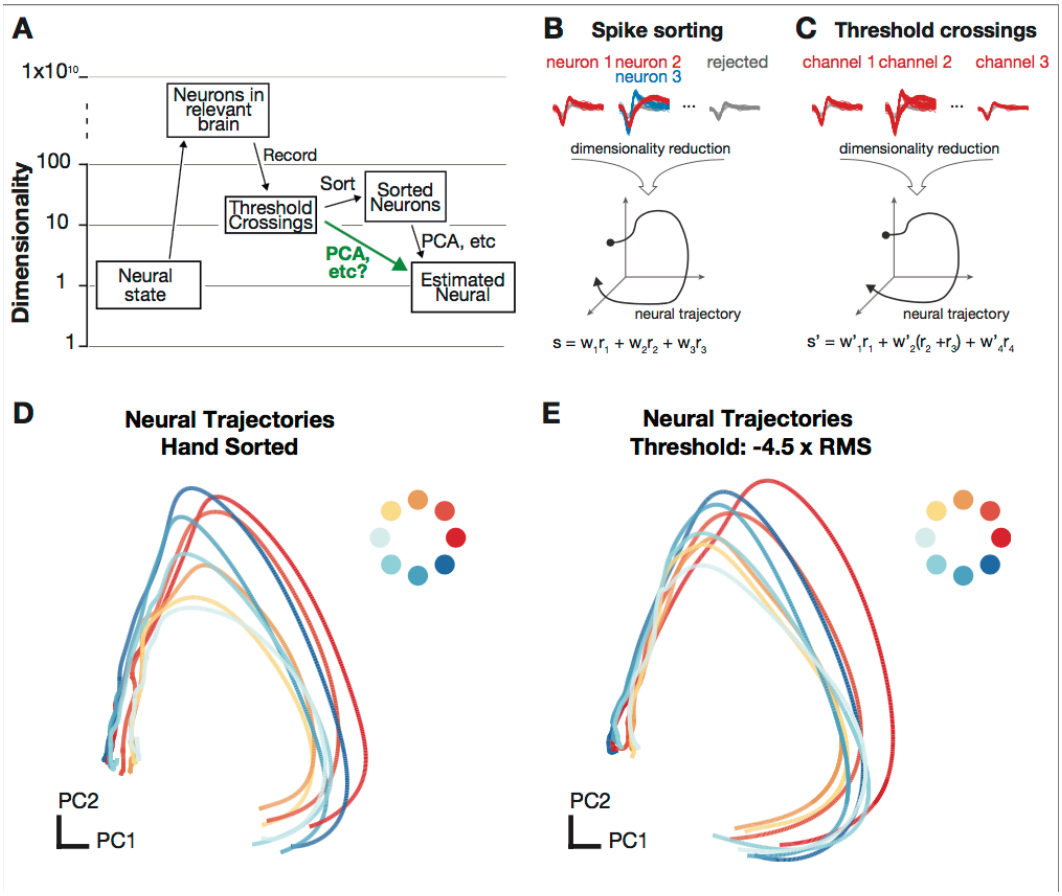
Simons Foundation

**Title:** Accurate recovery of neural population dynamics without spike sorting

**Authors:** ***E. TRAUTMANN**[1], S. D. STAVISKY[2], K. C. AMES[4], M. T. KAUFMAN[5], S. RYU[6], S. LAHIRI[3], S. GANGULI[7], K. V. SHENOY[8]
[1]Stanford Neurosciences, San Francisco, CA; [2]Neurosurg., [3]Stanford Univ., Stanford, CA; [4]Neurosci., Columbia Univ., New York, NY; [5]Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY; [6]Dept Neurosurg, Palo Alto Med. Fndn., Palo Alto, CA; [7]Stanford, Stanford, CA; [8]EE, BioE & Neurobio., Howard Hughes Med. Inst. - Stanford Univers, Stanford, CA

**Abstract:** A central goal of systems neuroscience is to relate an organism's neural activity to behavior. Neural population data analysis often begins by reducing the dimensionality of the raw neural data in order to focus on the neural population patterns that are most relevant to a given task. A major practical hurdle to data analysis is contending with the time consuming and inexact identification and separation of action potentials from individual neurons, and this problem is

growing rapidly as the number of neurons measured increases inexorably. Here we experimentally and theoretically investigate if `spike sorting' is necessary to accurately estimate neural population states and dynamics. Dimensionality reduction linearly combines neurons, suggesting that separating them prior to analysis may be unnecessary, and compressed sensing theory lends support to this hypothesis. We re-analyzed data from three previously published studies and found that it is straightforward to accurately estimate neural population states without spike sorting. Neural population dynamics and subsequent scientific conclusions are quite similar when multi-unit threshold crossings are used in place of isolated single neurons. This finding unlocks large repositories of existing data for new analyses without time-consuming manual sorting or error-prone automatic sorting, it enables scientific measurements using chronically-implanted electrode arrays that, due to their necessarily lower impedences, do not afford high quality spike sorting, and it informs the future design and use of electrode arrays for laboratory and clinical investigations.




**Disclosures:** **E. Trautmann:** None. **S.D. Stavisky:** None. **K.C. Ames:** None. **M.T. Kaufman:** None. **S. Ryu:** None. **S. Lahiri:** None. **S. Ganguli:** None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neuralink Inc., Cognescent, Scientific Advisory Board, Heal, Scientific Advisory Board.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.13/JJ24

**Topic:** E.05. Brain-Machine Interface

**Support:** UCSD ECE Medical Devices and Systems initiative

IBM Ph.D. Fellowship

**Title:** Methods for analyzing electrocorticographic signals during motor execution and imagery

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**Abstract:** Electrocorticography (ECoG) based brain-computer interfaces (BCIs) decode user intent from neural recordings taken from the cortical surface. Previous studies (Leuthardt, Eric C., et al.,2006) have shown that volitional cortical activities during kinesthetic imagery, i.e. imagining motor movement, can be used as control signals for BCIs. There is a significant difference in cortical activities during actual movement and motor imagination (Lotze, Martin, et al.,1999). In this work, we analyze ECoG data collected from 5 subjects who performed movement and imagery tasks (Miller, Kai J., et al.,2010). Our analysis is two-fold. Firstly, we present a deep learning approach to robustly decode tongue and hand movements. Secondly, we use unsupervised methods to find neurophysiological markers for imagery and actual movement. Most ECoG based BCIs use spectral powers in specific frequency bands in fixed time windows as features (Shenoy, Pradeep, et al., 2008). However, precise ranges of informative spectral bands vary across subjects and throughout the duration of task (Wander, Jeremiah D., et al.,2013). Decoding ECOG signals with good accuracy would require features that are more robust to timing and magnitude of changes in cortical activity. We use autoencoders to extract time-frequency(t-f) patterns that summarize cortical activity across brain areas and subjects. We show that this automated feature extraction method coupled with a deep neural network outperforms classifiers using spectral powers in specific frequency bands (Eg: 8-32 Hz and 76-100 Hz) as features by more than 15% across all subjects. Our approach eliminates the need for handpicking spectral bands and electrode channels with discriminable features and is more robust to changes in cortical activity that are hard to model.
We cluster the electrode channels based on activity during actual movement and imagery using low dimensional representations from autoencoders. The clustering of channels across cortical

areas is different for the two modalities suggesting different dominant activity patterns during imagery and movement.

**Disclosures:** **T. Pailla:** None. **K.J. Miller:** None. **V. Gilja:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.14/JJ25

**Topic:** E.05. Brain-Machine Interface

**Support:** NHMRC Grant 1062532

NHMRC Grant 1075117

Defence Health Foundation

**Title:** Chronic evaluation of the measured neural signal quality of a stent-based neural interface

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**Abstract:** Minimization of electrode insertion trauma, enhanced biocompatibility, and chronic, high-resolution functionality are critical for the success and clinical translation of emerging brain-computer interface systems. Penetrating microelectrodes and electrocorticography (ECoG) grids give access to high resolution neural signals. However, insertion of these devices requires craniotomy and direct contact with brain tissue, and a fibrotic response may degrade signal quality. We have developed a stent-based endovascular interface that is implanted within the brain via the vascular system through the jugular vein. Evaluation of the maximum neural information accessible and the long-term behavior of this device will strengthen the potential clinical applicability of the technology. Here, we aim to quantify the chronic signal quality recorded from an endovascular neural interface using visual evoked potentials and compare this with signals from a subdural grid implanted adjacent to the same cortical areas. Full-field visual flashes of light were delivered binocularly while neural signals were recorded with endovascular electrodes adjacent to the motor and visual cortices in awake sheep. The signal-to-noise ratio (SNR) was calculated on the filtered (2-400Hz) trial-averaged signal for each electrode, as the ratio of the root-mean-square values of the signal amplitude in two 130ms time-windows. One

window was centered at the peak of the response and the other in the baseline period (85ms pre-stimulus). The variability of the SNR was measured as the standard deviation across electrodes and animals. Electrochemical impedance spectroscopy was also measured to test the electrochemical properties of the stent in vitro and monitor electrode viability in vivo. Visual evoked responses were recorded, peaking at 60.94 ± 12.38 ms (mean ± std) after the visual stimulation. SNR values significantly greater than 1 were recorded from day 1 (8.516 ± 1.164 dB, p < 0.05) using the endovascular interface. The mean SNR was stable over recordings up to 57 days after implantation on both devices (linear fit, stentrode: slope = 0.0006 dB/day, CI = [-0.009:0.02] dB/day; subdural: slope = -0.02 dB/day, CI = [-0.004:-0.009] dB/day). The variability of the SNR decreased with time (days 1-30: 1.1077 ± 0.86 dB; days 30-57: 0.13 ± 0.136 dB, Wilcoxon Rank Sum test, p < 0.05). We have demonstrated the capability of electrodes placed within a cortical vessel to record visual evoked responses immediately after implantation. We also showed stability of the SNR over time, with a significant decrease in SNR variability beyond a month after implantation.

**Disclosures:** **G. Gerboni:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **S.E. John:** A. Employment/Salary (full or part-time):; Synchron Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **N.L. Opie:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Synchron Inc. **G.S. Rind:** A. Employment/Salary (full or part-time):; Synchron Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **S.M. Ronayne:** A. Employment/Salary (full or part-time):; Synchron Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **C.N. May:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **T.J. Oxley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Synchron Inc. **Y.T. Wong:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc. **D.B. Grayden:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Synchron Inc.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.15/JJ26

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA HAPTIX (Program Manager: Dr. Douglas J Weber)

**Title:** Long-term safety and performance of microelectrode arrays in rodent peripheral nerves

**Authors: *S. VASUDEVAN**[1], B. SHAFER[1,2], R. SHARMA[3], R. B. CALDWELL[4], L. RIETH[3,4,5,6], C. G. WELLE[7,1]
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**Abstract:** Peripheral Nerve Interfaces (PNIs) are medical devices that bridge between peripheral nerves and external hardware such as neuro-prosthetic devices. PNIs sense nerve impulses and/or evoke neural signals through electrical stimulation. There are PNIs currently under investigation for intuitively controlling neuro-prosthetic devices in amputees, and to restore motor and sensory function. To reap the maximum benefit, these devices should be safe and effective over long periods of time, ideally the lifetime of the patient. The current study aims to assess the chronic safety and performance of off-the-shelf Utah arrays and low-impedance Utah Arrays, all with a 4x4 set of electrodes that are 1.0 mm in length.
Off-the-shelf Utah arrays (UA) were purchased from Blackrock Microsystems and low impedance modified Utah arrays (mUA) were provided by Dr. Loren Rieth at the University of Utah. Arrays were implanted into the rat sciatic nerve along with custom EMG wire based arrays implanted into the gastrocnemius and tibialis anterior. Connectors from the arrays were secured to the lumbar fascia using 3D printed connector mounts. To record evoked potentials, two cranial screws were implanted over the somatosensory cortex. Weekly impedance measurements, recordings of spontaneous peripheral neural activity, and EMG evoked by electrical stimulation were recorded for 12 weeks. Nerve function tests were obtained bi-weekly to monitor motor and sensory function for up to 12 weeks. At 13 weeks, the animals were sacrificed to harvest the implanted nerve electrode for analysis under scanning electron microscopy (SEM). Tissues were harvested for histological assessment around the electrode shanks.
Spontaneous electrophysiological analysis indicated no difference in recording performance between UA and mUA arrays, while stimulation evoked responses indicated session-to-session variability. Walking track analysis and von frey tests indicated recovery of function around 2 weeks post-implantation. The surface morphology of the electrodes was characterized by SEM before implantation and after explantation, and showed fracture of recording tips in some cases. Further assessment is under investigation to establish the chronic safety and efficacy of these electrode arrays.
**DISCLAIMER:** The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.
Loren Rieth was a partial employee of Blackrock Microsystems during a portion of this study, and has moved to the Feinstein Institute for Medical Research.

**Disclosures:** **S. Vasudevan:** None. **B. Shafer:** None. **R. Sharma:** None. **R.B. Caldwell:** None. **L. Rieth:** A. Employment/Salary (full or part-time):; Partial employee of Blackrock Microsystems during a portion of this study. **C.G. Welle:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.16/JJ27

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA HR0011-15-2-0017

**Title:** Utah electrode arrays for cortical neural recording in rat

**Authors: R. RIHANI**, C. FREWIN, A. KANNEGANTI, B. J. BLACK, B. CHAKRABORTY, R. AYUB, *J. J. PANCRAZIO, S. F. COGAN
Univ. of Texas At Dallas, Richardson, TX

**Abstract:** Utah electrode arrays (UEAs) are used chronically for high signal-to-noise ratio (SNR) recordings. However, there have been few studies involving UEAs implanted in rat cortex and have only shown a limited capabilities in chronic recordings [12]. Therefore, we have investigated the stability of UEAs for long-term single unit cortical recordings *in vivo* in a rat. 4x4 UEAs with sputtered iridium oxide (SIROF) electrodes were implanted into the barrel cortex of a Long-Evans rat using a pneumatic insertion tool. The electrode array was connected by a flexible cable to a connector mounted on the rat's head. Weekly 10-minute recordings were collected 2 weeks post-implantation. Animals were lightly anesthetized via 2.5% Isoflurane to minimize movement artifacts. Raw signals were band-pass filtered (250-7000 Hz). Using a threshold set at 4σ of the calculated root mean square (RMS) noise, spikes were identified and manually sorted offline. An active electrode was defined as a site with at least one well-defined unit. SNR was calculated from, $SNR = (RMSs/RMSn)^2$
Where RMSs and RMSn are the RMS amplitudes of the signal and noise, respectively. Fluctuations in the number of active electrodes were apparent on throughout 111 days. Out of 16 electrodes, the maximum number of active electrodes (12) was obtained on week 2 (Fig. 1A). SNR increased after day 38, and reached maximum values of 16-18 between days 74 and 103. SNR values decreased after day 106 and remained low until the end of the study (Fig. 1B).
Our initial *in vivo* characterization of UEAs suggests viability of chronic cortical recordings in rats. The reduced number of active electrodes at the end of the study and their lower SNR values, suggests degradation via either biotic or abiotic mechanisms. More experiments are underway to examine the stability of single unit recordings and relationship to electrode failure mechanisms.
**References**

1. Ward M P, Rajdev P, Ellison C, Irazoqui P P. *Brain Res* **1282:** 183-200 (2009).
2. Nolta N F, Christensen M B, Crane P D, Skousen J L, Tresco P A. *Biomaterials* **53:** 753-762 (2015).

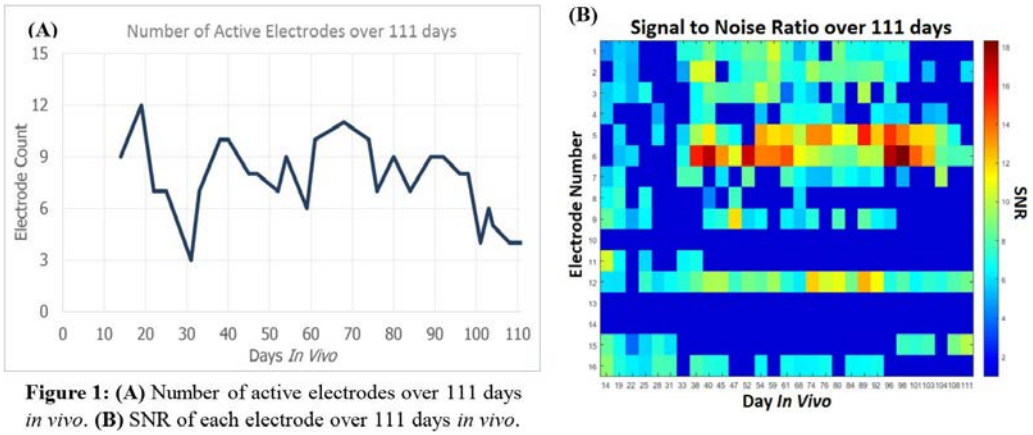


**Figure 1: (A)** Number of active electrodes over 111 days *in vivo*. **(B)** SNR of each electrode over 111 days *in vivo*.

**Disclosures: R. Rihani:** None. **C. Frewin:** None. **A. Kanneganti:** None. **B.J. Black:** None. **B. Chakraborty:** None. **R. Ayub:** None. **J.J. Pancrazio:** None. **S.F. Cogan:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.17/JJ28

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH R01 NS089679

Galvani SRA to TMO

**Title:** The Nanoclip: A microscale interface for recording and manipulating activity in small nerves

**Authors: *T. M. OTCHY**, C. MICHAS, T. J. GARDNER
Boston Univ., Boston, MA

**Abstract:** There is growing evidence of the therapeutic benefit of targeted modulation of the peripheral nervous system (PNS). Positive clinical trials are reported for vagal nerve stimulation to treat inflammatory diseases, depression, and epilepsy, and beyond these initial results, it is thought that a broad range of chronic diseases may be treatable through precise modulation of the autonomic PNS.
However, many large peripheral nerves, like the vagus, contain fascicles that innervate a variety

of organs, and hence bulk stimulation is often accompanied by undesirable off-target effects due to incidental stimulation of axons unrelated to the targeted organ. Fortunately, the organization of the PNS, whereby functional branches diverge and become more specific and homogenous as they near their target organ, can be exploited to avoid these off-target effects. These terminal nerve branches can be very small: the carotid sinus nerve, a possible treatment target for hypertension, is 400μm in diameter in humans and only 50μm in pre-clinical disease models. Thus, to advance basic research in bioelectronic medicine, highly miniaturized nerve interfaces will be necessary; however, the scale, fabrication method, and implant process of many currently available devices limit their consistency, reliability, manufacturability, and in vivo performance. Here, we present a nanoclip nerve interface for recording and stimulation that overcomes these limitations. Using two-photon direct laser writing to structure an acrylic photopolymer, we can tailor with micron resolution the geometry of the device to match the dimensions of the target nerve. The integration of thin film polyimide electrodes allows for precise and robust manufacturing of the electrical interface. In addition, fine design details were tuned to develop a device that is compact, easy to handle and implant, and capable of securely latching onto the nerve without disrupting nerve function.

We tested the polyimide-integrated nanoclip interface in acute and chronic preparations for efficacy in both recording and stimulation of functionally relevant nerve activity. We made single and multiunit recordings from whole nerves and found activity to be stable at chronic timescales. In addition, we stimulated nerves, using both bulk and current-steering techniques, to assess the precision, specificity, and repeatability of effects. The results of these tests indicate that multicontact polyimide-integrated nanoclips can be used as a robust, reliable, and highly manufacturable interface for peripheral nerves. Future research aims at adapting the current design for a variety of PNS targets.

**Disclosures:** **T.M. Otchy:** 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.; Galvani Bioelectronics. **C. Michas:** 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.; Galvani Bioelectronics. **T.J. Gardner:** 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.; Galvani Bioelectronics.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.18/JJ29

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA Grant N66001-11-1-4010

**Title:** 3D visualization of cellular biomarkers surrounding MicroProbes/NeuroNexus/Blackrock "hybrid" arrays implanted chronically in cats

**Authors: *N. NOLTA**, M. HAN
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**Abstract:** Intracortical microelectrode arrays allow for direct communication between the brain and external devices such as sensory or motor prostheses. It is widely believed that healthy, functioning neurons within the recording radius of microelectrode recording sites are critical for successful long-term performance. Neuronal density and other biomarkers have traditionally been assessed using 2D serial histological sections. However, the recording radius, the layered structure of cerebral cortex, and the foreign body response are all part of a 3D system that is difficult to accurately visualize and quantify with 2D methods. The problem becomes even more severe for large, complex, clinically-relevant devices that create anisotropic cortical tissue loss, compression, and fibrosis, and that often become tilted in brain tissue. In this work, we used 2D serial horizontal sections to build 3D reconstructions of the tissue surrounding “hybrid” microelectrode arrays implanted in cat sensorimotor cortex up to two years. The hybrid arrays consist of MicroProbes, NeuroNexus, and Blackrock microelectrodes assembled together into one monolithic, functional device. A variety of image processing techniques were employed to improve the quality, consistency, and automation of the 3D reconstruction process compared to our previous efforts. Finally, we show how this technique provides unique insights into the mechanisms of recording success/failure that 2D histology might otherwise overlook.

**Disclosures:** **N. Nolta:** None. **M. Han:** None.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.19/JJ30

**Topic:** E.05. Brain-Machine Interface

**Support:** HHMI

NIH

DARPA

Simons Foundation

**Title:** Direction and distance decoding accuracy from plan activity in monkey motor cortex

**Authors: *B. B. SHEFFER**[1], N. EVEN-CHEN[6], S. VYAS[2], S. RYU[7], K. V. SHENOY[8,3,4,2,5]
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**Abstract:** Brain machine interfaces (BMIs) can assist people with paralysis and improve their independence (e.g., by restoring communication or motor function). Discrete decoders, which classify the most likely movement endpoint from a discrete set of possible endpoints, show promising results for communication (e.g., Shenoy et al. 2003; Musallam et al. 2004; Santhanam et al. 2006). However, the spatial-accuracy limits of this approach have not yet been explored. To investigate these limits we quantified the accuracy of direction and distance classification using data from a high-density target reaching task. We trained a rhesus macaque to reach to a visual target after an instructed delay period (400 - 800 ms) in a center-out-and-back task. Using delay activity recorded from two 96-electrode arrays in primary (M1) and pre-motor (PMd) cortex (-4.5 RMS threshold crossings used), we classified the direction and the distance of the reach with SVMs. To quantify the accuracy of predicting direction, we used 24 uniformly-distributed targets arranged radially around an 8 cm circle. For distance, we used 12 targets spaced 1 cm apart in a line extending from the center of the screen going rightward (or leftward). For the radial task, we found that 95% of trials were classified within 38.9 degrees of their true target location. For the line configuration, 95% of trials were classified within 3.9 cm of their true target location. To see whether the target distance influences direction decoding (and vice-versa), we used 48 targets arranged in 3 concentric circles with radii 4, 8, and 12 cm, where each

ring had 16 evenly spaced targets. While distance decoding was consistent across directions, classifying the direction of reaches to each separate ring yielded accuracies of 28.1%, 45.5%, and 51.7% respectively (6.25% chance level when classifying the most likely target), increasing significantly with distance ($p < 0.01$). In designing communication BMIs an important measure of throughput is the information transformation rate (ITR) which is a function of success rate, number of targets and the neural activity integration time. To maximize ITR we calculated offline the effect of varying the number of targets in each configuration. In the ring configuration, the maximum ITR (4.63 bps) was achieved with a 6 target configuration (60 degrees between targets). For the line, the maximum ITR (1.1 bps) was achieved with 6 cm target spacing (2 targets in a given direction). Higher ITR may be achieved with target configurations that combine direction and distance or using larger radii. Such results can provide benchmarks that inform viable task environments for preparatory-activity based clinical BMIs.

**Disclosures: B.B. Sheffer:** None. **N. Even-Chen:** None. **S. Vyas:** None. **S. Ryu:** None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neuralink Inc., consultant, Cognescent, Scientific Advisory Board, Heal, Scientific Advisory Board.

**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.20/KK1

**Topic:** E.05. Brain-Machine Interface

**Support:** Paralyzed Veterans of America Research Foundation #3039

UPMC Rehab Institute

**Title:** Motor and pre-motor cortical activity during attempted, observed, passive and overt movements

**Authors: *S. V. HIREMATH**[1,2], W. WANG[6,2], R. M. RICHARDSON[3], A. ALHOURANI[3], W. LIPSKI[3], E. C. TYLER-KABARA[3,2,4,5], M. L. BONINGER[2,4,5,7]
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**Abstract:** Introduction and objective: Brain Computer Interface (BCI) technology has the potential to benefit individuals with severe functional impairments. Previously, researchers have proposed the use of action observation paradigms for neural decoder training - involving

watching videos of hand and arm movement or a neuroprosthetic arm movement and attempting the same movement. However, the time required by an individual with paralysis to learn and use the action observation paradigm may vary from a few days to several weeks. In this study, we evaluated the motor and pre-motor cortical activity for various neural decoder training paradigms including attempted, observed, passive, and overt movements in an individual with intractable epilepsy.
Methods: Testing was performed in an individual with intractable epilepsy. A high-density electrocorticography (ECoG) array was placed on the pre-motor and motor cortices of the participant. The participant performed four video-cued hand/arm movements: attempting, observing, passive movement by an experimenter while the participant was blindfolded, and overt movements. The videos included 13 body movements of shoulder, elbow, wrist, and fingers. Neuronal data included high-gamma band features. The decoding analysis used Naïve Bayes algorithm to predict the movements.
Results and Discussion: Attempted, visual, somatosensory, and overt movements induced motor and pre-motor cortical activity. Ten-fold classification analysis indicated that 10 channels within each paradigm of attempted, observed, passive, and overt movements resulted in an accuracy of 95.8% (chance level is 7.7%), 94.2%, 89.9%, and 87.7%, respectively. The top ten ECoG electrodes, from the high-density array, that contributed to the highest classification accuracy within each paradigm varied for the four paradigms. Developing a neural decoder for data transformed from one paradigm to another (e.g. passive to attempted movements) resulted in a reasonably high accuracy (82.0%). Based on the results, we postulate that various types of paradigms including visual and somatosensory can potentially be used for neural decoder training.
Support: Paralyzed Veterans of America Research Foundation #3039, UPMC Rehab Institute

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**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.21/KK2

**Topic:** E.05. Brain-Machine Interface

**Title:** Asynchronous detection and classification of spoken phonemes using mouth motor neural correlates in high-density ECoG

**Authors: *Z. V. FREUDENBURG**[1], J. BEREZUTSKAYA[2], M. J. VANSTEENSEL[3], E. J. AARNOUTSE[3], N. F. RAMSEY[3]

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**Abstract:** Recently, we presented a first case where a woman who was Locked-In due to late-stage ALS could successfully use a BCI to communicate in daily life using a simple click-based letter selection approach involving attempted hand movements (UNP system [Vansteensel et al. 2016]). Neural signals were recorded with subdural electrode strips and an implantable amplifier, making the UNP easy to use and available 24/7. Next generation BCI implants for communication aim to decode attempted speech. Promising results have been obtained with high-density ECoG grids in patients monitored for epilepsy, in decoding the smallest elements of speech, phonemes, from sensorimotor cortex [Mugler et al. 2014, Freudenburg et al. 2013]. These results motivate the use of chronic HD-ECoG implants as a platform for an intuitive multi-degrees of freedom communication-BCI using attempted speech production. However, the decoding studies involved labeled events, whereas the real use of a BCI would require continuous decoding of unlabeled events, to be able to translate attempted speech to computer-generated speech. In this study we investigated whether neural events generated by spoken phonemes can be detected and classified asynchronously.ECoG signal was collected from 5 intractable epilepsy patients who had high density-ECoG grids (3-4 mm center-to-center) implanted alongside standard clinical ECoG electrodes. While coverage and grid size varied across subjects, the mouth sensorimotor cortex was well covered in all 5 subjects. The subjects were visually cued to pronounce the phonemes /p/, /k/, /u/, and /aː/ or rest and fixate on the screen. Including only electrodes that displayed a response to the task, the ECoG gamma (65-95 Hz) response from a 0.5s time period around peak activity was used to build spatial-temporal match filters (STMF) on a subset of labelled trials (80% repeated 20-fold). Decoding of test trials (20%) was done by simply sliding the STMFs in time and detecting a neural event when the STMF fit of any phoneme was larger than the STMF fit for rest. The detected event was then given the class of the best fit STMF. Our results demonstrate that four spoken phonemes can be detected and decoded at a mean level of 51.6% over five subjects within 0.5s of the start of neural activity. While this level of accuracy is not sufficient for a reliable 24/7 BCI system it does encourage further work for improvement.

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**Poster**

**594. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Recording**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 594.22/KK3

**Topic:** B.09. Physiological Properties of Neurons

**Support:** NIH EB018783

W911NF-14-1-0440

Fondazione Neurone

**Title:** Amplitude bias in low-frequency oscillatory activity: Experimental evidence and implications

**Authors: *A. VATO**[1], G. SCHALK[2]
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**Abstract:** Several studies have shown that low-frequency oscillatory activity plays an important role in neural communication across brain regions by differentially modulating excitability across the cortex. These observations are commonly based on analysis methods that extract oscillatory power and phase from band-pass filtered signals in canonical frequency bands. The recently proposed Function-through-Biased-Oscillations (FBO) hypothesis [1] suggests that oscillatory voltage amplitude of low-frequency oscillations, rather than power or phase, is the most direct measure that reflects cortical excitability. It also suggests that changes in the peak-to-peak amplitude of oscillatory activity does not affect the peaks and troughs of oscillatory activity the same way, i.e., oscillatory activity does not symmetrically vary about a mean and, instead, has an amplitude bias that depends on oscillatory power. Even though this amplitude bias has substantial implications for optimal analysis of oscillatory activity, experimental evidence for it has been scarce.

In our study, we comprehensively evaluated the presence of biased oscillations in electrocorticographic (ECoG) signals recorded from 28 patients with intractable epilepsy who underwent temporary placement of subdural electrode grids. We extracted oscillatory activity in the alpha range (7-12) Hz (i.e., a measure of cortical excitability) and broadband activity in the gamma band (70-170) Hz (i.e., a measure of cortical excitation). Using data from a motor and auditory task, we established the presence of an amplitude bias in alpha oscillations in 88% of 126 task-related locations. Our results also suggest that this amplitude bias has important implications on the traditional interpretation of the relationship between oscillatory activity with cortical excitability, and may also help to explain a range of time-domain phenomena often observed in scalp-recorded EEG. Overall, our study provides experimental evidence to demonstrate that the instantaneous voltage amplitude of biased oscillations can be used as an alternative to the more traditional measurements of power and phase, and pinpoints areas in which those traditional measurements can be misinterpreted.

[1] Schalk G. *A general framework for dynamic cortical function: the function-through-biased-oscillations (FBO) hypothesis*. Frontiers in Human Neuroscience. 2015;9(352).

**Disclosures: A. Vato:** None. **G. Schalk:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.01/KK4

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA Contract N66001-15-C-4017

NIH Grant 1R43EB018200-01A1

DARPA - Subcontract to University of Texas at Dallas - ElectRx

**Title:** Lifetime improvements for penetrating electrodes in chronic clinical investigations

**Authors: *L. RIETH**[1], R. B. CALDWELL[2], B. BAKER[3], R. SHARMA[4]
[1]Electrical and Computer Engin., [2]Bioengineering, Univ. of Utah, Salt Lake City, UT; [3]Electrical and Computer Engin., Univ. of Utah, University of Utah, UT; [4]Electrical and Computer Engin. Dept., Univ. of Utah, Salt Lake City, UT

**Abstract:** Maintaining high performance bi-directional interfaces for periods > 1 year continues to be a challenge that limits the clinical impact of high density neural interfaces. This includes interfaces that are able to decode single unit activity, and able to safely evoke selective and graded sensory or motor responses through stimulation. As part of the DARPA HAPTIX program we have performed the longest implant of a Utah slanted electrode array (USEA) to date with 11 months of implantation to date. This report presents the performance of the electrodes arrays, identifies their failure modes, and communicates the improvements made to the arrays to mitigate these failure modes and improve performance and lifetime. The three primary aspects addressed include the percutaneous lead, encapsulation, and tip metallization. The percutaneous lead has previously been reported to be the source of significant failures for arrays used with human subjects. Arrays with the second-generation lead have been implanted in a subject for 11 months, and > 75% of the wires appear to have broken based on measurements of impedance and electrophysiological performance. We have some evidence that the wires fracture in the extracorporeal segment of the lead. We have developed a third generation lead for the USEA, and will report the performance of this lead. With regard to encapsulation, we have used advanced SEM and materials characterization techniques to investigate degradation of Parylene-C in-vivo. These results show degradation that commonly occurs with this encapsulation, and systematically analyze the surface morphology and character of the degradation. Evidence from chemical analysis suggests that oxidative mechanisms are a strong component of the degradation. This was further supported by use of an in-vitro degradation model reported in a separately. In addition, degradation of the tip metallization due to extended

stimulation pulsing was investigated. We continued to find that damage to the silicon under the tip metallization is the main degradation mechanism for the tip metallization. This research has enabled us to make substantial improvements to the lead, encapsulation, and tip metallization of the arrays, improving their lifetime and performance.

**Disclosures:** **L. Rieth:** A. Employment/Salary (full or part-time):; Blackrock Microsystems. **R.B. Caldwell:** None. **B. Baker:** None. **R. Sharma:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.02/KK5

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA HR0011-15-2-0017

**Title:** Investigation of blackrock microelectrode arrays chronic electrical and recording performance in rat motor cortex

**Authors: *B. CHAKRABORTY**[1], A. KANNEGANTI[2], R. RIHANI[2], B. J. BLACK[2], F. DEKU[2], R. AYUB[2], C. FREWIN[2], A. JOSHI-IMRE[2], J. J. PANCRAZIO[2], S. F. COGAN[2]
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**Abstract:** Blackrock microelectrode arrays (MEAs) are used in intracortical recording and stimulation devices employed in FDA-approved clinical trials involving brain-machine interfaces. Pre-clinical studies in small and large animal models have evaluated various aspects of these devices including optimal implantation techniques, electrode coatings, insertion trauma, foreign body response, and *in vivo* electrochemical properties. These studies were performed in a variety of animal models over different implantation times and with different experimental methods. Herein, we report on a comprehensive characterization of Blackrock MEAs implanted chronically in rat motor cortex for up to 120 days. We selected rat as an animal model for this study because of the established disease models in these animals and their comparatively low cost. The electrode coating used was low-impedance sputtered iridium oxide (SIROF). Electrochemical characterization of implanted electrodes included impedance spectroscopy (EIS), cyclic voltammetry (CV), and voltage transients during current pulsing. The neural recording performance of the MEAs was characterized by single-unit yield per electrode, unit amplitudes, and signal-to-noise ratio (SNR). Observations from pre-implantation saline measurements and weekly *in vivo* monitoring showed the expected increase in impedance over pre-implantation values with no significant subsequent changes observed in the 1 kHz impedance

over five weeks. Electrode charge storage capacities (CSCs), calculated from CV measurements over a 50-50,000 mV/s sweep rate range showed differential changes as a function of sweep rate, which were attributed to different rate-dependent contributions to the CSC. The capability of the electrodes to deliver stimulation pulses was determined by measuring voltage transients in response to 4nC/phase current pulses. Considerable variability in the polarization of the pulsed electrodes was observed and correlations between pulsed, CV and EIS behavior investigated in an effort to identify sources of the variability. Distinguishable single units were observed across multiples electrodes in anesthetized recording sessions with an array yield of 42% at five weeks' post-implantation. Average root mean square noise calculated for 350Hz to 6kHz signal window showed no significant change over time. In summary, the study investigated the changes in electrochemical properties and neural recording behavior of Blackrock MEAs in chronic rat preparations over a 120 days implantation period. The rat proved to be a convenient and inexpensive model for studying the chronic performance of the MEAs.

**Disclosures: B. Chakraborty:** None. **A. Kanneganti:** None. **R. Rihani:** None. **B.J. Black:** None. **F. Deku:** None. **R. Ayub:** None. **C. Frewin:** None. **A. Joshi-Imre:** None. **J.J. Pancrazio:** None. **S.F. Cogan:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.03/KK6

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH Grant 5R01NS062019

NIH Grant 1R01NS094396,

NIH Grant 1R01NS089688

**Title:** *In vivo* 2-photon microscopy mapping of acute mechanical damage due to neural electrode array implantation

**Authors: *J. R. ELES**[1], A. VAZQUEZ[2], Q. YANG[3], T. D. KOZAI[4], T. CUI[3]
[2]Radiology, [3]Dept. of Bioengineering, [4]Bioengineering, [1]Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:** Intracortical neural electrode arrays provides the ability to resolve high-frequency electrophysiological activity from single neurons, enabling high-fidelity brain-computer interfaces, surgical brain-mapping, and neuroscience research. Implantation of these devices, however, is a traumatic process. Post-mortem histological studies have demonstrated that this acute insertion injury is associated with neuronal death and gross tissue deformation [1]. We

extend these findings by mapping the extent of neuronal injury and deformation during implantation with high spatial and temporal resolution *in vivo* 2-photon microscopy. By tracking the positions of blood vessels during array insertion, we generated a map of mechanical strain in the neural tissue caused by implantation. Immediately following probe insertion, we observed a spike in $Ca^{++}$ transients in transgenic GCaMP+ neurons within 200μm of the implantation site. Using our implantation strain maps, preliminary data showed that 95% of this neural activity occurs where mechanical strains are > 0.3 at a strain rate of 0.075 $s^{-1}$, suggesting that mechanical deformation plays a role in neuronal activation. Similar strain values have been shown to be lethal to neurons *in vitro* [2]. By 2 hours, we noted the formation of hypertrophic, spherical regions on axons, which may indicate axonal degeneration. Using oligodendrocyte reporter mice (CNP-eGFP), we further quantified that cortical layer I axons surrounding the implanted device were oriented parallel to the array's face, while axons in healthy layer I had no orientation preference. This suggests that axons stretch to accommodate neural probes during insertion.
In sum, our findings indicate that mechanical forces during electrode array implantation are associated with aberrant neural activity and changes in axonal morphology. Future studies will elucidate how altering the magnitude of insertion strain (via variation of implant design and insertion methods) will alter neuronal outcomes. While the functional consequence of these changes has yet to be determined, this work provides a framework to study the interplay of implantation mechanics and long-term neural viability.
[1] Purcell et al (2009) *J. Neural. Eng.* 6: 049801
[2] Bar-Kochba et al (2016) *Sci. Rep.* 6: 30550

**Disclosures:** **J.R. Eles:** None. **A. Vazquez:** None. **Q. Yang:** None. **T.D. Kozai:** None. **T. Cui:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.04/KK7

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA-BAA-14-30

DARPA-BAA-16-24

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McKnight Brain Institute - University of Florida

University of Florida Pre-eminent Start-up Funds

**Title:** Lightsheet and two-photon imaging of a CLARITY processed regenerative peripheral nerve implant

**Authors: *E. ATKINSON**[1], J. B. GRAHAM[1], E. NUNAMAKER[1], B. SPEARMAN[1], V. H. DESAI[3], R. WACHS[4], C. SCHMIDT[1], J. W. JUDY[2], K. J. OTTO[1]
[2]Nanoscience Inst. for Med. and Engin. Technol., [1]Univ. of Florida, Gainesville, FL; [3]Dept. of Bioengineering, Univ. of Texas At Arlington, Arlington, TX; [4]Univ. of Nebraska-Lincoln, Lincoln, NE

**Abstract:** A growing body of research has demonstrated that there exists the potential to create a chronic interface between technological devices and aspects of the nervous system. Such interfaces can provide a clinical benefit to patients with a wide range of conditions including deafness, blindness, and nervous system disorders. A common goal shared between CNS and PNS interface research is the improvement of implantable devices ranging from modifications of material formulas to completely novel device designs. Making any such improvements, however, necessitates a firm understanding of how the biological tissues surrounding implanted devices interact with the device itself. This understanding is critical to making informed modifications to future devices.

The traditional method for examining the biological response to implanted devices is to use a tissue-sectioning approach, which segments the tissue into thin slices that can then be examined via microscope with further techniques. This approach has many drawbacks, however. For example, the device may need to be completely removed from the organism before the tissue can be examined. If devices are left in during sectioning they may become dislocated from the tissue, making further analysis less informative. Also, tissue slices can easily be physically distorted when placed onto glass slides. This physical distortion varies from slide to slide which can make serial slide analysis complicated.

This study demonstrates the application of a whole-tissue histological approach based on a modified version of the CLARITY protocol, originally developed by Kwanghun Chung and Karl Deisseroth. The samples examined are a novel tissue-engineered electrical nerve interface (TEENI) implanted into the rat sciatic nerve and explanted for analysis after six weeks of regeneration. The multiphoton and lightsheet imaging data presented shows how this whole-tissue approach was used to identify specific proteins of interest to inform future design changes. While this technique is proving useful for investigating the tissue-device interface of peripheral nerves, it may also be useful to other researchers investigating PNS structure and function.

**Disclosures:** **E. Atkinson:** None. **J.B. Graham:** None. **E. Nunamaker:** None. **B. Spearman:** None. **V.H. Desai:** None. **R. Wachs:** None. **C. Schmidt:** None. **J.W. Judy:** None. **K.J. Otto:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.05/KK8

**Topic:** E.05. Brain-Machine Interface

**Support:** DARPA #HR0011-17-2-0019

**Title:** Modification of perineuronal nets following implantation of intracortical neural interface devices

**Authors: *J. B. GRAHAM**, J. GAIRE, E. ATKINSON, K. J. OTTO
Univ. of Florida, Gainesville, FL

**Abstract:** Intracortical devices have the capacity to transduce neurochemical and electrical signals from excitatory neurons into recordable data with the goal of driving complex prosthetic devices. While acute recordings are often described as clean and replicable within the first few weeks following implantation, signal strength often decreases over time. Several studies have revealed that the foreign body response and subsequent glial scarring may be responsible for decreased signal to noise ratio and increased impedance yet direct histological evidence is circumstantial and rarely correlates with recorded data variability. Although additional studies have shown slight reductions in reactive astrocytes, microglia, and neuronal death by adjusting the device dimensions, stiffness, or adding protective coatings, temporal diminishing of recordable signals persists indicating that additional factors are involved in signal reduction. Local disruption to the blood brain barrier and subsequent micro hemorrhagic events are inevitable during device implantation. Although most resolve themselves, evidence of blood induced damage has been reported up to several millimeters from the electrode tips. Furthermore, persistent irritation and movement of the device relative to the local brain tissue can exacerbate disruption of the microvasculature over time. The resulting cascade of cytokine production could disrupt the local extracellular matrix through upregulation of matrix metalloproteinases known to target proteoglycans, a major component of perineuronal nets. While perineuronal net disruption has been described following ischemic stroke and traumatic brain injury, very little evidence has been reported on how the injury resulting from device implantation affect the local tissue. Interestingly, experimental disruption of perineuronal nets located within the visual cortex with chondroitinase ABC corresponds to a transient opening of the critical period where increased plasticity and hyperexcitability modify optical dominance. Additionally, reformation of perineuronal nets correlates with reduction of induced hyperexcitability. This led us to hypothesize that device implantation may cause disruption of local perineuronal nets and alter recorded excitatory signals. Additionally, the cyclical

reconstitution of nets may contribute to observed signal reduction. Here we investigated the spatial temporal modifications of perineuronal nets following implantation of a intracortical device. To preserve the device/tissue interface, we incorporated advanced histological techniques using Clarity and light sheet microscopy.

**Disclosures:** **J.B. Graham:** None. **J. Gaire:** None. **E. Atkinson:** None. **K.J. Otto:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.06/KK9

**Topic:** E.05. Brain-Machine Interface

**Support:** This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) BTO under the auspices of Dr. Doug Weber through the DARPA Contracts Management Office. Grant/Contract: Inter Agency Agreement with U.S. Food and Drug Administration

**Title:** RAA 2.0: an automated and high throughput reactive accelerated aging (RAA) system to evaluate performance of neural implants

**Authors: *M. G. STREET**[1], C. G. WELLE[2], P. A. TAKMAKOV[1]
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**Abstract:** The explosive growth in number and variety of new neuromodulation therapies is partly accelerated by device miniaturization made possible by novel fabrication techniques and materials that do not have a history of clinical use. Pilot clinical studies with such novel devices demonstrated that limited in vivo device lifetime is a major bottleneck for translation of this technology to patients. Lifetime evaluation of different neural implants in animal models requires lengthy experiments to establish their safety and performance on clinically relevant timescales. To accelerate testing of innovative materials and device configurations, and with a future goal to supplement or replace chronic animal testing, we developed a reactive accelerated aging (RAA) technique to rapidly simulate chronic device performance. RAA incorporates elevated temperature and reactive oxygen species (hydrogen peroxide) to simulate foreign body response contributing to implant degradation. To overcome laborious maintenance and performance variability inherent in our first system design, we implemented automation, modularity and multiplexing in RAA 2.0. First, to enable real-time automatic closed-loop control of hydrogen peroxide concentration, we used direct in-line optical (UV) and electrochemical measurement of hydrogen peroxide. Secondly, we developed an electrical heating system to

maintain desired temperature. Thirdly, we fully automated the system operation with a Raspberry Pi based controller that allows us to perform in situ optimization of operating parameters and data logging. These solutions for RAA 2.0 allow us to operate several modules simultaneously to expose neural implants to different conditions, such as temperature and hydrogen peroxide concentration to obtain a more comprehensive picture of device failure modes.

**Disclosures:** **M.G. Street:** None. **C.G. Welle:** None. **P.A. Takmakov:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.07/KK10

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH Grant 5R01NS062019

NIH Grant 1R01NS094396

NIH Grant 1R01NS089688

**Title:** CLARITY based 3D histology assessment of neural electrodes with antifouling coating implanted in mouse cortex

**Authors: *Q. YANG**[1,2], J. R. ELES[2], B. WU[2], A. VAZQUEZ[3], T. D. KOZAI[2], T. CUI[2]
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**Abstract:** Intracortical implants enable multiple dimensional control and precise feedback of Brain Machine Interface by providing high quality single-cell resolution signals. However, foreign body responses to the device is a challenging problem that attenuate recorded signals over time. Post-mortem histology has been widely used to evaluate inflammatory responses of brain tissue to invasive implants. However, conventional section and staining approaches are low in output efficiency and prone to lose or damage samples. In many cases, the implants need to be removed before tissue processing, which inevitably destroy the device-tissue interface. Two-photon microscopy allows direct imaging of neural electrodes in live animal, but usually requires special transgenic strains and the imaging depth is limited to a few hundreds micrometers. Tissue clearing techniques like CLARITY have revolutionized the way of visualizing tissue structure by making tissue transparent. Here we developed and optimized CLARITY based 3D imaging method for assessing histology of intact brain tissue around the neural implants. Multiple stains were applied after tissue clearing, allowing for visualization of types of cells in the same tissue block. These methods were used to evaluate tissue response to neural electrodes modified with a zwitterionic coating following implantation into a transgenic mouse model with GFP expression

in microglia. We also quantified tissue deformation caused by clearing procedures through comparing CLARITY cleared tissue with in vivo two-photon microscopy results. In summary, with optimization, CLARITIY provides a new way for analysis of the foreign body response to intracortical neural electrodes and offer information complimentary to conventional histology and two-photon microscopy.

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**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.08/KK11

**Topic:** E.05. Brain-Machine Interface

**Title:** Self-assembled neural microtissue as a relevant, reliable, and high throughput *In vitro* model of the device-tissue interface

**Authors: *E. ATHERTON**[1], J. SEVETSON[2], D. HOFFMAN-KIM[3,4,6], D. A. BORTON[5,6,7]
[1]Biotech., [2]Neurosci., [3]Dept. of Mol. Pharmacology, Physiology, and Biotech., [4]Ctr. for Biomed. Engin., [5]Sch. of Engin., Brown Univ., Providence, RI; [6]Brown Inst. for Brain Sci., Providence, RI; [7]Dept. of Veterans Affairs, Providence Med. Center, Ctr. for Neurorestoration and Neurotechnology, Providence, RI

**Abstract:** Chronically implanted cortical electrodes are the leading technology for collecting high resolution spatiotemporal recordings from the brain and are essential for enabling neural decoding for both research and clinical applications. However, acute and long-term interactions at the device-tissue boundary often lead to production of inflammatory molecules, glial encapsulation, electrode material damage, and neuronal cell death, all contributing to long term recording instability. While acute tissue responses to devices are easily tested in vivo, many of the long-term complex biomarkers correlated with the foreign body response pathology are difficult and time consuming to test in animals. We propose that a physiologically relevant in vitro model may be a more efficient platform for assessing the device-tissue interface for development and screening of novel biocompatible materials and neuroimmune modulating drug treatments. Currently, the most complex in vitro models of CNS foreign body response to devices approximate the device-tissue interface by a monolayer of cells co-cultured with microwires. Such models produce only a subset of the in vivo hallmarks of foreign body response. Here we leverage physiological interactions between cortical cells in a newly developed 3D primary rat neural microtissue culture implanted with a microelectrode as a foreign body. The self-assembled tissue better models the in vivo microenvironment with

migration of immune cells and diffusion of cell signalling molecules. The microtissue-electrode platform has the potential for high throughput testing of tissue response to novel biocompatible materials and neuroimmune modulating drug treatments. We hope to have a broader impact on the optimization of signal quality and functional recording life of chronically implanted microelectrodes.

**Disclosures: E. Atherton:** None. **J. Sevetson:** None. **D. Hoffman-Kim:** None. **D.A. Borton:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.09/KK12

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH R01 NS095875-01

**Title:** Increasing base permeability of the utah electrode array improves integration in cortical tissue

**Authors: *B. VELAGAPUDI**, M. D. POLEI, P. A. TRESCO
The Univ. of Utah, Salt Lake City, UT

**Abstract:** Electrophysiological recordings from cortical neurons using the Utah Electrode Array (UEA) can support a broad range of clinical applications including neuroprosthetic control. However, long-term recording inconsistency, which is attributed partly to the brain's foreign body response (FBR), limits clinical potential. Recent studies indicate that FBR associated dural fibrosis surrounding UEAs in non-human primate cortex is sufficient to change the position of the implanted UEAs from their original trajectory, and in some cases, pull the device out of brain tissue. Other studies indicate that the FBR is a neuroinflammatory reaction mediated by proinflammatory cytokines released from immune cells at the biotic abiotic interface surrounding the implanted devices. Therefore, approaches that reduce the proinflammatory cytokine production may reduce the FBR and its associated negative sequela. We hypothesized that a hydrogel base coating added to the underside of the UEA may lower the brain's FBR by sequestering secreted molecules within the hydrogel while better matching the mechanical properties of brain tissue. To test this approach, finite element models were used to define the specifications of the base coating. To validate the models' predictions, adult Sprague Dawley rats were implanted with unwired control UEAs (n=7) and unwired UEAs with a 500μm thick, permeable hydrogel coating underneath the array (n=7). Each cohort also had four bone screws and an acrylic headstage to mimic a recording scenario. 12 weeks after implantation, both

cohorts were transcardially perfused. We observed that the craniotomy edges grew to surround the hydrogel coated arrays but remained similar to the original size in the solid base (control) cohort. Moreover, the hydrogel coated cohort was better integrated into rat cortex with significantly thinner dural tissue surrounding the implant site suggesting a lower level of inflammation during the indwelling period. A more detailed histological analysis comparing the two cohorts is ongoing and will be presented at the meeting. These findings suggest that a simple modification to the UEA that may improve biocompatibility and long-term recording performance.

**Disclosures: B. Velagapudi:** None. **M.D. Polei:** None. **P.A. Tresco:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.10/KK13

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH R01 59312810

**Title:** The effect of headstage tethering on the orientation of high density electrode arrays implanted in rat cortex

**Authors: *M. POLEI**[1], B. VELAGAPUDI[3], P. A. TRESCO[2]
[1]Bioengineering, [2]Univ. of Utah, Salt Lake City, UT; [3]The Univ. of Utah, Salt Lake City, UT

**Abstract:** Available evidence indicates that untethered microelectrode arrays have a reduced foreign body response (FBR) compared to devices that are fixed to the skull either directly or with a head stage. Many of these early studies were performed using single shank Michigan style microelectrode arrays where the brain volume displacement, device surface area and insertion trauma are considerably less when compared to more clinically relevant and higher density devices like the UEA or microwire arrays. Moreover, several recent publications indicate that the FBR following chronic implantation of UEAs in non-human primate cortex results in significant dural fibrosis sufficient to change the position of the implanted UEAs from its original trajectory, and in some cases pull the device out of brain tissue. In these studies, the devices were not attached from above and were described as free floating being only secured by the trailing wire bundle. To gain insight into the mechanism of UEA movement following implantation, we compared unwired 4x4 Utah electrode arrays left free-floating (untethered) to a cohort tethered from above to an acrylic headstage. In both cases, unwired UEAs were implanted similarly into adult male Sprague Dawley rat cortex, covered with an *in situ* curing silicon elastomer (Kwik-Cast), but only one group was fixed in place from above with a UV-curable acrylic (Dymax

1187-m) and bone screws. The other was left free floating in cortical tissue. 12 weeks after implantation, rats were transcardially perfused and the device orientation and FBR was investigated. All tethered devices maintained their original implantation position and orientation (N=7) while five of the six free floating UEAs had orientations that deviated from their original implantation trajectory and position. One device had shifted 90 degrees medially relative to its original position with half of the microelectrode shafts being lifted above the cortical surface with the others ranging from 20 to 45 degree changes in position relative to vertical. The observations indicated that FBR tissue remodeling that follows implantation is sufficient to move the array and may be a significant mechanism that underlies changes in recording performance with untethered approaches. A more detailed histological analysis is ongoing and will be presented at the meeting. These findings have important implications on the design and future use of untethered, unwired high density stimulating and recording arrays.

**Disclosures:** **M. Polei:** None. **B. Velagapudi:** None. **P.A. Tresco:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.11/KK14

**Topic:** E.05. Brain-Machine Interface

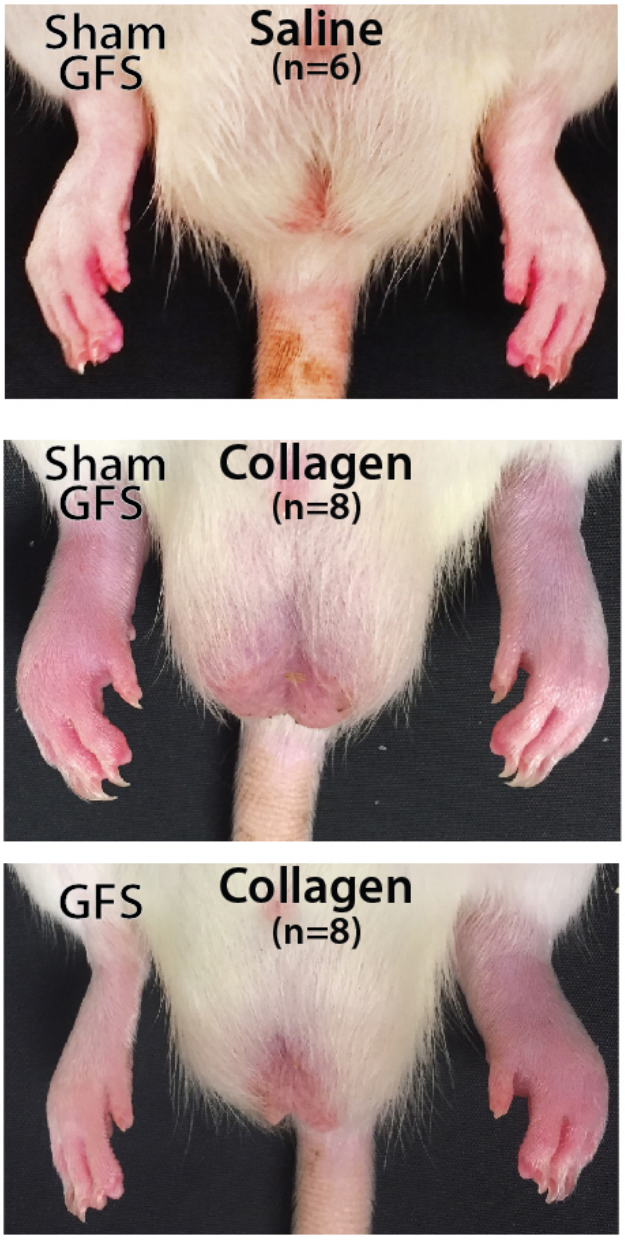
**Title:** Drg stimulation prevents inflammatory arthritis in the rat CIA model

**Authors: *Q. H. HOGAN**, Z. ZHANG, B. PAN
Med. Col. of Wisconsin, Milwaukee, WI

**Abstract:** Electrical field stimulation of the dorsal root ganglion (i.e. ganglionic field stimulation, GFS) is a neuromodulation modality that has recently been established for controlling pain. Its mechanism in uncertain, but electrophysiological recordings from DRG neurons [1] indicate that GFS blocks transmission of pain signals through the DRG. We hypothesize that GFS initiates impulse trains in C-type neuronal somata that propagate down the stem axon to the T-junction, where peripheral and central processes join. There, use-dependent inhibition then causes failure of impulse transmission through the T-junction [2]. This predicts that GFS should block not only afferent impulse trains that generate pain but also block efferent impulses on sensory neurons, which support neurogenic inflammation underlying inflammatory arthritis [3]. We therefore applied GFS, according to our established rat model [4], in rats subjected to the collagen-induced arthritis (CIA) model of rheumatoid arthritis (RA), in which intradermal collagen type II immunization causes inflammation of distal extremity joints. We show that GFS limits development of arthritis ipsilateral to stimulation (Figure - top panel: control with saline rather than collagen immunization; middle panel: CIA but only sham GFS,

i.e. stimulator in place at lumbar 4 DRG but inactive; bottom panel: active GFS). Measurement showed that GFS diminished paw volume, paw width, and ankle width on the side treated with GFS. Arthritis score decreased with GFS, as did cold sensitivity (acetone), and mechanical allodynia (von Frey) and hyperalgesia (pin) on the treated side. We conclude that GFS may be a neuromodulation approach for controlling clinical RA and other forms of inflammatory arthritis.

1. Neuromodulation 2013; 16: 304
2. J Physiol 2013; 591: 1111
3. Levine J Rheumatol 1985; 12:406
4. J Pain 2016; 17: 1349




**Disclosures:** **Q.H. Hogan:** None. **Z. Zhang:** None. **B. Pan:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.12/KK15

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH Grant R01NS030853 (RJN)

DoD CDMRP W81XWH-10-1-0742 (RJN)

**Title:** Intracortical stimulation affects synaptogenesis and long-term potentiation in the sensorimotor cortex

**Authors: *J. NGUYEN**[1], D. J. GUGGENMOS[2], S. BARBAY[3], J. D. MAHNKEN[4], R. J. NUDO[2,3]
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**Abstract: Background:** Activity-dependent stimulation (ADS) uses recorded neural activity to trigger stimulation of the brain. Open-loop stimulation (OLS) is independent of neural feedback, thus relying on a machine-generated pattern of stimulation. A previous study found that ADS and OLS both promote fine motor control recovery in a rat model of traumatic brain injury in the primary motor cortex. ADS enhanced recovery of motor function compared to OLS. Investigation of the underlying mechanisms driving motor recovery in response to ADS and OLS is needed.
**Methods:** Six healthy, adult male rats were implanted with recording electrodes in the premotor cortex (PM) and stimulating electrodes in the somatosensory cortex (S1). Three of the rats were treated with ADS; action potentials (spikes) recorded in the premotor cortex triggered stimulation in S1. Three rats were treated with random OLS mimicking the same rate of stimulation as ADS. After 21 days of stimulation, brain tissue was processed for evidence of morphological differences between the two types of stimulation. Immunohistochemistry was used to label sections for synaptophysin, BDNF, GluR1, and GluR2. Densitometry was used for semiquantitative analysis.
**Results:** As this was a pilot study with a small sample size, analyses were exploratory. Observed synaptophysin and GluR1 immunoreactivity (IR) averages were greater in ADS rats compared to OLS rats, whereas BDNF and GluR2 lacked such trends. In all rats the stimulated hemispheres expressed significantly more synaptophysin ($p$= 0.0132) and GluR1 ($p$=0.0062) than the non-stimulated hemispheres. BDNF and GluR2 expression were significantly lower in the stimulated hemispheres ($p$=0.0030 and $p$=0.0054 respectively).
**Conclusions:** The data suggests that ADS and OLS both enhance synaptogenesis and LTP

induction. At least qualitatively, ADS appears to induce greater synaptogenesis and LTP than OLS. This pilot study elucidates the impact of intracortical stimulation on synaptic plasticity in the cerebral cortex.

**Disclosures: J. Nguyen:** None. **D.J. Guggenmos:** None. **S. Barbay:** None. **J.D. Mahnken:** None. **R.J. Nudo:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.13/KK16

**Topic:** E.05. Brain-Machine Interface

**Support:** Department of Defense W81XWH-13-C-0157

**Title:** Soft and elastomeric electrodes for muscle and nerve interfaces

**Authors: *X. S. ZHENG**[1], K. M. WOEPPEL[1,2], M. J. LOOKER[5], E. CHANG[5], B. CLAPSADDLE[5], A. M. ARAL[3], V. GORANTLA[3,6], L. E. FISHER[4,2], X. T. CUI[1,2,6]
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**Abstract:** Functional electrical stimulation of the peripheral nervous system (PNS) has the potential to restore functions of amputees and to treat neuromuscular atrophy. Electrodes that are chronically implanted in the PNS use conventional conductive materials such as stainless steel (e.g.Cooner wire) and platinum wires, which are significantly stiffer than neural tissue and cause inflammatory tissue response and performance failure. Many efforts have been made to develop flexible electrodes for PNS interfaces, such as the polyimide based thin film longitudinal intrafacicular electrode (Navarro et al, 2007) and the polydimethylsiloxane based flat interface cuff electrode (Tyler et al, 2002). We have developed a soft and elastomeric electrode capable of electrophysiological recording and stimulation for the brain (Kolarcik et al, 2015; Du et al, 2017). The soft electrode consists of a blend of a PEG-modified PEDOT conducting polymer and polydimethylsiloxane elastomer and utilizes an electrically-insulating fluorosilicone coating. This composition had a Young's modulus of 974kPa, and showed excellent chronic tissue integration with healthy neurons at the interface and reduced BBB leakage and gliosis. To translate this technology to the more dynamic and mechanically demanding peripheral environment, carbon nanotubes have been incorporated into the conducting elastomer core to enhance electrical properties of the composition while maintaining favorable mechanical properties. In acute *in vivo* evaluations, electrical stimulation is achieved through implanting a

stimulating soft wire electrode (90 um) in the rat's sciatic nerve, and two recording soft wire electrodes (180 um) in the rat's gastrocnemius muscle. The 90 um soft wires successfully elicited muscle twitch at 2 uA (biphasic pulse, 500 uS pulse width, 50uS interphase delay), and resulted in a graded increase in compound muscle action potential of the rat gastrocnemius measured by the 180 um soft wires. For recording, a 90 um soft wire was implanted in the tibial nerve, and manual brushing of the posterior hind limb elicited multiunit activity and sortable single units. Chronically, the soft wires implanted in the muscle remained intact and demonstrated efficacy in eliciting muscle twitch one month after implantation. Post mortem histology showed decreased fibrotic scarring around the soft wire implant compared to the stiff wire control implants. Our soft wires have the potential to improve the interface with the peripheral nervous system, and to improve the control of prosthetic limbs for research and clinical applications.

**Disclosures: X.S. Zheng:** None. **K.M. Woeppel:** None. **M.J. Looker:** None. **E. Chang:** None. **B. Clapsaddle:** None. **A.M. Aral:** None. **V. Gorantla:** None. **L.E. Fisher:** None. **X.T. Cui:** None.

**Poster**

**595. Neurophysiology: Implanted Electrodes and Direct Interactions With Neurons - Tissue Reactions**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 595.14/DP10/KK17 (Dynamic Poster)

**Topic:** E.05. Brain-Machine Interface

**Support:** NIH Grant R01NS094396

NIH Grant R01NS094404

**Title:** Multi-scale, multi-modal analysis of the brain tissue-implant interface reveals new depths of the biological research field at the neuroelectronic interface

**Authors: *T. D. KOZAI**[1], A. L. VAZQUEZ[2], J. R. ELES[1], N. J. MICHELSON[1], X. CUI[1], J. J. WILLIAMS[3]
[1]Bioengineering, [2]Radiology, [3]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:** Intracortical electrode arrays suffer from reliability and variability issues that impact their long-term performance. The large variability in performance has led to a growing number of questions regarding the relationships between the recorded signal and the neurons around the implant. Previous attempts to correlate recording and histological outcomes have led to results that are sometimes contrary to expectations. We show that the challenges of correlating recording performance to impedance and histological outcomes are due to several sources of error and artefacts that need to be addressed. Electrophysiological recording and GCaMP

imaging demonstrate that neurons in resting state are largely quiescent compared to driven activity. This has led to increased studies in awake, free-moving, non-behaving, animals. However, while motion artefacts in NHP are easy to identify, motion artefacts in rodents have time-constants like action potentials often leading to misclassification even with common average referencing. Visual stimulation to head-fixed subjects is used to drive activity in V1 while eliminating motion artifacts. Visually evoked current source density analysis is used to identify cortical layers. Then, the electrophysiological layer map is compared to a histological layer map based on neural morphology, and neural density. However, good histology coupled with loss of recorded signal revealed that several modes of material failure can impact performance. Even when electrically and mechanically intact arrays were examined, poorly performing recording sites were identified with good histological outcomes. Functional intrinsic blood flow imaging and GCaMP activity around implants demonstrated a dramatic decrease in activity over the first two weeks, without a substantial decrease in GCaMP expression. Detailed two-photon analysis revealed axonal protrusion injuries, oligodendrocyte myelinosome injuries, and a decrease in blood flow perfusion around the array. These tissue responses may impact the neural network activities around the implant. Our findings revealed our limited understanding of the biology governing the host tissue responses and their impact on longitudinal electrophysiology, and highlight the need for a new level of neurobiology research at the neuroelectronic interface.

**Disclosures:** **T.D. Kozai:** None. **A.L. Vazquez:** None. **J.R. Eles:** None. **N.J. Michelson:** None. **X. Cui:** None. **J.J. Williams:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.01/KK18

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH GRANT MH051393

NIH GRANT NS070583

NIH GRANT NS066587

**Title:** Network degeneracy and the dynamic of task switching in the feeding circuit in *Aplysia*

**Authors: *Y. WANG**, *Y. WANG, M. CAMBI, K. R. WEISS, E. C. CROPPER
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**Abstract:** Degeneracy in network function has been described in several model systems leading to speculation as to why it occurs (e.g., Prinz et al., 2004). We utilize a multi-tasking network-the feeding circuit in *Aplysia*, to address this issue. The feeding circuit generates egestive and ingestive motor programs. Egestive activity can be triggered using two paradigms, egestive repetition priming and positive biasing. We hypothesize that circuit parameters differ in the two situations. Experiments here probe this idea and the idea that degeneracy in this context has functional consequences for task switching. In the feeding circuit, motor activity is in part reconfigured by changing the phasing of activity in motor neuron B8. This can happen either by changes in B8 excitability or alterations in B8 synaptic input. Here we show that B8 excitability is decreased after egestive repetition priming. Presumably, this is at least in part due to the induction of a persistent outward current that we demonstrate develops as priming progresses. In contrast, in the positive biasing paradigm, we find that the persistent current in B8 is either inward, or if it is outward, very little current is induced. Previously we have shown that after egestive repetition priming, stimulation of the ingestive command-like neuron CBI-2 triggers egestive activity—i.e., there is a task switch cost. The persistent outward current that develops during egestive repetition priming would be expected to contribute to this phenomenon. Additionally, previous studies have demonstrated that egestive priming decreases the excitability of B40, an interneuron that produces an ingestive firing pattern in B8. We now demonstrate that the B40 excitability remains elevated after positive biasing. We hypothesized that egesitive to ingestive switches would occur more readily after positive biasing in part due to the fact that virtually no outward current develops in this situation. Further we postulated that there would be less suppression of B40 activity. Consistent with this hypothesis, we demonstrate that if we induce positive biasing and then stimulate CBI-2, programs are ingestive (instead of egestive as they are after repetition priming). Taken together, these data suggest that degeneracy in network function is observed in the feeding network. In addition, our results support the idea that this degeneracy is of physiological importance due to its implications for the dynamic of task switching.

**Disclosures:** **Y. Wang:** None. **M. Cambi:** None. **K.R. Weiss:** None. **E.C. Cropper:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.02/KK19

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** National Natural Science Foundation of China (Grants 31671097, 31371104)

National Institutes of Health (NS066587, NS070583, and MH051393)

**Title:** Synaptic and circuit mechanisms for variability in motor programs elicited by a command neuron in a feedforward network

**Authors: T.-T. CHEN**[1], G. ZHANG[1], K. YU[1], W.-D. YUAN[1], S.-Y. YIN[1], S.-A. CHEN[1], D.-D. LIU[1], E. C. CROPPER[2], K. R. WEISS[2], *J. JING[1,2]
[1]Nanjing Univ., Jiangsu, China; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY

**Abstract:** When one repeats a motor act, the executions vary as a result of variable expression in the activity of the behavior mediating neurons. Although there is a debate as to its functional significance, variability must originate, at least in part, from mechanisms in the corresponding network. Previously, there are some computational and molecular studies on network variability. Despite these efforts, circuit and synaptic mechanisms are not well understood. In part, this is a reflection of the fact that it is inherently difficult to study phenomena that are variably expressed. We study variability in the *Aplysia* feeding motor system, where behavioral and motoneuronal variability has been well characterized. Here, we report the identification of a command-like neuron, cerebral-buccal interneuron-10 (CBI-10), that is active during feeding behavior. We demonstrate that CBI-10 drives motor programs that are more variable than programs driven by a previously-identified command-like neuron, CBI-2. The difference in variability is partly attributable to differences in the strength of synaptic connections between command-like neurons and pattern-generating interneurons that excite motoneurons. One of these interneurons, B34, fires at a relatively low frequency when programs are triggered by CBI-10. In contrast, the B34 firing frequency is higher when programs are triggered by CBI-2. Importantly, stimulation of CBI-10 together with subthreshold depolarization of B34 makes programs less variable, and more similar to programs evoked by CBI-2. This suggests an obligatory role of B34 in generating variability when programs are induced by CBI-10. Our results establish how variability may arise through specific mechanisms in a single feedforward network. These findings are likely to be broadly relevant. Many neural circuits, including cortical circuits, are feedforward and show various degrees of variability.

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**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.03/KK20

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant GM117582

NIH IRP ZIAMH002800

**Title:** Imaging spontaneous motor neuron activity and hormone-induced reorganization

**Authors: *A. D. ELLIOTT**[1,2], F. DIAO[3], Y. WU[4], R. SCOTT[3], H. SHROFF[4], B. H. WHITE[5]
[1]NIH/NIMH, Bethesda, MD; [2]Nigms, [3]NIMH, [4]NIBIB, NIH, Bethesda, MD; [5]Labor Molec Bio, NIMH, Bethesda, MD

**Abstract:** Resting state activity that occurs in the absence of any obvious stimulus is a feature of all nervous systems from humans to fruit flies. Little is known, however, about the purpose of this activity or its relationship to directed brain activity elicited in response to environmental or homeostatic signals. To characterize the relationship between resting state and elicited brain activity, we are analyzing a hormonally-induced state transition in the nervous system of the fruitfly, Drosophila melanogaster. Using GCaMP6s to image activity specifically in the motor system of animals at the pupal stage, we observe that rhythmic spontaneous activity throughout the central nervous system (CNS) is rapidly reconfigured by exposure to Ecdysis Triggering Hormone, ETH. ETH governs the execution of a developmentally essential behavioral sequence called the pupal ecdysis sequence, and previous work has shown that ETH can induce a fictive pupal ecdysis sequence in an isolated CNS. The pupal ecdysis sequence consists of three serially executed motor programs defined by distinct abdominal movements and in the excised CNS, ETH induces three stereotyped patterns of motor neuron activity that replace resting state activity. Because there are no known behavioral correlates of the resting state activity, we sought to determine if the motor neuron activity was communicated to muscles. We expressed GCaMP6s in the muscles and, surprisingly, observed spontaneous activity along the long body axis prior to the onset of the pupal ecdysis sequence. This spontaneous muscle activity—as well as spontaneous motor neuron activity in the excised CNS—remained even when neurons in the pupal ecdysis central pattern generator were suppressed, a manipulation that eliminates the ecdysis-specific reorganization of motor activity in both the excised brain and the muscles. We are currently using a custom light-sheet microscope to interrogate the brainwide dynamics of this system at cellular resolution with and without ETH stimulation. Using two-color imaging, we are comparing the activity of a subset of motor neurons implicated in ecdysis-specific movements with that of all motor neurons using RCaMP1f and GCaMP6s. Our goal is to identify the cellular determinants of spontaneous and ecdysis-specific motor activity and the effects of ETH on each.

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**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.04/KK21

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH NINDS 1 R01 NS085006 to R.L.C.

**Title:** Output variability in a motor system: From CPG to motor plant

**Authors: *A. WENNING**[1], B. J. NORRIS[2], R. L. CALABRESE[1]
[1]Biol., Emory Univ., Atlanta, GA; [2]California State Univ., San Marcos, CA

**Abstract:** Rhythmic behaviors vary across individuals. We interrogated a feedforward motor control system – leech heartbeat – to determine the extent of this output variability for each level, from the CPG to the heart motor neurons and the heart muscles and potentially identify its sources.

While bilaterally symmetrical in its layout, burst and beat patterns are inherently left/right asymmetrical. The same CPG orchestrates different intersegmental coordinations for the two hearts: rear-to-front peristaltic on one side and synchronous on the other. Superimposed on this ongoing activity is periodic switching of the coordination side to side about every 15 to 60 burst and beat cycles (70-350 s). The system thus alternates episodes of two states: left synchronous/right peristaltic and right synchronous/left peristaltic.

We considered four types of variability: 1) inherent variability owing to the stochastic nature of biological processes (quantifying cycle-to-cycle variabilities within a coordination mode episode), 2) population variability including, but not limited to, genetic variability and variability in individual experience (comparing across animals), 3) repetition variability taking advantage of the unique feature of the leech heartbeat system as the same function is performed multiple times by the same elements (comparing across episodes), and 4) developmental variability (comparing bilaterally homologous elements). We compare these types across levels and coordination modes. As a metric to characterize the two coordination modes, we used the difference between the activity phases of two segments.

Cycle-to-cycle variability within a switch episode is low indicating a precise system. Across individuals, variability is high. Interestingly, variation at one level is not predictive of variation on the next and, on the same level, differed between coordination modes. In seeking to elucidate the sources of this population variability, we show that repetitions of a coordination vary little and thus contribute little. On the other hand, we show that when bilaterally homologous neurons and muscles perform the same motor act, variability can be as large as in the population itself, again depending on level and coordination mode.

Across levels, phase variation was either similar (synchronous) or varied non-monotonically (peristaltic): high for the CPG and hearts but low for the motor pattern. We argue that the mechanisms that transform CPG output to motor neurons, namely, the bursting pattern and synaptic output of the CPG interneurons, and the properties of the motor neurons, may limit the range of output variation of the motor pattern.

**Disclosures:** **A. Wenning:** None. **B.J. Norris:** None. **R.L. Calabrese:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.05/KK22

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant MH046742

**Title:** Variation across network output, excitatory post synaptic potentials, ionic conductances, and ion channel and receptor mRNAs within motor neurons of the crustacean cardiac ganglion

**Authors: *D. R. KICK**[1], B. J. LANE[2], J. L. RANSDELL[3], S. S. NAIR[4], D. J. SCHULZ[1]
[1]Div. of Biol. Sci., Univ. of Missouri, Columbia, MO; [2]Brandeis Univ., Waltham, MA; [3]Departments of Developmental Biol. and Med., Washington Univ., Saint Louis, MO; [4]Electrical & Computer Engin., Univ. of Missouri Columbia, Columbia, MO

**Abstract:** Central pattern generators (CPGs) are critical in producing many behaviors including locomotion, respiration, and swallowing. Despite the consistency of network output, a significant degree of variation has been documented in the magnitude of ionic conductances and the abundance of ion channel mRNAs. Not only has this variation been observed between animals, but within the neurons of a single CPG as well. Several mechanisms have been proposed to explain how appropriate output can be reached despite underlying dissimilarities including activity independent regulation, synaptic scaling, and correlated mRNA abundances. In this study we seek to indirectly address whether regulation of mRNA abundances, ionic conductances, synaptic excitation, or network output is more tightly constrained through a population level approach. We have examined a crustacean CPG, the *Cancer borealis* cardiac ganglion at these levels and report our findings below.
We report that while there is low variability in network activity for a single animal the same cannot be said between animals, where we have identified a nearly 6 fold range in cycle period. We find higher variability in the duration of the pace making interneuron small cells (scs) burst duration and the burst duration of the large cell motor neurons (Coefficient of variance (cov) 0.80 and 0.70 respectively). Less variable burst characteristics were the duty cycle and termination of the scs and LCs (duty cycle coefficient of variance 0.30 and 0.40, termination coefficient of variation 0.27, 0.30). We have used a resampling method to determine the simulated median fold range and cov. The scs EPSP were found to have a 5 fold range in regard to peak amplitude (cov 0.50). At 0mV we report a 13 fold range in the magnitude of the A-type potassium ($I_A$) and an 8 fold range in the high threshold potassium current. The latter is a composite of the calcium activated potassium current and the delayed rectifier ($I_{Kd}$) which had fold range of 7 and 5.9 respectively (cov 0.53, 0.48, 0.43, 0.37 respectively). The mRNA abundances for channels contributing to $I_A$, *shal* and *shaker* varied by 1.74 and 6.26 fold range.

The genes of the large and small calcium activated potassium currents varied by 31.90 and 6.18 fold range respectively. Finally, those genes encoding $I_{Kd}$ channels *shab, shaw1,* and *shaw2,* varied by 5.99, 5.51, and 8.07 fold range. From these results we suggest that the tolerable variability is lower at the network level and higher at the level of currents and mRNAs. This finding aligns with previous computational and experimental work that multiple and varied solutions exist to arrive at a CPG's target output.

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**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.06/KK23

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Title:** Locomotion in insects (cockroaches and ants): Waves and discrete states of neural activity in modules for central pattern generation

**Authors: J. F. GOMEZ-MOLINA**[1], A. L. GOMEZ-MOLINA[1], *U. M. RICOY[2]
[1]Intl. Group of Neurosci. (IGN), Medellin, Colombia; [2]Biol., Northern New Mexico Col., Espanola, NM

**Abstract:** INTRODUCTION. 1. An important function of neurons is to generate a coordinate movement. Locomotion is one of the simplest forms of movement with elementary modes of rhythmicity (oscillatory modes). Because basic microcircuits of interneurons in cortical modules might reuse similar mutual inhibition mechanisms as those present in central pattern generators (Gomez-M 2003), the study of insect ganglia and the control of legs and antennae give us a good opportunity to understand robust mechanisms of coordination of neural systems. 2. Grooming of various body parts is organized in particular sequences (Sachs 1988). Sequential motion during turning behavior has been also described in many species. 3. Based on previous experimental work (Griego, Salazar, Ricoy 2017; Wu, Liu, Chen, Wang, Bai, Li and Ren 2014; Gomez-Molina 2007), we present additional analysis for locomotion responses to sugar and turning behaviors in cockroaches and ants. -METHODS. Theoretical and experimental (videorecordings) methods. Sensors for locomotion.--PRELIMINARY CONCLUSIONS. 1. Regardless of the frequency of oscillation, the total number of cycles can be a good indication of local activity in neural modules and systems. 2. One important advantage of these models is that the states of activation (alert-like, sleep-like and relax-like states) have direct correlates in the observed behavior of the animal (e.g. running, walking and resting for example). 3. Sugar increases locomotor activity, as can be expected given the positive correlation between activation states and glucose consumption (Fig. 1). 4. For cockroaches walking in a flat terrain, small differences

in phase between oscillations in small turnings (Fig 2) can be interpreted as general and robust mechanisms of waves for sequential activation in multiple types of neural modules (Gomez JF, 2007, 2015). 5. Recording of insect behavior is a good way to test for robust associations between rhythmicity, activation, total number of cycles and wave-like neural communication (Griego, Salazar, Ricoy 2017).

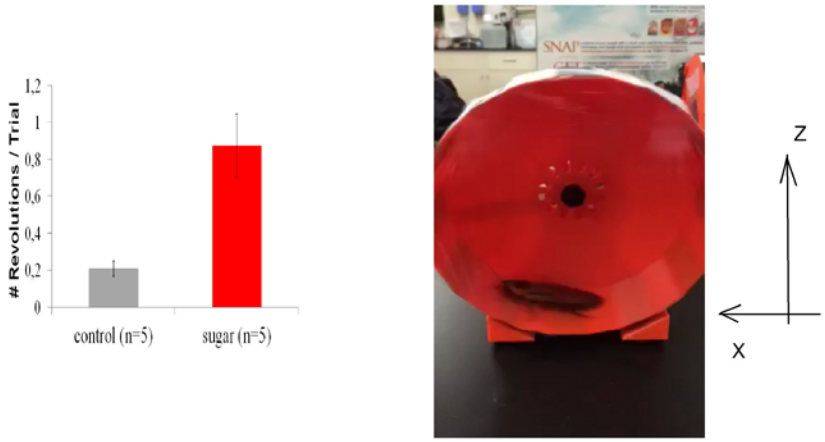


Figure 1. Relation between sugar and locomotor activity (running behavior in cockroaches, Griego et. al. 2017). Cockroaches were tested one by one in a running wheel, and their level of activity was recorded.

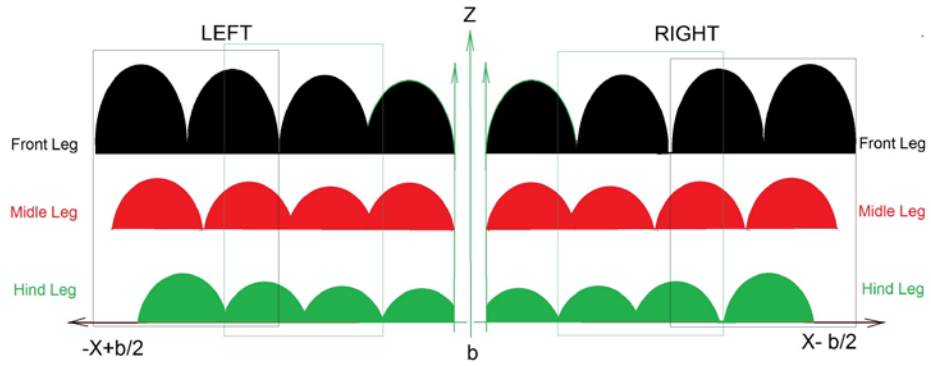


Figure 2. Graphical Analysis (not real data) for the movement of legs in insects (based on Wu et al. 2014 and Gomez-M 2007).

**Disclosures:** **J.F. Gomez-Molina:** None. **A.L. Gomez-Molina:** None. **U.M. Ricoy:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.07/KK24

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant R35 NS097343

NIH Grant 2T32NS007292-31

**Title:** Short and long-term effects of high potassium ion concentration saline on the crab pyloric rhythm

**Authors: *L. HE**, E. JAMES, D. POWELL, M. KAR, E. MARDER
Brandeis Univ., Waltham, MA

**Abstract:** The pyloric circuit of the stomatogastric ganglion (STG) of the crab Cancer borealis produces the triphasic rhythmic output pattern that drives filtering of food. This circuit robustly maintains consistent output for years despite ongoing neuromodulation, membrane protein turnover, and fluctuating environmental conditions. To better understand the specific regulatory mechanisms underlying the robust output, we searched for an appropriate perturbation to study, settling on studying the effects of superfusing saline with varying amounts of potassium ion (K+) concentration (1.5x, 2x, 2.5x, 3x) on the pyloric rhythm. In a series of extracellular recordings, we found that high [K+] saline produced a short term increase in rhythmic frequency followed by a decrease and sometimes complete loss of activity. Subsequently, the rhythm recovered, despite continued superfusion of high [K+] saline. At higher [K+] concentrations, a greater percentage of preparations lost activity within the first few minutes of superfusion of high [K+] saline. In baseline conditions, intracellular recordings of the Pyloric Dilator (PD) neurons showed regular slow-wave bursting activity. In the superfusion of 2x [K+], the neurons depolarized by 10-12 mV, and, after an initial transient, the amplitudes of the slow wave in the PD waveform decreased and the amplitude(s) of the spike(s) increased. The neurons transitioned from firing robust bursts to firing tonically or to firing single spike bursts with small slow waves at the depolarized resting membrane potential. Over the next 10-15 minutes in 2x [K+] saline, the neurons slowly repolarized and resumed stereotypical slow-wave bursting activity. This recovery of rhythmic activity appears indicative of a homeostatic process that regulates the pyloric neurons' resting potential and thus the neurons' ability to generate rhythmic slow wave bursts and action potentials. When the preparations were returned to control saline, the rhythms reverted to activity patterns that were nearly indistinguishable from those recorded in the baseline condition.

**Disclosures:** **L. He:** None. **E. James:** None. **D. Powell:** None. **M. Kar:** None. **E. Marder:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.08/KK25

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** R35 NS 097343

**Title:** Neuromodulators differentially affect the motor output of the stomatogastric ganglion across temperature

**Authors: *S. A. HADDAD**[1], E. E. MARDER[2]
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**Abstract:** As global temperatures increase, the question of how organisms will respond to this change arises. How do neuromodulators affect the persistent and essential pyloric rhythm of the marine invertebrate, *Cancer borealis*, as temperature is increased? Previous work showed that the triphasic phase relationships of the pyloric neurons were maintained as temperature increased within a permissible range. Above this range, the neuronal activity patterns decay and do so differently across individuals, revealing underlying differences in network parameters. The stomatogastric ganglion, which contains the cell bodies of the pyloric motor neurons, is modulated by substances that are released locally from descending inputs and ascending sensory feedback, as well as hormonally from substances in the hemolymph. We made extracellular recordings from the motor axons of the pyloric activity at temperatures from 11°C-31°C. When the descending modulatory inputs were silenced the pyloric rhythm became more temperature sensitive. To convey the variety of activity patterns observed across individuals as the rhythm becomes unstable, we devised a categorization-based system. When preparations become unstable, they transition between activity patterns. In the absence of modulatory inputs, the number of transitions increases as temperature increases, significantly by 27°C and 31°C. The types of activity patterns present are also less robust (least like the standard triphasic rhythm) at higher temperatures. When the decentralized preparations are superfused with saline that contains $10^{-6}$M proctolin or $10^{-5}$M oxotremorine, both which activate the modulatory inward conductance $I_{MI}$ on different subsets of pyloric neurons, the rhythms are more robust and temperature invariant. In both modulatory conditions, a phase invariant triphasic rhythm is maintained through a temperature range of 11°C-31°C and the preparations do not transition across activity patterns. When the superfused saline contains $10^{-5}$M serotonin the triphasic rhythm becomes more temperature sensitive. Serotonin increases the variability of activity patterns observed across decentralized preparations. Preparations transition across activity

patterns significantly more than their decentralized counterparts at 19° and 23°C. When modulatory inputs are intact, the presence of serotonin does not produce this effect and the number of transitions and activity patterns observed are similar to the intact preparation at comparable temperatures in normal saline.

**Disclosures: S.A. Haddad:** None. **E.E. Marder:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.09/KK26

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH MH060605

**Title:** Neuromodulators stabilize the activity phase of neurons in an oscillatory network

**Authors: *H. ANWAR**[1], D. M. FOX[3], F. NADIM[2]
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**Abstract:** Neurons in oscillatory networks often fire at a constant activity phase, yet the underlying mechanisms for such phase stability are unknown. Our previous work in the pyloric network of the crab stomatogastric ganglion showed that activity phase can be stabilized if the maximal conductance and peak phase of synaptic conductance changes in a frequency dependent manner, for example through short-term synaptic plasticity (STSP). In addition to STSP, neuromodulators also alter synaptic properties. We aimed to determine the respective roles of STSP and neuromodulators in stabilizing the activity phases of neurons in the pyloric rhythm. We explored whether pyloric pacemakers used STSP to communicate changes in rhythm frequency to the follower neurons by modifying the shape of the IPSC. For this, we recorded the oscillatory waveform of the pacemaker PD neuron, suppressed the network activity, and played back the PD waveform into the voltage clamped PD neuron periodically at different cycle frequencies ($F$ = 0.5-4 Hz). We recorded the resulting IPSC in the follower LP neuron. As $F$ increased, the peak IPSC amplitude decreased and the peak phase of the IPSC was delayed, confirming our hypothesis.
To find out whether this change in IPSC shape was sufficient to stabilize the activity phases of follower neurons as $F$ changed, we removed the neuromodulatory inputs by decentralizing the preparation. As above, we drove the PD neuron with its prerecorded waveform and measured the activity phase of the LP and PY follower neurons relative to the PD burst onset. The activity phases of the LP and PY neurons did not remain constant across different $F$s, indicating that short-term synaptic dynamics are not sufficient to stabilize the activity phase.

Finally, we investigated whether activity phase becomes more stable in the presence of neuromodulators. We repeated the above protocol in the presence of the endogenous modulatory neuropeptide proctolin (bath applied at $10^{-7}$M). We found that proctolin significantly stabilized the activity phases as *F* changed. To understand if this effect is due to changes in synaptic dynamics, we examined the effect of proctolin on the peak amplitude and peak phase of the IPSC. We found that proctolin increased the peak amplitude but did not alter the peak phase at all frequencies. We are exploring whether the increased stability in activity phases is due to the direct modulation of the synapse or the modulation of excitability of the postsynaptic neurons. Together, our results show that neuromodulators can stabilize the activity phases of neurons in oscillatory networks across different frequencies.

**Disclosures: H. Anwar:** None. **D.M. Fox:** None. **F. Nadim:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.10/KK27

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant R35 NS097343

**Title:** Variability in modulator innervation of the stomatogastric ganglion

**Authors: *J. PIPKIN**[1], E. E. MARDER[2]
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**Abstract:** The synaptic strengths and conductance parameters that constrain neuronal computations are constantly influenced by neurochemical modulation. Modulation entails flexibility, and can be employed to either maintain or alter circuit activity patterns in response to environmental changes without any change to circuit connectivity. In the stomatogastric ganglion (STG) of the crab, *Cancer borealis*, a slew of neuromodulators ensure the robustness of the pyloric and gastric rhythms, two motor activity patterns that govern the filtering and chewing of food respectively. The STG, which sits in a blood sinus, is influenced by modulators released broadly into the circulatory system and those that are locally-released by descending and ascending projections into its neuropil. Of this latter class, immunohistochemical staining and electron microscopy reveal that many modulators are packaged into both large and small vesicle-packed boutons that widely innervate the neuropil. Staining patterns vary between different modulators, and even the same modulator may be stained slightly differently from animal to animal. To address these areas of variability quantitatively, we sought to measure the size and number of immuno-stained modulator-containing boutons in the STG. To be enable us to differentiate all boutons, in some cases we employed expansion microscopy to enhance effective

imaging resolution. When stained for allatostatin B (AST- B), preliminary findings indicate that the number of boutons greater than 1.5 microns in diameter vary across a 3-fold range from animal to animal. Very large boutons (those greater than 4 microns in diameter) vary across a 5-fold range. We further explore several modulators in expanded and unexpanded tissue, adding to our understanding of the complex and variable neuromodulatory system of the STG.

**Disclosures: J. Pipkin:** None. **E.E. Marder:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.11/KK28

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** NIH Grant T90 DA032435

NIH Grant R35 NS097343

**Title:** An *In vivo* exploration of cardiac and pyloric activity in Cancer borealis

**Authors: *D. KUSHINSKY**, J. HALEY, E. MARDER
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**Abstract:** To simultaneously examine the *in vivo* motor output of the cardiac and pyloric rhythms, which generate the heart contractions and filtering behaviors in the marine crustacean *Cancer borealis*, respectively, we developed a technique using photoplethysmography (PPG). In this method we attach an infrared sensor to the carapace of the animal with dental wax and record movement as deflections in the signal. Local and hormonal neuromodulation and sensory inputs onto these CPGs play a significant role in the actual responses to perturbations in an animal, but are usually lost or removed during *in vitro* studies. Over long term, 24 hour recordings, animals appear to have a 3 hour cycle of increase and decrease in frequency. In the population tested (n = 54), cardiac rhythms were bimodal with means at 0.7 Hz ±0.1 and 1.3 ±0.24 Hz. The pyloric rhythms were normally distributed with a mean of 0.9 ± 0.4 Hz. To investigate how these rhythms behave in response to perturbations, we conducted temperature ramps on *in vivo* animals by heating or cooling the saline in tanks while recording both the cardiac and pyloric muscle movements. The change in frequency with temperature was lower in intact animals than those previously studied *in vitro* (Tang et al., 2010), consistent with previous *in vivo* experiments using extracellular recordings (Soofi et al., 2014). Critical temperature, defined as the temperature that rhythmic motor movements were no longer apparent in PPG recordings, were determined for both the pyloric and cardiac rhythms of 8 animals. The critical

temperatures of the cardiac and pyloric rhythms were significantly different (29.2 °C and 22.2 °C, respectively, $p < 0.01$, pairwise t-test).

**Disclosures: D. Kushinsky:** None. **J. Haley:** None. **E. Marder:** None.

**Poster**

**596. Motor Systems, Variability, and Stability**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 596.12/KK29

**Topic:** E.07. Rhythmic Motor Pattern Generation

**Support:** Eazysense Nanotechnologies, Inc.

Indian Angel Network

Broderick Brain Foundation

**Title:** Neuromolecular imaging and BRODERICK PROBE® nanobiosensors reveal temporal synchrony patterns between neurotransmitter concentration and movement in the physiologic state and temporal asynchrony between neurotransmitter concentration and movement in the pathologic state

**Authors: *L. WENNING**, P. A. BRODERICK
Dept. of Molecular, Cell. and Biomed. Sci., City Col. of New York, CUNY Sch. of Med., New York, NY

**Abstract:** Temporal synchrony describes the simultaneous, rhythmic regularity between neurotransmitter concentration and movement frequency. Using neuromolecular imaging (NMI) and BRODERICK PROBE® nanobiosensors, a point-by-point temporal synchrony was imaged between the synaptic concentration of 5-HT released from dorsal striatum $A_9$ terminals and natural open-field behaviors such as ambulations, fine movements, and rearing in freely moving, male, Sprague Dawley rodents. Indeed, 5-HT synaptic concentration increased and decreased in parallel with the rise and fall of open-field behavior frequency. Thus, temporal synchrony occurs when the symphony of physiologic neurochemistry and behavior is uninterrupted by drugs, disease, or injury. In contrast, neurotransmitter concentration and movement frequency are not in concert when monitoring psychostimulant-induced behaviors. After cocaine administration, NMI and BRODERICK PROBE® nanobiosensors revealed temporal asynchrony between endogenous 5-HT release at $A_{10}$ terminals and both cocaine-induced ambulations and fine movements in freely moving, male *Rattus norvegicus*. Intriguingly, the psychostimulant effect of cocaine on dopamine $A_{10}$ somatodendrites in the ventral tegmental area remained rhythmic and episodic but not synchronous. Thus, psychostimulant effects on temporal synchrony appear to occur more intensely in somatodendrites compared to nerve terminal nuclei. Temporal asynchrony was also

imaged during *in vivo* studies of anterior temporal lobe epilepsy in human patients. After EEG monitoring, 6 to 10 recordings with γ-irradiated (11.6-12.7 kilograys), laminar biocompatible carbon-based BRODERICK PROBE® laurate biosensors were taken at a cortical depth of microns to less than 2 mm for 20-30 minute intervals. Results of these studies revealed the presence of L-tryptophan (L-TP) and peptides in high concentrations in the neocortex of epilepsy patients during surgery. Therefore, L-TP and peptides may serve as markers for pharmaco- and/or gene therapy for neurodegenerative processes. In this manner, temporal patterns may be used to create a dynamic data profile in the clinical setting. Although static neurotransmitter levels are currently the standard, these static parameters become more valuable when empirically studied within the context of movement rather than solely focusing on whether a neurotransmitter level has increased or decreased. Not only does this dynamic data provide a more complete portrait of the temporal harmony of physiology, but it may also be used to distinguish the intensity of disease or drug-induced temporal cacophony on specific parts of microneuroanatomy.

**Disclosures: L. Wenning:** None. **P.A. Broderick:** None.

**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 597.01/KK30

**Topic:** E.10. Motor Neurons and Muscle

**Support:** R01 5R01AI093504

**Title:** A novel sublethal mouse model of botulism using running wheel activity as an indirect readout of respiratory function

**Authors: *A. B. BRADFORD**, M. J. STENSLIK, E. J. VAZQUEZ-CINTRON, M. C. MANGKHALAKHILI, M. EISEN, D. L. NGUYEN, C. A. ONDECK, P. M. MCNUTT
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**Abstract:** Botulinum neurotoxins (BoNTs) are exceptionally potent poisons that cleave presynaptic proteins essential for neurotransmission. The resulting impairment of neurotransmitter release manifests as long-lasting muscle paralysis, leading to death by respiratory collapse at lethal doses. Although botulism victims can be maintained with supportive care, such as mechanical ventilation and parenteral feeding. Treatments to reverse paralysis and accelerate recovery of neuromuscular function are critically needed. However, testing of potential antidotes in rodent models is complicated by the rapid progression from symptomatic onset to mortality. An alternative approach has been to measure longitudinal changes in muscle function in gastrocnemius or inguinocrural muscles following local

administration of paralytic doses of toxin. However, these treatments fail to evaluate the effects of intoxication and recovery on the diaphragm, which is the clinically relevant target of botulism, and involve profound paralysis of the injected muscle, which presents a therapeutically challenging model. Therefore, we sought to establish a sublethal model of botulism that enabled evaluation of therapeutic treatments on diaphragm function. Here we demonstrate that systemic administration of a sublethal dose of BoNT/A caused robust changes in running wheel activity (RWA) that closely correlated with *ex vivo* measurements of diaphragm function. Following establishment of stable RWA, mice were administered a single intraperitoneal injection of BoNT/A that resulted in botulism symptoms in ~70% of mice, but caused less than 10% mortality. Symptomatic mice exhibited a greater than 95% reduction in RWA that lasted for up to 7 d, followed by a gradual, linear recovery to baseline levels over the subsequent 14 d. In contrast, mice that remained asymptomatic or received antitoxin concomitant with BoNT/A exhibited no significant differences in RWA. To determine if changes in RWA were correlated to diaphragm impairment, we measured nerve-elicited twitch and tetanic contraction in isolated hemidiaphragms from symptomatic mice at 3, 7, 14 and 21 d post-intoxication. Force-frequency relationships demonstrated that longitudinal changes in diaphragm function were closely correlated to RWA, suggesting that voluntary running behavior can serve as a simple, reliable correlate of diaphragm paralysis in mice. This model is currently in use to evaluate lead candidate therapeutics for botulism, including intraneuronal delivery of single chain antibodies (Cyto-111). Our studies indicate antidotal efficiency of Cyto-111 at all stages of disease and in multiple animal models.

**Disclosures:** **A.B. Bradford:** None. **M.J. Stenslik:** None. **E.J. Vazquez-Cintron:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cytodel, LLC. **M.C. Mangkhalakhili:** None. **M. Eisen:** None. **D.L. Nguyen:** None. **C.A. Ondeck:** None. **P.M. McNutt:** None.

**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 597.02/KK31

**Topic:** E.10. Motor Neurons and Muscle

**Title:** The effect of exercise on aging spinal cord and peripheral nerves

**Authors: *E. GIORGETTI**[1], M. PANESAR[2], M. RONCO[2], C. LAMBERT[3], M. OBRECHT[3], N. ACCART-GRIS[3], N. BECKMANN[3], S. BRACHAT[4], D. SHIMSHEK[5], M. BIDINOSTI[2], M. NASH[2]
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**Abstract:** Denervation and motor neuron degeneration are key features of aging and motor neuron diseases. Gradual loss of motor neurons is observed after 60 years of age and old age is itself the biggest risk factor in many neurodegenerative diseases. A similar decline in functioning motor units is observed in mice, where a decline in motor units begins at 12 months. Moreover, there is a slower recovery following sciatic nerve crush in old mice compared to young, highlighting the impairment of regenerative mechanisms with aging. Preventing this decline in motor neuron function may be fundamental to improve mobility and overall life quality in the elderly population.
The beneficial effects of exercise have been extensively documented. Here we describe the effect of exercise on Motor Unit Number Estimation (MUNE) and gene expression in spinal cord. Interestingly, age-related MUNE decline is prevented by exercise in mid-age mice, and even restored to levels observed in young mice, suggesting that nerve-muscle communication can potentially be re-established at that age. Moreover, muscle and spinal cord samples were analyzed by RNA sequencing. Exercise was able to reduce muscle atrophy and to promote a shift towards a slower-oxidative muscle fiber phenotype. On the other hand, pathway analysis in aged spinal cord revealed upregulation of genes related to microglia and, surprisingly, the same set of genes was downregulated by exercise. The results were validated by RT-qPCR.
These data prompted us to assess whether any correlation exists between the over-activation of microglia in aged spinal cord and the decline in MUNE and whether motor unit loss could be reduced or slowed by depleting harmful microglia in the CNS. To first prove a link between over-activation of microglia in spinal cord and motor unit loss, we performed a sciatic nerve crush study in young mice. Interestingly, at 1 and 2 weeks post-surgery a partial activation of microglia was observed in spinal cord. These data suggested that motor neuron damage could trigger microglia activation in spinal cord. Over-activated microglia, in turn, could be harmful to many cell types, especially neurons: in fact, a large body of evidence suggests that abnormally activated microglia in the aged CNS can be neurotoxic. Therefore, a study with a CSF1R inhibitor to deplete microglia and reduce their migration/proliferation in the CNS, in mid-aged mice is ongoing and we aim to demonstrate a beneficial effect on MUNE. The study will potentially open new therapeutic opportunities for the treatment of the aging neuromuscular system.

**Disclosures: E. Giorgetti:** None. **M. Panesar:** None. **M. Ronco:** None. **C. Lambert:** None. **M. Obrecht:** None. **N. Accart-Gris:** None. **N. Beckmann:** None. **S. Brachat:** None. **D. Shimshek:** None. **M. Bidinosti:** None. **M. Nash:** None.

**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** CIHR MOP 130373

ANR-10-IAIHU-06

AFM Téléthon

**Title:** Biophysical characterization of two Nav1.4 mutations identified in patients with cold-induced myotonia or periodic paralysis

**Authors: H. POULIN**[1], S. VICART[2], K. HABBOUT[3], D. STERNBERG[4], S. GIULIANO[3], B. FONTAINE[5], S. BENDAHHOU[3], S. NICOLE[4], *M. CHAHINE[1]
[1]Medecine, IUSMQ Ctr. de recherche CERVO, Québec, QC, Canada; [2]Hôpital de la Pitié Salpêtrière, Paris, France, Paris, France; [3]UMR7370 CNRS, LP2M, Labex ICST, Univ. Nice Sophia-Antipolis, Nice, France; [4]INSERM, U1127, Paris, France, Sorbonne universités, UPMC Univ. Paris 6, UMR S1127, Paris, France; [5]NSERM, U1127, Paris, France, Sorbonne universités, UPMC Univ. Paris 6, UMR S1127, Paris, France

**Abstract: Introduction:** Mutations in the *SCN4A* gene, which encodes $Na_v1.4$, the skeletal muscle voltage-gated $Na^+$ channel, underlie several skeletal muscle ion channelopathies with two major phenotypes: enhanced excitability (myotonia) and transient loss of excitability (periodic paralysis). Alternations between these two conditions cause a severe loss of function (myasthenia). We report here the functional characterization of two dominant *SCN4A* missense mutations targeting the R1451 residue located in transmembrane segment S4 of DIV composing the voltage sensor domain and resulting in distinct biophysical phenotypes: The R1451L is a novel mutation found in two unrelated patients. The first patient was diagnosed with SMC, and the second with a mixed hyperPP-hypoPP phenotypes. The second mutation (R1451C) was previously reported by our group in one patient with a single attack of PP induced by glucocorticoids. **Methods:** To elucidate the mechanism underlying the phenotypes caused by the R1451C/L mutations and their roles in different types of sodium channelopathies, we used the whole-cell patch-clamp technique to study tsA201 cells expressing WT, R1451C or R1451L channels. **Results:** Our results showed that both mutations shifted the steady-state inactivation to hyperpolarized potentials, reduced the current density, slowed the recovery from slow inactivation and exhibited a slow of the overall kinetics of fast inactivation. Cooling further enhances the abnormalities of fast inactivation kinetics in R1451L channels. Homology modeling revealed a dissimilar disruption of hydrogen bonds in the VSD of R1451C/L mutant channels. **Conclusion:** We concluded that the altered biophysical properties of R1451C/L channels account for the clinical phenotypes seen in our patients but that additional factors are likely to play a role in the diversity of symptoms.

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**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** KAKENHI/16K00380 to R.K.

KAKENHI/17H01810 to I.T.

**Title:** Real time monitoring system for lactic acid released from *Ex vivo* preparations

**Authors: *K. SHOTO**[1], S. NEGISHI[1], I. TAKASHIMA[2], H. KUDO[1], R. KAJIWARA[1]
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**Abstract:** The lactic acid is well known substance involved in muscle fatigue. However, recent report suggested that the lactic acid can improve the function of depolarized muscles. In our previous study, we have constructed an continuous LA monitoring system using a micro-fluidic dual-analyte (LA and glucose) (Negishi et al, 2016). The system is suitable for organ level experiments in conjunction with the recording chamber for ex vivo preparations. This system is expected to be free from the effects of movement, breathing, or interactions with other organs or larger organ systems, and allow a physiological experiment under special conditions that cannot be conducted in in vivo. In the present study, we applied the LA monitoring system to various recording chamber, and measured the change of micro amount LA released from the biological organs such as nerve-muscle and brain tissue preparations. Our system consists of a multi-analyte (LA and glucose) biosensor, poly vinyl chloride (PVC) adhesive sheet with micro flow channel (width: 500μm) and polydimethyl siloxane (PDMS) sheet with inlet/outlet tubes for supplying artificial cerebrospinal fluid (aCSF) and experimental solutions. We used polyethylene terephthalate (PET) as a substrate material of the biosensor and printed carbon graphite electrodes on the substrate by conventional screen printing method. The biosensor utilizes the redox reaction of osmium-wired horseradish peroxidase (Os-HRP) by enzymatic reaction. Owing to the Os-HRP redox reaction, the sensor is operated under the low potential of 0 V vs. silver / silver chloride electrode, which is possible to reduce the effect of interferences such as Ethanol. The biosensor has four electrodes (working electrode 1 on which lactate oxidase (LOD) is immobilized, working electrode 2 on which glucose oxidase (GOD) is immobilized, silver / silver chloride reference electrode and counter electrode). LA and glucose measured by amperometric method as a change of hydrogen peroxide which is produced by enzymatic reaction of LOD and GOD. The ex vivo monitoring of LA from nerve-muscle preparations was performed using the our system. We placed the 1cm nerve fiber of the mouse gastrocnemius muscles to the recording chamber, and supplied the aCSF via the inlet cannula. The LA concentration in the experimental solution was continuously monitored from the outlet port of

the chamber. By perfusing the high-[K+] solution, we observed the increase of the LA level. This increase should be caused by the neuromuscular activity of the preparation.

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**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 597.05/KK34

**Topic:** E.10. Motor Neurons and Muscle

**Title:** Evidences of mitochondrial dysfunction in the muscle biopsy of patients with lipid storage disorders

**Authors: *D. BANDOPADHYAY**
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**Abstract:** Lipid storage disorders are a group of inherited metabolic disorders in which lipid accumulates in cells and tissues. Defects in lipid metabolism affect either the mitochondrial transport and oxidation of exogenous fatty acid or the catabolism of endogenous triglycerides. These disorders impair mitochondrial bioenergetics in skeletal muscle causing progressive myopathy with muscle weakness. However, a comprehensive study involving histology, biochemical and proteomic approaches in human ipid storage disorders is lacking. We assessed the mitochondrial function in clinically evaluated and histopathologically confirmed cases of lipid storage disorders (n=5) vs control (n=5). Enzyme histochemistry (COX-SDH staining) on these samples indicated the presence of COX deficient fibres suggesting mitochondrial dysfunction. Ultrastructure analysis revealed presence of cytoplasmic lipid droplets, disarray of myofibril, Z-band streaming and loss of connectivity of myofibril. Apart few mitochondria showed presence of lipid droplet and electron dense material. Mitochondrial dysfunction was indicated by lower activity of respiratory complex (I, II, III & IV) and elevated ADP/ATP ratio. At 10 plex proteomic experiment of mitochondrial extract from 5 lipid storage disorder and 5 controls revealed a upregulation of 38 and downregulation of 60 mitochondrial proteins. Most of the differentially regulated proteins were part of respiratory complexes and tricarboxylic acid (TCA) cycle. Enzyme assay of selected proteins validated the proteomic data. Together we propose that mitochondrial dysfunction play a role in lipid storage disorders and similar metabolic disorders. On a therapeutic note, addressing mitochondrial protection via targeted drugs could be a treatment modality in such metabolic diseases.

**Disclosures: D. Bandopadhyay:** None.

**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

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**Topic:** E.10. Motor Neurons and Muscle

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**Title:** Comparison of small ankryin 1 and sarcolipin regulation of SERCA1 in myocytes to SERCA2B in neurons

**Authors: *A. LABUZA**, P. F. DESMOND, J. MURIEL, M. L. MARKWARDT, A. E. MANCINI, M. A. RIZZO, R. J. BLOCH
Physiol., Univ. of Maryland Baltimore, Baltimore, MD

**Abstract:** Sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase (SERCA) regulates intracellular $Ca^{2+}$ concentrations by clearing cytosolic $Ca^{2+}$ into the lumen of the endoplasmic reticulum, and, in muscle, the sarcoplasmic reticulum (SR). Three major isoforms of SERCA include SERCA1, primarily expressed in skeletal muscle, SERCA2A, primarily expressed in cardiac muscle, and SERCA2B, which is ubiquitously expressed. SERCA1 is known to be inhibited by sarcolipin (SLN), a small transmembrane protein. We have recently shown that SERCA1 activity is also inhibited by small ankyrin 1 (sAnk1), a ~20kDa transmembrane protein that also binds to the cytoskeletal protein, obscurin. We have demonstrated SERCA1, SLN, and sAnk1 can form a three-way complex.
SERCA2B is known to be expressed within the ER of glial cells and neurons, where it is found particularly in dendrites and spines. However, it is unknown if sAnk1 regulates SERCA2B in brain tissue. Here, using qPCR we show mRNA expression of sAnk1, SLN, and SERCA2B in cortex and cerebellum. SERCA2B expression is at least 5 fold higher than the SERCA1 or SERCA2A isoforms in cortex. SERCA2B expression was lower than SERCA1 and SERCA2A in muscle and heart, respectively. We are now determining if, like SERCA2B, SLN and sAnk1 are expressed in neurons, and if so, if they regulate the activity of SERCA2B in the same way that they affect SERCA1. These results have significant implications for the development of therapeutic approaches to treat a variety of diseases linked to calcium misregulation such as muscular dystrophies and neuropathies.

**Disclosures: A. Labuza:** None. **P.F. Desmond:** None. **J. Muriel:** None. **M.L. Markwardt:** None. **A.E. Mancini:** None. **M.A. Rizzo:** None. **R.J. Bloch:** None.

**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 597.07/KK36

**Topic:** E.10. Motor Neurons and Muscle

**Support:** NCCR Grant 565488

**Title:** Morphological of peripheral axons for ultrasound neuromodulation

**Authors: *T. LEMAIRE**[1], S. MICERA[2]
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**Abstract:** Ultrasonic Stimulation (US) is an emerging technology that has the potential to achieve reliable and selective neuromodulation of central and peripheral neural structures from a distance. However, in order for US to become a reliable neuromodulation technology, we need a deeper understanding of the fundamental mechanism(s) by which ultrasonic waves can modulate neural activity. In this study, two morphological models were developed to predict the behaviour of myelinated and unmyelinated peripheral axons subject to US. For that purpose, the recent model of *Neuronal Intramembrane Cavitation Excitation (NICE)* has been implemented, and a new optimization method has been developed. The previously bidirectional coupling of the electrophysiological and cavitation mechanics parts has been broken up and further pre-computation allowed to derive a new set of Hodgkin-Huxley coefficients grasping the net effect of mechanical oscillations on the membrane dynamics with a virtually constant capacitance. This approach accelerated the resolution time by more than two orders of magnitude without significant impact on the results. Compartmental cell models were then developed in the *NEURON* simulation environment, and resonant sonophores structures were modelled both as continuous distributions and stochastic occurrences in specific compartments. Membrane dynamics was defined using the pre-computed Hodgkin-Huxley coefficients. Myelin was modelled as a mechanical resistor that could either partially or totally block the transmission of ultrasonic waves to the membrane. Models were evaluated with simple acoustic amplitude distributions along the axon, and fundamental differences in the responses of myelinated and unmyelinated axons were characterized. We plan to couple these morphological models with computational acoustic models of nerve anatomy to evaluate the behaviour of the framework in more realistic conditions.

**Disclosures: T. Lemaire:** None. **S. Micera:** None.

**Poster**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement 665667 (call 2015)

Bertarelli Fondation

**Title:** Grasp smarter, not harder: Proportional control of an electromyographic prosthesis with a touch of automation

**Authors: *K. Z. ZHUANG**[1], N. SOMMER[2], E. FORMENTO[3], E. D'ANNA[3], A. BILLARD[2], S. MICERA[3]
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**Abstract:** Over recent decades, myoelectric prostheses have demonstrated their potential to vastly improve autonomy and quality of life for upper-limb amputees by restoring some lost mobility. However, current upper-limb myoelectric prostheses are very limited in their number of controllable degrees of freedom, often allowing only a few discrete grasp types, while also requiring substantial visual attention. Here, we demonstrate surface electromyogram decoding of individual finger joint angles in real time using a multilayer perceptron model. The decoder is able to achieve simultaneous high-accuracy predictions of these many degrees of freedom during numerous sessions with both able-bodied and amputee subjects. In addition, we provide a proof-of-concept of a shared-control scheme. In this mode, the user is able to control preshaping and grasp intention while an assistive compliant controller automates fine digit movements to maximize object contact during actual grasping. This combination of multi-degree-of-freedom proportional control and automated grasp tuning allows for both precise positioning as well as lower user attention. We believe from our encouraging results that the future of myoelectric prostheses will lie within such a synergy between robotic automation and user intention.

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**Poster**

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**Program#/Poster#:** 597.09/LL2

**Topic:** E.10. Motor Neurons and Muscle

**Support:** Wyss Center for Bio and Neuroengineering

**Title:** Rostrocaudal activation patterns in the human cervical spinal cord identified by fMRI during upper limb motor tasks

**Authors: *N. KINANY**[1], E. PIRONDINI[2], R. MARTUZZI[3], L. MATTERA[3], D. VAN DE VILLE[1], S. MICERA[1]
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**Abstract:** The spinal cord is the main interface between the brain and the periphery. Rather than acting as a simple relay, it actively participates in the sensorimotor processes. However, the nature of the underlying spinal mechanisms remains to be investigated. In this regard, non-invasive imaging of the spinal cord stands as a promising tool. Nevertheless, many challenges (e.g., small cross-sectional area, field inhomogeneities or also physiological noise) have hindered the development of functional magnetic resonance imaging (fMRI) approaches for the spinal cord. Although new technological advances in terms of equipment and pre-processing have allowed to circumvent these constraints, a thorough characterization of the potential and limitations of the technique is still lacking. Notably, the precision and the reliability of spinal cord fMRI are still debated.
Here we aimed at assessing whether spinal activity can be reliably detected in different segments of the spinal cord during upper limb motor tasks. We performed spinal cord fMRI in right-handed healthy volunteers using a T2*-weighted gradient-echo echo-planar-imaging sequence on a 3.0 Tesla MRI system. We probed blood oxygenation level-dependent signal changes in the cervical spinal cord during a task-based experiment involving alternating periods of rest and upper limb movements. The movements were selected to include different myotomes, in order to potentially elicit activations in different spinal segments. Electromyographic recordings were also employed to relate muscular and spinal activities. Finally, physiological signals (pulse oximetry and respiration) were acquired over the course of the experiment and further used for noise removal.
Task-related activity was observed at the individual and group level. More specifically, the selected movements were characterized by specific patterns of activation, distributed rostrocaudally and in distinct segments consistently with anatomy. Our results suggest that the recorded fMRI signals stem from a neural origin and reflect the underlying activation of the

motoneuron pools innervating the task-related muscles. Spinal cord fMRI thus offers the prospect of a novel tool to study motor processes, as well as their modification following neuro-motor disorders.

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**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 597.10/LL3

**Topic:** E.10. Motor Neurons and Muscle

**Title:** Effect of tibialis anterior fatigue on the recruitment curve of the soleus h-reflex

**Authors: *I. E. JESUS MAGALHÃES**[1], R. B. NETO[1], M. BOTTARO[2], R. A. MEZZARANE[1]
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**Abstract:** Fatigue is a reduction in the maximal voluntary force that occurs after constant muscle activation. Changes in the excitability of reflex pathways indicate neurophysiological effects of the fatigue. The analysis of parameters obtained from sigmoidal fitting of the soleus (SO) H-reflex recruitment curve (RC) was presently used to evaluate the neuronal adaptations in different populations of motor units followed fatigue of tibialis anterior (TA) muscle. Such adaptations have already been reported in previous works, however, there are scarce information about the fatigue effect of the antagonist muscle on the excitability of the stretch reflex pathway. Therefore, the aim of the present study was to investigate the possible effects of fatigue in TA on the recruitment pattern of the SO H-reflex. Seventeen healthy and physically active male subjects, between 18 and 35 years, participated in the present study. The recruitment curve (32 points) of the right SO H-reflex was built by changing the electrical stimulus in seven different moments: at rest, after a maximal isometric voluntary contraction (MIVC) of the anterior tibialis in the isokinetic dynamometer, immediately after the MIVC, and 5, 10, 15 and 20 minutes after the fatigue protocol. It was asked the subjects to maintain a contraction level between 40 and 60% of the MIVC, with visual feedback from the dynamometer software. Fatigue was determined as the incapacity to maintain the selected level of force for 5 consecutive seconds. Parameters from a sigmoid function fitted to the RC values were obtained. An ANOVA of repeated measures was used to detect differences in these parameters across conditions. The significance level was set at $p<0,05$. There was no statistical difference in parameters extracted from the RC, despite a tendency of increased amplitude of the threshold H-reflex (from the first motor units being recruited), as compared to the remaining conditions. This result could suggest a higher excitability of the first recruited low threshold motor units after fatigue of the antagonist muscle. Additional experiments will be performed to confirm or refute this hypothesis.

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**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

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**Program#/Poster#:** 597.11/LL4

**Topic:** E.10. Motor Neurons and Muscle

**Support:** Swartz foundation

**Title:** Primary motor cortex TMS-evoked ERPs decomposed using ICA

**Authors: *M. MIYAKOSHI**[1], M. R. BORICH[2], T. MULLEN[3], S. MAKEIG[4]
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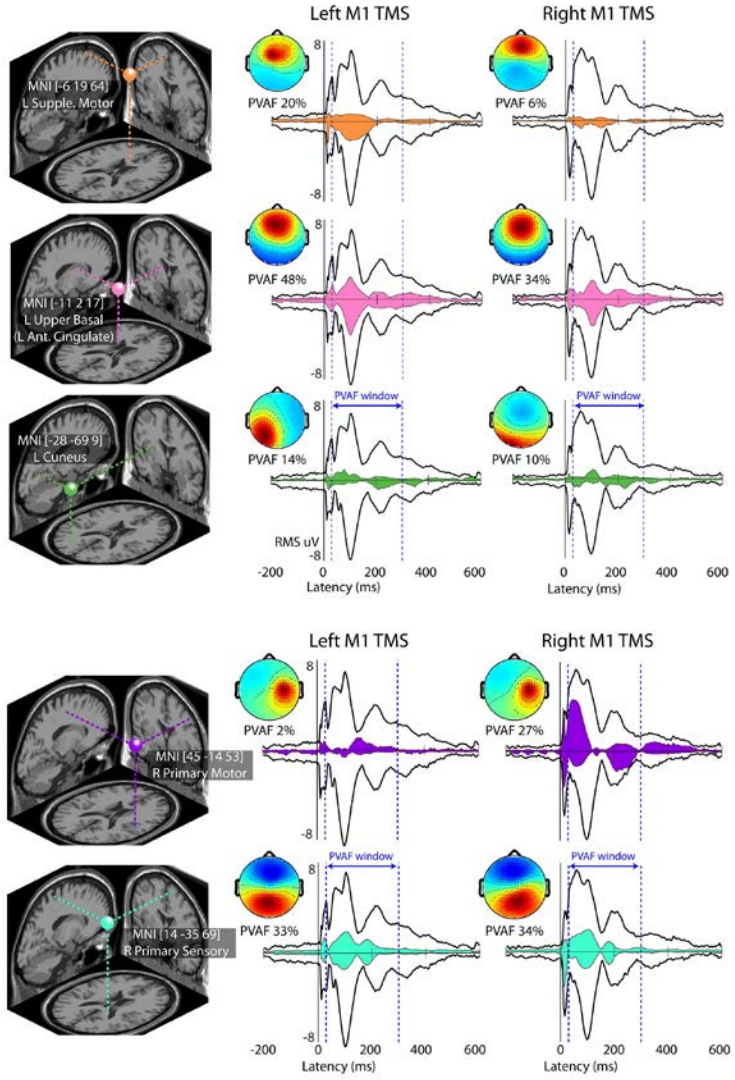
**Abstract:** To test whether high-density encephalographic (EEG) recording during transcranial magnetic stimulation (TMS) allows effective source-resolved imaging of localized cortical activities that are broadly projected to the scalp by volume conduction, we investigated the cortical sources of event-related potential (ERP) responses to TMS stimulation over primary motor cortex. Twelve healthy participants participated in the study. Single TMS pulses were delivered at 120% of resting motor threshold (RMT) over right or left primary motor cortex (M1) during eyes open rest. We replaced pulse-contaminated data points (0-10 ms) with autoregressively-extended EEG that we then blended into the original data (10-20 ms). Preprocessed EEG data were decomposed by adaptive mixture independent component analysis (AMICA). Resulting independent component (IC) processes were clustered across subjects based on dipole locations. Results indicated: 1) the same small set of IC clusters dominated both the early (10-30 ms) and late (30-300 ms) ERP time courses; 2) the largest difference between right- and left-stimulation ERPs (near 60 ms) was earlier than first major ERP peak (near 100 ms); 3) the primary effective sources of the ERPs were in primary sensorimotor, anterior cingulate, and cuneus; 4) primary motor sources showed strongest side-of-stimulation response differences. Thus, source-resolved EEG analysis of TMS-evoked responses can identify, localize, and quantify the activities of major ERP generators in cortical locations that were here largely in common across subjects. Source-resolved EEG imaging before, during, and after TMS could improve both our understanding and therapeutic targeting of abnormal cortical activities associated with many neurologic conditions.




**Disclosures:** **M. Miyakoshi:** None. **M.R. Borich:** None. **T. Mullen:** None. **S. Makeig:** None.

**Poster**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** Supported by a Xunta de Galicia Grant (Consolidación y Estructuración de Unidades Competitivas), Spain.

**Title:** Primary motor cortex (M1) inhibition increases during fatiguing repetitive movements but force and central drive to the muscle are preserved

**Authors: P. ARIAS**[1], E. MADINABEITIA[1], A. MADRID[1], *J. CUDEIRO[2,3]
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**Abstract:** Muscle fatigue has central and peripheral origins. While the central origin of muscle fatigue induced by isometric activities is well defined, little is known about the central determinants of fatigue for repetitive movements. Perhaps for this reason, the gold-standard reference to determine central fatigue in clinical practice is the reduction in both, muscle force and central drive to the muscle during isometric activities. This reduction appears in parallel with an increase in inhibition of M1 and spinal cord circuits. In this work, we tested if force and central drive to the muscle are reduced after fatiguing repetitive movements; we also tested the state of inhibitory circuits in the motor cortex right at the end of the fatiguing activity.
Eleven young healthy subjects participated in different experimental blocks involving the execution of several sets of finger tapping (*ft*), all performed at the maximal possible rate for 30secs. Before and immediately after *ft* we tested: i) muscle force ii) the level of central drive to the muscle; iii) the silent-period (SP) induced by transcranial magnetic stimulation which is a surrogate marker of the excitability of inhibitory M1-interneurons (GABAb-dependent).
The *ft* rate decrease rapidly, ≈18% at the end of the task ($p<0.001$); while SP increased (≈18%; $p<0.001$). Force and central drive to the muscle were not different before and after *ft*.
Neither, the reduction of force nor the central drive to the muscle are underlying factors of fatigue induced by repetitive movements. This type of fatigue presents a central component, which physiological mark is the increased inhibition of M1. These results might be of importance in the fields of sport, ergonomics, and in the study of diseases where fatigue is a clinical sign.

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**Poster**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** JSPS KAKENHI Grant Number 26750308

**Title:** Modulations in corticospinal excitability accompanying contraction mode conversion during joint movements

**Authors: *A. HIGASHIHARA**[1], K. NAKAGAWA[2,4], K. NAKAZAWA[3]
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**Abstract:** Lengthening (LEN) and shortening (SHO) contractions are the fundamental patterns of muscle activity underlying movement. Previous studies have shown that excitability of the corticospinal pathway is significantly lower during LEN than during SHO contractions, despite the similarity of their background electromyographic activity (BGA) levels (Abbruzzese et al., 1994; Sekiguchi et al., 2001, 2003; Uematsu et al., 2010). However, modulation of corticospinal excitability accompanying contraction-mode conversion during joint movements is unclear. The present study investigated phasic modulation in corticospinal excitability during wrist joint movements. Participants performed single, rapid wrist movements by resisting and lifting weights (20% maximal voluntary, isometric contraction force) connected to a dynamometer through a pulley system. Single transcranial magnetic stimulation (TMS) at 130% of the active motor threshold was applied to the left M1 at random timing before and after wrist movement conversion. Motor evoked potentials (MEPs) were measured as the peak-to-peak amplitude value from the right flexor carpi radialis muscle (FCR). The BGA of the FCR was averaged in a 20-ms window just before the TMS was delivered. The timing of the TMS was rearranged referencing to the timing at which the wrist movement converted, and each data were sorted into 5-degree bins and were averaged within each bin. A two-way repeated analysis of variance was used to compare the differences between before (LEN phase) and after (CON phase) wrist movement conversion for corresponding symmetric angles. The results showed that the BGA of the FCR during the LEN phase was significantly larger than that of the CON phase, whereas there were no differences in the MEPs between two phases. These results suggest that during the LEN phase before the wrist movement conversion, the inhibitory component of the corticospinal pathway was relatively higher than that during SHO contractions.

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**Poster**

**597. Motor Neuron: Muscle Exercise and Movement**

**Location:** Halls A-C

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**Program#/Poster#:** 597.14/LL7

**Topic:** E.10. Motor Neurons and Muscle

**Title:** Influence of passive finger movement with & without duty cycle on corticospinal excitability

**Authors: *S. TSUIKI**[1,2], R. SASAKI[1,2], S. MIYAGUCHI[1], S. KOJIMA[1], K. SAITO[1], Y. INUKAI[1], M. MASAKI[1], N. OTSURU[1], H. ONISHI[1]

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**Abstract: Background/Objective:** Previous study reported corticospinal excitability decreases after passive movement (post-exercise cortical depression). However, there are also reports stating that corticospinal excitability increases or does not change after repeated passive movement. These conflicting results are likely due to the use of different intervention conditions. However, it is unclear whether corticospinal excitability is influenced by passive movement with duty cycle. Here we aimed to clarify the effect of passive finger movement with & without duty cycle on corticospinal excitability. **Methods:** Nine healthy subjects participated in this study. Motor-evoked potentials (MEPs) were used to evaluate corticospinal excitability before intervention (pre) & immediately (post0), 5 min (post5), & 10 min (post10) after intervention with transcranial magnetic stimulation (TMS). MEPs were recorded from the right first dorsal interosseous (FDI) muscle. The intensity of the stimulator output was adjusted to baseline recordings so that the average stimulus produced an MEP of 1 mV in the relaxed FDI muscle. TMS was delivered over 15 trials. For passive movement, repetitive abduction-adduction movements of the right index finger were performed for 10 min; movement ranged from a neutral position to 20° of abduction. Experiments were completed using three conditions: (1) movement velocity of 40°/s & consecutive repetitive passive movement (600 times); (2) movement velocity of 40°/s & intermittent repetitive passive movement (240 times) with a configured duty cycle of 4 s on/6 s off; & (3) movement velocity of 100°/s & intermittent repetitive passive movement (600 times) with a configured duty cycle of 4 s on/6 s off. All experiments were performed in the afternoon, and the three interventions were performed in a repeated measurement design in a random order with an interval of at least 3 days between each condition. Mean MEP amplitudes were calculated from peak-to-peak amplitudes. **Results:** A two-way repeated measures analysis of variance revealed a significant condition & time interaction ($p < 0.05$). MEP amplitudes elicited at post0 & post5 under condition 1 were significantly smaller than pre-intervention values ($p < 0.05$). However, MEP amplitudes at pre-intervention & post-intervention under conditions 2 & 3 were not significantly different. **Conclusion:** These results demonstrate that 10 min of consecutive repetitive passive movement, but not intermittent repetitive passive movement with a configured duty cycle, reduces corticospinal excitability.

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**Poster**

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**Location:** Halls A-C

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIH Grant NS091836

**Title:** Integration of sensory and motor inputs in spinal motoneurons

**Authors: *A. A. MAHROUS**, S. M. ELBASIOUNY
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**Abstract:** Motoneurons in the spinal cord represent the final common pathway for the motor commands processed in the nervous system. A motor intent that originates in the brain reaches spinal motoneurons through descending tracts (motor input). The motoneurons also receive feedback from their target muscles through the dorsal root ganglia sensory neurons (sensory input). Each of these two inputs has been separately investigated in many studies. However, the ability to perform precise movements and develop motor skills require closed-loop motor control in which both inputs are integrated. We investigated the interaction between motor and sensory inputs to motoneurons in the adult mouse spinal cord in vitro. Sensory inputs were induced by electrical stimulation of the dorsal roots, while motor inputs were induced by stimulation of the descending axons through an electrode placed on the surface of the cord. The two inputs were induced separately and then simultaneously at different frequencies, intensities, and neuromodulatory states. The motor output in response to stimulation was recorded from single motoneurons using sharp electrodes, or from ventral roots using bipolar wire electrodes. Our results show a non-linear fashion of summation of the two inputs at the cellular as well as the motor pool levels.

**Disclosures: A.A. Mahrous:** None. **S.M. Elbasiouny:** None.

**Poster**

**598. Motor Neuron: Development**

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**Program#/Poster#:** 598.01/LL9

**Topic:** E.10. Motor Neurons and Muscle

**Support:** DFG ZA 885/1-1

**Title:** Dissecting classical cadherins roles in motor neuron positioning

**Authors: *C. DEWITZ**[1], P. HACKEL[1], N. ZAMPIERI[1,2]
[1]AG Zampieri, Max Delbruck Ctr. For Mol. Med., Berlin, Germany; [2]Charité - Universitätsmedizin, Cluster of Excellence NeuroCure, Berlin, Germany

**Abstract:** Circuits controlling the activity of motor neurons are crucial for the execution of motor programs that underlie movement. The precise organization of different motor neuron subtypes into spatially distinct and conserved structures, termed pools, is a potential strategy to simplify the problem of wiring pre-motor circuits (Surmeli et al., 2011). Thus, it is of great interest to define the mechanisms controlling motor neuron positioning during development in order to better understand the processes regulating the assembly of motor circuits. Previous studies show that classical cadherin (type I and type II) adhesive signaling is a determinant of motor neuron spatial organization (Price et al., 2002; Demireva et al., 2011; Bello et al., 2012). However, the mechanisms and relative contributions of different cadherins are still unclear. Expression patterns, as well as phylogenetic and binding affinity analyses suggest that distinct subsets of type II cadherins conspire to generate an "adhesive code" driving accurate motor neuron positioning into pools. On the other hand, the type I N-cadherin, which is expressed by all motor neurons, may provide a basal level of adhesion necessary for type II cadherins to promote specific recognition. In order to test these hypotheses, we established a quantitative assay to analyze positioning of motor neurons in three dimensions and compared changes in spatial organization after eliminating different combinations of classical cadherins in the mouse spinal cord.

**Disclosures: C. Dewitz:** None. **P. Hackel:** None. **N. Zampieri:** None.

**Poster**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** CONACYT Grant 372422

**Title:** GABAB receptors modulate the synapsis between motoneurons and dorsolateral funiculus

**Authors: *C. X. DELGADO-RAMÍREZ**[1], C. CUELLAR-RAMOS[2], R. DELGADO-LEZAMA[2]
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**Abstract:** Spinal motoneurons integrate the central nervous system information and translate it into movement, requiring a control of its excitability to respond accurately to the requirements of the environment. GABA is an inhibitory transmitter that regulates neuronal excitability and neurotransmitter release via $GABA_B$ Receptors ($GABA_BR$). The aim of this work was to determine whether the $GABA_BR$ located in excitatory and inhibitory terminals of the Dorso

Lateral Funiculus (DLF) that synapse motoneurons are tonically activated by ambient GABA, inhibiting the transmitter release and regulating the excitability of the motoneurons. In slices of the spinal cord of *Trachemys sp* turtles, motoneurons intracellularly recorded were identified by their input resistance, time constant and adaptation of the action potential firing. The action of $GABA_BR$ was evidenced by applying the agonist baclofen, which inhibited synaptic potentials by ~ 50% action that was completely reversed by CGP 54626 ($GABA_BR$ blocker). Interestingly, after recovery of the synaptic potential about 15% facilitation was observed, suggesting a possible tonic inhibition of transmitter release mediated by $GABA_BR$, tonically activated by ambient GABA. In addition, it was found that the excitability of the motoneurons is also regulated by these receptors. Furthermore, blockade of the GABA transporters increased the depression of the synaptic potentials, which suggested that GABA of the extracellular medium does not come from the inversion of the transporters. Our results suggest, that $GABA_BR$ are tonically active by ambient GABA, regulate the strength of synaptic transmission between the DLF terminals and motoneurons and the excitability of these neurons.

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**Poster**

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**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIH-NINDS Grant P01NS057228

NIH-NINDS Grant R01NS57190

NSF GRFP Grant DGE-1444932

**Title:** Restoration of motoneuron KCC2 following peripheral nerve injury is dependent on successful muscle reinnervation

**Authors: *E. T. AKHTER**[1], A. W. ENGLISH[2], F. J. ALVAREZ[3]
[1]Cell Biol. & Physiol., [2]Cell Biol., [3]Physiol., Emory Univ., Atlanta, GA

**Abstract:** Following peripheral nerve injury (PNI), axotomized motoneurons undergo many changes within the spinal cord that are not well understood. One such change is the disappearance of the potassium-chloride cotransporter-2 (KCC2) from the membrane of motoneuron somata and proximal dendrites. In the dorsal horn, KCC2 is downregulated within interneurons due to microglial release of brain-derived neurotrophic factor (BDNF). However, this is not the case for motoneurons that are themselves axotomized after PNI. In this case

microglia-specific deletion of BDNF did not prevent the removal of KCC2 from the proximal somatodendritic membrane of motoneurons. We are investigating alternative mechanisms regulating motoneuron KCC2 after PNI, including a possible role of BDNF from non-microglial sources. We also illustrate that KCC2 is only restored in animals that have undergone successful nerve repair, not those that have had regeneration prevented by tightly ligating their axons. We confirmed these findings by correlating the degree of KCC2 restoration on motoneurons with the success of reinnervating neuromuscular junctions after a sciatic nerve transection followed by repair with fibrin glue. Based on these results we suggest that the regulation of KCC2 on motoneuron proximal cell membranes cannot be entirely explained by cell autonomous mechanisms or release of BDNF from microglia. Instead it is likely that a retrograde signal from the periphery plays an important role in regulating this significant intrinsic motoneuron property.

**Disclosures: E.T. Akhter:** None. **A.W. English:** None. **F.J. Alvarez:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 598.04/LL12

**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIH NINDS R01NS098509

NIH NINDS R01NS085331

**Title:** The distribution of motoneuron excitability among upper extremity motor pools

**Authors: *L. M. MCPHERSON**[1], J. M. WILSON[3], N. K. RENDOS[5,2], R. K. POWERS[6], C. HECKMAN[4], C. K. THOMPSON[7]
[1]Florida Intl. Univ., Miami, FL; [2]Physical Therapy, Florida Intl. Univ., MIami, FL; [3]Physical Therapy & Human Movement Sci., [4]Dept. of Physiol., Northwestern Univ., Chicago, IL; [5]Andrews Res. and Educ. Fndn., Gulf Breeze, FL; [6]Dept Physiol & Biophysics, Univ. Washington, Seattle, WA; [7]Spinal Neuromotor Lab., Temple Univ., Philadelphia, PA

**Abstract:** Differences in neural drive to motor pools of proximal vs. distal muscles of the upper limb likely reflect the distinct roles of these muscle groups in skilled motor control. Proximal muscles optimize arm stability to allow distal muscles to interact with the environment through fine, precise movements. While both proximal and distal muscles are innervated by corticospinal and brainstem-spinal motor pathways, proximal muscles receive a greater proportion from brainstem-spinal pathways, and distal muscles receive a greater proportion from corticospinal pathways. Both of these motor systems transmit motor commands that culminate as excitatory and inhibitory inputs alpha motoneurons. However, brainstem-spinal pathways also provide

crucial neuromodulatory input (via the release of serotonin and norepinephrine) that dramatically affect motoneuron excitability and thus change how motoneurons respond to excitatory and inhibitory input. One mechanism by which this occurs is through facilitation of persistent inward currents (PICs) that amplify and prolong the response of motoneurons to excitatory input. Presumably, this effect of PICs is beneficial for the strong, sustained contractions required of proximal muscles but detrimental to the fine, rapidly-changing activation characteristic of distal muscles.
The purpose of this study was to estimate the amplitude of PICs in deltoid, biceps, triceps, and extrinsic finger flexors and extensors in 5 individuals without neurological impairment. Using motor unit discharge decomposed from high-density surface EMG (Negro et al, 2016) recorded from each muscle, we quantified two aspects of motor unit discharge that are characteristic of PICs during slowly increasing and then decreasing triangle contractions: hysteresis of the firing rate at recruitment vs. de-recruitment (quantified using the delta-F technique), and saturation of the firing rate after the initial recruitment acceleration (quantified using the rate modulation slope). We expected that delta-F would be high in proximal muscles and low in distal muscles, with rate modulation slopes showing the opposite pattern. Group mean values for the DELT, BIC, TRI, FF, and FE for delta-F were 6.4, 3.9, 6.2, 3.6, and 3.2 pps, respectively, and values for rate modulation slopes were .01, 0.38, -0.07, 0.18, and 0.28 pps/%MVT. Values for both metrics were similar for DELT and TRI and fit our expectation; however, BIC was similar to more distal FF and FE. Across all muscles, there was a significant correlation between individual mean delta-F and rate modulation slopes ($r = -0.52$, $p = 0.03$), demonstrating a relationship consistent with activation of PICs.

**Disclosures: L.M. McPherson:** None. **J.M. Wilson:** None. **N.K. Rendos:** None. **R.K. Powers:** None. **C. Heckman:** None. **C.K. Thompson:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 598.05/LL13

**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIH Grant 1 R15 GM119099-01

**Title:** A nova-dependent microexon splicing program controls neuromuscular junction in the tunicate ciona robusta

**Authors: *M. HOSSAIN**[1], A. STOLFI[3], L. CHRISTIAEN[3], M. RUGGIU[2]
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**Abstract:** Alternative splicing (AS) is a major driving force in amplifying the coding capacity of eukaryotic genomes. By generating multiple transcript variants from a single gene, AS produces proteome diversification, modulates protein function, and contributes to tissue specificity. The central nervous system comprises the tissues and cells with the highest rate of AS in the body, and RNA-binding proteins play a major functional role in neurons. Microexons -that is, exons smaller than 50 nt- are of particular interest for nerve cell biology as more than 90% of regulated microexons show the highest rate of inclusion in neurons. Moreover, 1/3 of all neuron-specific AS events conserved between mouse and human are microexons, and microexons engage in critical functions in neurons. The large proteoglycan agrin is a major player at the neuromuscular junction (NMJ), a synapse between nerve cells and muscle cells, and the best-studied model synapse. Interestingly, NMJ function is completely regulated at the level of AS: while agrin is transcribed in every cell of the body, only neurons make a splice variant -termed Z agrin- that is responsible for the formation, development, and maintenance of the NMJ. Interestingly, agrin undergoes AS at 3 different sites termed X, Y, and Z, and all these splice variants encode for microexons, making agrin a unique model gene to study evolution and function of biologically-relevant microexons. In mammals, there are two microexons at the Z site, termed Z8 and Z11 as they encode for 8 and 11 amino acid (AA) peptides, respectively. The Z exons are also of particular interest as they are potential mutation sites in congenital neuromuscular diseases. By using the tunicate *Ciona robusta* as our animal model, here we show that a neural-specific microexon program is conserved between tunicates and mammals, and is regulated by the paraneoplastic neurologic degeneration antigen Nova. We cloned both Nova and agrin from *Ciona* and discovered that the Z exons are even smaller in tunicates, encoding for only 6 and 5 AA respectively. Nova is a neuron-specific splicing regulator with three RNA-binding domains of the KH type. While in mammals Nova appears to regulate splicing via its third KH domain, in *Ciona* Nova requires the first two KH domains to mediate Z exon inclusion, and it does so via a bipartite intronic splicing enhancer downstream of exon Z5. Our work establishes the tunicate *Ciona robusta* as a model organism to study microexon function and its relevance to synapse biology and neurodegenerative diseases.

**Disclosures:** **M. Hossain:** None. **A. Stolfi:** None. **L. Christiaen:** None. **M. Ruggiu:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

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**Program#/Poster#:** 598.06/LL14

**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIEHS/NIH grant R01 ES024064

**Title:** Acute MeHg exposure alters immunofluorescence of the Renshaw Cell Area in the C57BL/6J mouse model

**Authors: *M. RIOS CABANILLAS**, W. ATCHISON
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**Abstract:** Renshaw cells (RCs) are spinal cord inhibitory interneurons that receive excitatory collaterals from alpha motor neurons mediated through cholinergic transmission. RCs send inhibitory axons that synapse back on the same motor neuron through both GABAA- and glycinergic neurotransmission. This completes the negative-feedback circuit named “recurrent inhibition”. Methylmercury (MeHg) is an environmental contaminant that easily binds to cys-loop ligand-gated receptors and it accumulates on spinal cord motor neurons. MeHg neurotoxicity leads to calcium dysregulation and hyperexcitability, which may lead to motor neuron cell death and loss of recurrent inhibition. Chronic MeHg treatment of SOD1 G93A mice hastens the onset of ALS-like phenotype. RCs have been reported to degenerate in both ALS rodent models and patients. Thus, disruption of RC function, or their loss by MeHg could contribute to the hastened development of ALS-like phenotype in the SOD1 mouse model. The goal of this study is to determine if acute (1hr) in situ MeHg exposure alters the expression of proteins associated with RCs (calbindin, gephyrin and ChAT), suggesting loss of RCs which could, in turn affect recurrent inhibition, thereby leading to enhance motor neuron excitability in the lumbar region of the C57BL/6J mouse. The RCs area is determined by the characteristic presence of gephyrin clusters, calbindin d28k immunoreactivity and the presence of cholinergic contacts. Immunohistochemistry demonstrate MeHg toxicity effects on the RCs area measured by the presence of ChAT, calbindin d28k and gephyrin primary antibodies. Staining of all three antibodies was markedly decreased at 5μM MeHg by at least 40 fold from control (ChAT 90%, calbindin d28k 36% and gephyrin 24%). At higher MeHg concentrations, there was first an increase and then decrease in staining intensity. All experiments $n \geq 4$. Research supported by NIEHS/NIH grant R01 ES024064.

**Disclosures: M. Rios Cabanillas:** None. **W. Atchison:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 598.07/LL15

**Topic:** E.10. Motor Neurons and Muscle

**Title:** Comparison of mu rhythm ERS and ERD for cranial nerve and spinal cord related activities

**Authors: *H. BAGHERZADEH**, Q. XIE, K. DEMMERLE, D. GUPTA, F.-S. CHOA
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**Abstract:** It is believed that conscious brain may go through higher level brain hubs to indirectly influence lower level subconscious circuits, particularly those in the deep brain regions, like the roots of cranial nerves and brainstem. It would be interesting and beneficial to understand the mechanisms employed by such activity. One of the potential paths to study these mechanisms is through mirror neurons, which play critical roles in linking doing, seeing, imagination, mind reading (theory of mind), social cognition, language and speech processing, and neural disorders like Autism. Mirror neuron activities usually can be monitored by observing Mu wave suppression at motor cortex. Mu rhythm is an electroencephalography (EEG) brain wave between 8 and 13 Hz recorded by scalp electrodes over the sensorimotor cortex in non-active states. To understand mirror neuron's role in activating subconscious brain circuits, we would like to first quantitatively calibrate a person's mind reading ability. We also would like to separate their influence over spinal cord path and cranial nerve path. Mu wave ERS (Event-Related Synchronization) and ERD (Event-Related Desynchronization) have been proposed as signatures of voluntary movements. Various techniques such as MEG, EEG, and fMRI have been deployed to detect mu wave. In the current study, mu rhythm suppression was investigated using EEG in response to speaking (cranial nerve activity), fist making (spinal cord activity), and their corresponding imagination. Four subjects performing these tasks were studied. Referential (monopolar) EEG montage was obtained using 18 electrodes spread over the scalp including 2 electrodes on sensorimotor cortex (C3 and C4) with each action responding to contralateral hemisphere brain structures. Since left hand was used to press time marker (right brain hemisphere activity), only the signals from the left hemisphere electrodes were analyzed. As expected, significant mu wave suppression was observed in sensorimotor cortex in response to the performed tasks. While the imagination of speaking and fist making could be affected by different neurons, the suppression of mu rhythm on both cases was observed. The signals on the electrodes close to the C3 electrode also changed to a lesser degree during the mu wave suppression. The data show that the mu wave suppression on speaking and fist making action may employ the same mechanism and not separable even though the degree of the mu wave suppression of imagination of speaking is slightly less than that of first making. The results also imply that EEG and mu wave detection can be quantified and possibly used to calibrate a person's mind reading ability.

**Disclosures: H. Bagherzadeh:** None. **Q. Xie:** None. **K. Demmerle:** None. **D. Gupta:** None. **F. Choa:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

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**Program#/Poster#:** 598.08/LL16

**Topic:** E.10. Motor Neurons and Muscle

**Title:** A unique adult insect muscle with polyneuronal innervation displays distinct domains of innervation from different segmental nerves

**Authors: *M. J. VALESKY**, G. W. SMITH, R. J. BAYLINE
Washington & Jefferson Col., Washington, PA

**Abstract:** Motor neuron innervation patterns can heavily influence adult muscle development in a variety of insects. Specifically, innervation can influence myoblast proliferation and accumulation in both *Drosophila melanogaster* and *Manduca sexta*. In the moth *Manduca sexta,* diverse muscles types such as the dorsolongitudinal flight muscle, thoracic leg muscles, and abdominal body wall muscles all require innervation for proper proliferation and growth. One muscle, the tergosternal muscle (TSM), does not show an absolute requirement for innervation. The TSM is unique in that its appearance and development differs from other adult skeletal muscles in *Manduca*. The TSM develops later than other skeletal muscles, beginning accumulation and proliferation 2-3 days later than other abdominal muscles, and forms a sheet of parallel fibers positioned dorosoventrally along the lateral epidermis surrounding the spiracle. In contrast to other adult *Manduca* skeletal muscles that typically receive innervation from single motor neurons, the TSM receives innervation from eight motor neurons with axons in both the dorsal and ventral segmental nerves. Considering that the TSM is the only abdominal muscle to receive innervation from multiple neurons via different nerves in the adult, single TSM fibers may be polyneuronally innervated via both the dorsal and ventral nerves. If true, this would suggest that the mechanism by which motor neurons target individual muscles fibers may differ for the TSM when compared to other abdominal muscles. Anterograde nerve fills reveal that motor neurons derived from the dorsal nerve innervate the anterior two-thirds of the TSM, and motor neurons derived from the ventral nerve innervate the posterior third. Initial physiological recordings support the findings from histological data that the domains of innervation of the TSM from the ventral and dorsal nerve are completely segregated. Taken together, these data reveal that, although the TSM has a unique pattern of development and reduced dependence upon innervation during development, the innervation that it receives is restricted to different domains that is not distinct from other abdominal muscles.

**Disclosures: M.J. Valesky:** None. **G.W. Smith:** None. **R.J. Bayline:** None.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

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**Program#/Poster#:** 598.09/LL17

**Topic:** E.10. Motor Neurons and Muscle

**Support:** NIH R15HD075207

NDF IIS1608147

**Title:** Changes in spinal motor networks when cocultured with astrocytes or striated changes in spinal motor networks when cocultured with astrocytes or striated muscle

**Authors: *A. THARANEETHARAN**, M. A. HARRINGTON
Delaware State Univ., Dover, DE

**Abstract:** Co-cultures are a traditional method for studying the network properties of cell to cell interactions among different cell types. How cellular properties in these multicellular synthetic systems vary from monocultures are of particular interest. Understanding these changes in cell behavior can provide new insights into *in vivo* systems and how to develop models that better reflect physiological conditions - something of paramount importance to the progress of synthetic biology. Through synthetic biology positive social impact can be delivered by the development of future biomedical applications.
*In vitro* models of spinal motor neurons have been customarily studied as a monoculture, and the overwhelming consensus is that in culture they are different in nature from their *in vivo* counterparts. All vertebrate motor neurons are cholinergic, but under common cultured conditions they are primarily glutamatergic. As a control we used E7 embryonic chick spinal ventral horns cultured on a hydrogel matrix substrate, we compared them with those co-cultured with spinal astrocytes or striated muscle also from embryonic chicks.
We studied the electrophysiological properties of these networks using 64 channel multielectrode array (MEA) systems. An increase in synchronous firing was observed when ventral spinal motor neurons were cocultured with astrocytes. Cholinergic receptor activity in spinal motor neurons was notably increased in striated muscle cocultures. We also examined levels of ChAT, GFAP, HB9, VACht, and neurofilament via in the cultured neurons with western blot and immunocytochemistry. Particular attention was given to changes in ChAT, HB9, and VAChT in striated muscle cocultures; while changes in GFAP levels were noted in astrocyte cocultures. Traditional culturing techniques involving a monolayer of uniform cell type might not be the best way to mimic *in vivo* systems. A synthetic ecosystem of various cell subtypes is beneficial to replicating cell behavior *in vitro*, thus is a necessary refinement to the commonly used technique of cell culture. With a more accurate model system, the questions that we ask about interacting systems can be addressed with greater accuracy.

**Disclosures:** **A. Tharaneetharan:** A. Employment/Salary (full or part-time):; Delaware State University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R15HD075207, NDF IIS1608147. **M.A. Harrington:** A. Employment/Salary (full or part-time):; Delaware State University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R15HD075207, NDF IIS1608147.

**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 598.10/LL18

**Topic:** E.10. Motor Neurons and Muscle

**Title:** Gene expression analysis in spinal motoneurons of defined subtypes

**Authors: *N. ISOO**[1], N. MURABE[1], S. FUKUDA[1], M. TANIGUCHI-IKEDA[2], S. TERAMOTO[3], S. KWAK[3], M. SAKURAI[1]
[1]Univ. of Teikyo, Tokyo, Japan; [2]Kobe Univ. Grad. Sch. of Med., Kobe, Japan; [3]Univ. of Tokyo Grad. Sch. of Med., Tokyo, Japan

**Abstract:** Some cells in the motor-related cortices directly control muscles via spinal motoneurons (MNs) by monosynaptic connection in higher primates. It is generally accepted that this direct connection in primates is essential for dexterous hand movements. We previously showed that corticospinal (CS) synapses are once formed throughout the spinal cord, but are eliminated from the ventral horn during development in rodents, raising the possibility that CS axons transiently make direct connections with MNs located in the ventral horn of the spinal cord. Indeed, it was disclosed that MNs innervating distal forearm muscles receive monosynaptic inputs from CS axons electrophysiologically by whole cell recordings and with optogenetic stimulation of CS axons in mice. We then employed a genetically modified rabies virus that spreads only monosynaptically and demonstrated that CS neurons make direct connections with MNs innervating distal forearm muscles in juvenile mice, but lose them by postnatal day (P) 22. Based on these findings, here, we used genetic approaches to elucidate the molecular mechanisms for which MNs transiently make direct connections with CS neurons in juvenaile stage and lose them late in the development. We payed special attention to identify defined MN subtypes as follows. MNs innervating distal forearm muscles of mice were retrogradely labeled via intramuscular injection of fluorescent cholera toxin B subunit to ascertain the location of their MN pool in the spinal cord. Thereafter MNs located in the MN pool of distal forearm

muscles stained with 0.1% toluidine blue, were isolated by laser microdissection from spinal cord slices of P10 and P21 mice. Gene expression profiling of these two samples using microarray was performed and we compared the obtained array data. Among the 22,341 genes surveyed, 278 displayed an increase of more than 2-fold expression and 725 displayed a decrease of less than half in MNs of P10 mice. Of these, genes involved in synaptic function and cell adhesion accounted for 61 and 35 genes, respectively. These findings would contribute to elucidating the molecular mechanisms for emergence of direct connections between CS axons and MNs, which serve as the basis for dexterity in primates.

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**Poster**

**598. Motor Neuron: Development**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 598.11/LL19

**Topic:** E.10. Motor Neurons and Muscle

**Title:** The functional role of activity-dependent plasticity in motoneurons during development revealed by metabotropic glutamate receptor activation

**Authors: *S. S. BERTRAND**, J.-R. CAZALETS, M. SOURIOUX
INCIA CNRS UMR5287, Bordeaux, France

**Abstract:** In neuronal networks, synaptic strength is not constant but depends on the past activity of the synapse. This change in synaptic efficacy called activity-dependent synaptic plasticity (ADSP) is paramount to synaptic processing and maturation. Using spinal cord slices from mice at two developmental stages, 1-4 and 8-12 postnatal days (P1-P4 ; P8-P12), we have previously shown that high-frequency stimulation of presumed reticulospinal neuron axons in the ventrolateral funiculus (VLF) induced either a long-term depression (LTD), a short-term depression (STD) or no synaptic modulation in limb MNs depending on both spinal cord developmental stage and functional flexor or extensor MN subtype.
In the spinal cord, metabotropic glutamate receptors (mGluRs) modulate synaptic transmission and undergo subtype-specific regulation of their expression and localization during development. In the present study, we investigated the impact of mGluR activation on ADSP expression in P1-P3 and P8-P12 MNs. We found that mGluR agonists differentially and selectively modulated ADSP at VLF-MN synapses in a developmentally regulated manner. We then used mGluR activation as a tool to indirectly access the functional role of high-frequency-induced-synaptic plasticity at VLF-MN synapses in locomotor pattern generation. For this purpose, the effects of mGluR agonists were analysed during VLF-induced fictive locomotion in the *in vitro* spinal cord preparation and *in vivo* during swimming episodes recorded from newborn mice.

**Disclosures:** **S.S. Bertrand:** None. **J. Cazalets:** None. **M. Sourioux:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 599.01/LL20

**Topic:** F.01. Neuroethology

**Title:** A novel learning and extinction algorithm enhances goal-directed foraging decisions in simulation

**Authors: E. D. GRIBKOVA**[1], *R. GILLETTE[2]
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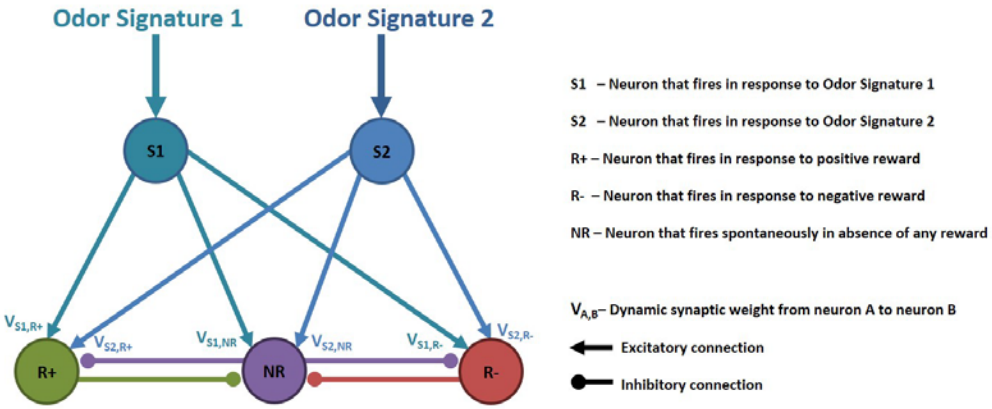
**Abstract:** A wide variety of learning algorithms are known that can establish simple associations between sensory stimuli and rewards. However, few have explored extinction and recovery of these learned associations using the delicate balance of excitation and inhibition often observed in natural neuronal circuits. We used our agent-based foraging simulator, Cyberslug™, to compare three different algorithms for learning: the Rescorla-Wagner model, a temporal difference learning (TDL) model, and a novel learning and extinction circuit. In particular, for this novel learning and extinction circuit we hypothesized that extinction and spontaneous recovery of learned associations can occur primarily due to the spontaneous activity of a central inhibitory neuron. The learning circuit consists of 1) sensory neurons that monitor the agent's perceived odor signatures, 2) reward neurons that monitor the agent's received positive and negative rewards, and 3) a spontaneously active "no-reward" neuron that is in reciprocal inhibition with the reward neurons. Associations between sensory and reward or no-reward neurons are learned through a simple Hebbian learning rule that includes eligibility traces for sensory and reward neuron activity. Forgetting is implemented as a constant and steady decay of learned association strengths. When compared to the other algorithms, this learning and extinction circuit appears to adapt more quickly to changes in environment, including arrival and departure of different types of prey that may express the same odor signatures but provide very different rewards.




**Disclosures:** **E.D. Gribkova:** None. **R. Gillette:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 599.02/DP11/LL21 (Dynamic Poster)

**Topic:** F.01. Neuroethology

**Support:** WRF Innovation Post-Baccalaureate Fellowship

**Title:** Computational strategies underlying arm coordination of Octopus rubescens during naturalistic foraging

**Authors: *D. M. SIVITILLI**[1], V. GOPAL[2], A. SEMINARA[3], J. A. SISNEROS[1], D. H. GIRE[1]
[1]Psychology, Univ. of Washington, Seattle, WA; [2]Physics, Elmhurst Col., Elmhurst, IL; [3]Lab. de Physique de la Matière Condensée, CNRS, Univ. Nice Sophia Antipolis, Nice, France

**Abstract:** The ability to locate food has been one of the main selective pressures during the evolution of cognition. We studied *Octopus rubescens* to define how an evolutionarily distant yet cognitively sophisticated species of animals forages for food under semi-naturalistic conditions. Despite their high degree of visual acuity, octopuses are primarily nocturnal hunters, using the highly concentrated chemoreceptors located in the suckers on their arms to detect and discriminate odors emanating from food sources, and can distinguish objects by their chemical composition alone. This sensitivity is crucial for nocturnal hunting and navigation strategies through the use of chemical trails and plumes. While their chemosensory abilities rival that of macrosmatic mammals, octopuses have evolved divergent behavioral and neural mechanisms to process chemical information. Each arm of the octopus is highly autonomous neural processing within peripheral ganglia along the arms allows the octopus to locally integrate large amounts of sensory information in parallel. In fact, more of the octopus nervous system is located in the

periphery than in the central brain. To study how this distributed nervous system processes chemical information to guide natural behavior we have developed a food foraging task for the octopus and combined this task with semi-autonomous arm tracking algorithms. We placed food items as well as inert objects at a distance (75cm) from the octopus's den in an aquarium (120x30x45cm). The octopus then foraged for these food items, discriminating between them to locate its preferred food. Octopus behavior was imaged at a high rate (220 Hz) and automated segmentation was performed to quantify the trajectory of the animal and its arm configuration. We found that during nocturnal foraging octopuses alternated between sampling and movement. A typical trajectory involved pausing at a distance from the food source while maximizing the area sampled by its arms, retracting the arms back toward the animal and then moving toward the food source. We compared these trajectories to odor plumes imaged from the food locations (obtained with laser-induced fluorescence of fluorescein) and found that the octopus obtained odor information almost entirely during the sampling phase and not during movement. Taken together, these data suggest that octopuses are capable of extracting directional information from odor plumes by using their arms to sample large areas of a plume, and that this information directs movement toward the probable location of the odor source. Further work will define the algorithms used to integrate odor information across the arms during this behavior.

**Disclosures: D.M. Sivitilli:** None. **V. Gopal:** None. **A. Seminara:** None. **J.A. Sisneros:** None. **D.H. Gire:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

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**Program#/Poster#:** 599.03/LL22

**Topic:** F.01. Neuroethology

**Support:** NSF, grant No. IIS-1065489

**Title:** Robotic platform for understanding peristaltic locomotion

**Authors: *A. KANDHARI**[1], K. A. DALTORIO[1], H. J. CHIEL[2], R. D. QUINN[1]
[1]Mechanical and Aerospace Engin., Case Western Reserve Univ., Cleveland, OH; [2]Case Western Res. Univ., Cleveland, OH

**Abstract:** Soft-bodied animals, such as earthworms, are capable of locomotion in diverse environments. Duplicating these behaviors for robotics would be valuable in constrained-space applications (such as burrowing, exploration and, search and rescue). However, soft-bodied locomotion is a challenging problem for robotics and biological insights can be valuable in both the control and mechanical design. Here, we present a robotic platform for exploring the biomechanics and neurobiologically inspired control of soft-bodied peristaltic locomotion. Our

initial focus in determining the roles of circumferential and longitudinal stiffness for locomotion and turning. The robotic platform is inspired by earthworm locomotion. Earthworms locomote using traveling waves of segment contraction and expansion. Because of the hydrostatic coupling, as the diameter of a segment increases, its length decreases. Segments can also bend. The mechanics of the body result in large range of body shapes which both comply with the environment and contribute to directed locomotion. This is true for both animals and our new twelve DOF soft robotic platform: Compliant Modular Mesh Worm robot with Steering (CMMWorm-S). The CMMWorm-S is a mesh-based robot that uses cables for actuation. The mesh, composed of 3D printed rigid pieces and flexible tubes, allows us to interchange components easily to vary the stiffness of the robot. On this robotic platform, we show that locomotion efficiency is highly sensitive to body stiffness. High longitudinal stiffness improves turning locomotion, whereas higher circumferential stiffness improves straight-line locomotion. However, softness is advantageous to comply with its surroundings in order to access tight spaces, getting traction from irregular surfaces and recovering from external perturbations. Earthworms could be adjusting body stiffness to suit to their particular task based on sensory feedback. In future work, to better model the animals' responsiveness, mechanosensors, such as pressure and stretch receptors, could provide input to neurobiologically inspired control circuits.

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**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

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**Topic:** F.01. Neuroethology

**Support:** NSF IOS-1555933

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Klingenstein-Simmons Fellowship to K.N.

Sloan Fellowship to K.N.

NIH R01MH109690

**Title:** Algorithms underlying olfactory navigation in walking fruit flies

**Authors: *E. ALVAREZ-SALVADO**, K. NAGEL
Neurosci. Inst., NYU Langone Med. Ctr., New York, NY

**Abstract:** In order to locate food sources in the complex, natural environments, fruit flies rely on multi-sensory integration, using odors and wind cues simultaneously. This combined use of environmental information applies beyond the olfactory system, and it is critical for many other organisms. We want to understand the computations necessary to turn complex sensory input into a goal-directed, successful behavior.
To achieve this goal, we have developed a high-throughput assay to monitor olfactory-driven behavior in walking flies, while precisely controlling wind and odor stimuli. Using this apparatus we have identified the major behavioral components that underlie the search for an attractive odor's source. In the presence of odor, flies move faster and straighter towards upwind ("odor-ON"), and following odor offset, a slower searching pattern dominates flies' movements ("odor-OFF"). Surgically blocking the ability of flies to sense wind direction precisely eliminated any directional specificity to these responses, showing that wind is exclusively determining flies' orientation. However, changes in speed and straightness/tortuosity of movements were still observable independently of wind-sensation, therefore being triggered by odor onset/offset alone. These results suggest parallel processing of different sensory cues and provide evidence of cross-modal interaction.
We are currently investigating the intra- and inter-individual variability observed in the different parts of this behavior. Our goal is to test whether there are consistently different patterns of response between individual flies and also across genotypes. These can be expressed in uniquely shaped trajectories or different probabilities of exhibiting odor-ON or OFF responses.
Based on our experimental measurements, we developed a computational model that is capable of reproducing the behavior of real flies. We use this model to show that the algorithms described are sufficient to solve more complex and realistic tasks. Furthermore, our model provides insight into the specific roles played by each of the different components of the flies' behavior.
Together, our data and model help us understand the computations taking place in the fly brain during olfactory search behavior, and represent a first step towards dissecting the neuronal substrates of these computations.

**Disclosures: E. Alvarez-Salvado:** None. **K. Nagel:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 599.05/LL24

**Topic:** F.01. Neuroethology

**Support:** JSPS Grant-in-Aid for JSPS Fellows Grant Number 14J06037

**Title:** The role of neural activities in AIY interneurons for controlling behavior of *Caenorhabditis elegans*

**Authors: *H. MORI**[1], H. SHIDARA[2], K. ASHIDA[3], T. NIKAI[1], K. HOTTA[1], K. OKA[4]
[1]Ctr. for Biosci. and Informatics, Keio Univ. Sch. of Fundamental Sci. and, Yokohama / Kanagawa, Japan; [2]Dept. of Biol. Sciences, Sch. of Sci., Hokkaido Univ., Sapporo, Hokkaido, Japan; [3]Dept. of Biosci. & Informatics, Keio Univ., Yokohama-Shi, Japan; [4]Keio Univ., Yokohama, Kanagawa, Japan

**Abstract:** Interneurons integrate information from sensory neurons, and calculate motor commands for behavioral control. It is known that behavioral strategies are organized by the neural processing in *Caenorhabditis elegans*. For example, when the worms are transferred from on-food plates to off-food ones, they travel in local area at first. Then after 15 minutes, they travel and search in global area. Several interneurons contribute this travel and search behavior, demonstrated by their ablation. AIY interneurons also involve in several behaviors: head movement, reversal initiation and run speed [Kocabas *et al*., 2012; Li *et al*., 2014]. Interestingly, AIY has been known to show sporadic $Ca^{2+}$ responses when worms are stimulated by odor under restricted condition in microfluidic devises [Chalasani *et al*., 2007]; however, it has not been revealed what circumstance induces such sporadic $Ca^{2+}$ responses, and whether it correlates with behaviors or not. In this study, we measured neural activities of AIY in free-moving worms, and investigated the role of sporadic $Ca^{2+}$ responses and $Ca^{2+}$ peaks with high amplitudes.
To visualize neural activities, we used a $Ca^{2+}$ indicator, G-CaMP6 [Ohkura *et al*., 2012]. As previously reported, $Ca^{2+}$ changes in AIY were detected only in neurites, not in soma. To measure the $Ca^{2+}$ level in AIY and behaviors of worms at the same time, we used a home-made tracking system. The system detected AIY locations from binarized fluorescent images, and traced worms by controlling a x-y stage driven by stepping motors. In our experiment, we investigated two pre-conditioned worms; one condition was named ‘control’ - worms just transferred from on-food plates to off-food plates, and the other condition was ‘starving’ - worms left 30 minutes after transferred to off-food plates. In both conditions, some worms showed sporadic $Ca^{2+}$ responses and $Ca^{2+}$ peaks with high amplitudes, which exceed average + 5SD of the basal activities. We investigated differences between animals with and without $Ca^{2+}$ peaks in each condition. Without regard to the control and starving, AIY activities have positive correlation with the speed of forward run in worms with $Ca^{2+}$ peaks. However, worms without peaks in starving, AIY activities have no correlation with the forward speed. These results indicate that sporadic $Ca^{2+}$ responses are important for control of forward speed, especially in starved worms.

**Disclosures: H. Mori:** None. **H. Shidara:** None. **K. Ashida:** None. **T. Nikai:** None. **K. Hotta:** None. **K. Oka:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 599.06/LL25

**Topic:** F.01. Neuroethology

**Title:** Innate locomotor bias in larval zebrafish influences behavioral choice

**Authors: *E. J. HORSTICK**, Y. BAYLEYEN, H. A. BURGESS
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**Abstract:** Throughout the animal kingdom, bilateral organisms respond to their environment using asymmetric yet consistent motor strategies. A prominent example is human handedness, a behavior apparent early in life that effects social and physical interactions. Despite the commonality and impact of locomotor biases, the underlying neural bases and function remains poorly understood. Using three independent assays we demonstrate that larval zebrafish exhibit a persistent and state-dependent locomotor bias. First, we show that after loss of illumination larva respond with persistent same-direction turns. Individual larva, repeatedly tested over minutes or days demonstrated a preference for same direction turn use. Second, after loss of illumination, individual long-latency startle direction was biased. Third, larva presented with two equal intensity light spots preferentially phototaxed to either left or right-ward over repeated trials. Surprisingly, direction preference in all three assays was correlated for individual larvae. Collectively these data demonstrate larval zebrafish possess an innate locomotor bias that influences how larvae respond to environmental challenges in the absence of clear directional cues. We have excluded genetic inheritance, developmental sensory instruction, and retinal circuitry as the source of the lateralized bias. To locate circuitry driving this behavior we performed a circuit breaking screen using Gal4 lines. Lines that fail to maintain same-direction turning will help identify the neural basis of the behavioral asymmetry. Our data show that we have identified a novel persistently biased locomotor behavior in larval zebrafish, influencing how these larval engage their environment. By using this novel model we can determine how functional behavioral asymmetries are established in the nervous system.

**Disclosures: E.J. Horstick:** None. **Y. Bayleyen:** None. **H.A. Burgess:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

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**Program#/Poster#:** 599.07/LL26

**Topic:** F.01. Neuroethology

**Support:** RGP0040

N00014-12-1-0339

FA9550-14-1-0398

IOS1460149

**Title:** Natural echolocation sequences evoke target range selectivity of neurons in the inferior colliculus of the big brown bat (Eptesicus fuscus)

**Authors: *S. MACIAS**, J. LUO, C. F. MOSS
Dept. of Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

**Abstract:** Echolocating bats utilize precise auditory temporal computations in biosonar to estimate their distance to objects in the environment. Here, we report that response selectivity of neurons in the inferior colliculus (IC) of the big brown bat to pulse-echo delay, the neural representation of target distance, depends on the temporal patterning of sound elements in a natural echolocation sequence. Acoustic stimuli consisted of species-specific echolocation sound sequence of frequency-modulated (FM), containing both pulses and echoes from a moving target with dynamic spectro-temporal features. About 55 % (165/299) of IC neurons showed selective and facilitated responses to a subset of pulse-echo elements in the sequence, indicating selectivity to pulse-echo delay. This response selectivity was not evident when the same neurons were stimulated with isolated pulse-echoes presented randomly at 300 ms pulse intervals. In addition, the selectivity to specific pulse-echo delay appeared for pulse intervals below 50 ms in the natural sequence, but not when pulse intervals in the sequence were unnaturally expanded to 250 ms. Our data suggests that echo responses to objects at different distances are gated by the bat's active control over the temporal patterning of its sonar emissions.

**Disclosures: S. Macias:** None. **J. Luo:** None. **C.F. Moss:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 599.08/LL27

**Topic:** F.01. Neuroethology

**Support:** Air Force Office of Scientific Research grant no. FA9550-14-1-0398

**Title:** Midbrain responses to communication and echolocation sounds in big brown bats

**Authors: *A. SALLES**, S. MACIAS, M. WARNECKE, C. F. MOSS
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**Abstract:** Acoustic communication has been studied in many species, such as crickets, birds, amphibians and mammals, including humans. Yet our understanding of the neural mechanisms of sound communication is incomplete. Bats are auditory specialists that use sound to navigate the environment and to communicate with conspecifics. Important advances have been made in understanding the neural underpinnings of echolocation, but far less is known about the mechanisms of acoustic communication. In big brown bats (Eptesicus fuscus) call structure, along with behavioral context, appears to determine the function of acoustic signals. Past studies in other bat species have shown that neurons in the inferior colliculus (IC) encode specific spectrotemporal features of natural communication sounds through selectivity to FM features. This leads us to investigate the neural mechanisms that enable the big brown bat's discrimination of communication and echolocation calls, with FM components that differ largely in duration and sweep rate. Here we compare neural responses of single neurons in the IC to acoustic signals used by bats for spatial orientation and social communication. We recorded echolocation and communication signals of bats in flight and played back these sounds to awake, passively listening animals. IC recordings were taken with multichannel silicon probes, and single units were sorted off-line. Here we compare single unit responses to the bat's own echolocation and communication signals with neural activity evoked by the signals produced by conspecifics.

**Disclosures: A. Salles:** None. **S. Macias:** None. **M. Warnecke:** None. **C.F. Moss:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

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**Topic:** F.01. Neuroethology

**Support:** Human Frontiers/RGP0040

AFOSR/FA9550-14-1-0398

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**Title:** Clutter modulates midbrain SC activity in bats tracking sonar targets along the range axis

**Authors: *M. J. WOHLGEMUTH, III**[1], N. B. KOTHARI[2], C. F. MOSS[2]
[2]Dept. of Psychological and Brain Sci., [1]Johns Hopkins Univ., Baltimore, MD

**Abstract:** Echolocating bats often forage in cluttered spaces, where they must detect and track moving prey surrounded by other competing objects. This task requires that the bat localize and separate sonar objects in azimuth, elevation and range. The bat measures the time delay between sonar pulse emission and echo return to determine target distance, and adaptations in vocal behavior can therefore be analyzed to provide a measure of the bat's orientation along the range axis. The midbrain superior colliculus (SC) has been implicated in sensorimotor integration for species-specific orienting behaviors, with sensory signals related to the spatial position of an object converted into pre-motor commands for orienting towards a stimulus. In the bat SC, sensory neurons respond selectively to the 3D location of auditory objects, and premotor neurons are implicated in the generation of commands for sonar vocalizations, ear and head movements. Early research on the visuomotor orienting system of primates has shown that competitive stimulus interactions reduce SC sensory responses to an attended stimulus, and saccadic eye movement trajectories curve towards the competitive stimulus. In this study, we investigated competitive stimulus interactions between a moving prey item and stationary clutter objects positioned along the target's trajectory. Using 16-channel silicon probes, we recorded neural activity in the SC of a bat tracking a moving prey item, with and without clutter placed along the target's trajectory. We analyzed extracellular potentials off-line and determined whether sensory and motor activity in the SC of the echolocating bat was modulated by the presence of clutter objects. Our results show that distracting objects evoke adjustments in the bat's sonar orienting behaviors and alter activity of single SC neurons. This study provides new data on the role of the SC in spatial orientation in complex 3D environments.

**Disclosures: M.J. Wohlgemuth:** None. **N.B. Kothari:** None. **C.F. Moss:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

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**Topic:** F.01. Neuroethology

**Support:** HFSP LT000279/2016-L

NSF IOS-1010193

HFSP RGP0040

**Title:** Fast ripple in the inferior colliculus of big brown bat encodes precise timing of sonar vocalization

**Authors: *J. LUO**, S. MACIAS HERRERA, C. F. MOSS
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

**Abstract:** Echolocating big brown bats can discriminate echo delay differences in the microsecond range. Despite decades of research, the neural mechanisms mediating such remarkable behavioral performance are not well understood. Here we report that the first spike of multiunit activity in the inferior colliculus (IC) of awake big brown bat occurs as soon as four milliseconds after the onset of frequency-modulated sweeps stimuli and shows a sub-millisecond variability in response latency. The 1st spike of multiunit activity is topographically organized, with shorter response latency and lower latency variability in ventral regions of the IC. The 1st spike of some multiunit activity was frequency selective and/or shows amplitude-latency trading. We also recorded local field potentials to the same sound stimuli, and focused our analysis on the high frequency band (200-600 Hz), also known as fast ripple. The latency of the fast ripple onset depended on stimulus amplitude and frequency, showing parallels with multiunit response patterns at the same recording sites. It is noteworthy that the onset of the fast ripple showed shorter response latency and lower temporal jitter than single unit responses to the same sound stimuli. To test whether the observed fast ripple is an artifact of the electrostatic loudspeaker signal broadcast, we replaced the playback system with a vocalizing bat and again found fast ripple responses to sound stimuli. Further analysis revealed that the magnitude of fast ripple correlated positively with the number of neurons firing synchronously. Lastly, to better understand the contribution of single unit activity to the fast ripple, we developed a biophysical model. Through quantitative biophysical simulation, we show that the magnitude of the fast ripple depends directly on the number of neighboring neurons firing synchronously within a 5 ms window. Thus, we provide the first evidence that fast ripple can encode sound stimuli and could serve to register the precise timing of auditory events on a rapid time scale.

**Disclosures: J. Luo:** None. **S. Macias Herrera:** None. **C.F. Moss:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

**Location:** Halls A-C

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**Topic:** F.01. Neuroethology

**Support:** HFSP GRant RGP0040

AFOSR FA9550-14-1-0398

**Title:** Density of echo flow patterns guides navigation in echolocating big brown bats

**Authors: *M. WARNECKE**[1], B. FALK[2], S. MACIAS HERRERA[2], C. F. MOSS[2]
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**Abstract:** Navigation and orientation are fundamental to all living creatures, and organisms must adapt their locomotion in response to environmental stimuli received through different sensory modalities. For example, some visually-guided animals, such as bumble bees, adapt their flight paths and velocity with respect to optic flow patterns (Srinivasan et al., 1996; Baird et al., 2010). Similarly, recent studies have evaluated the navigation of echolocating bats guided by acoustic flow patterns (Warnecke et al., 2016). Echo flow, cascades of echoes arriving at the ears of the moving bat, varies with the animal's velocity, its head aim, and distance to objects in the environment. When echolocating big brown bats flew through a corridor whose walls were built from symmetrically spaced poles that returned equal echo flow patterns from opposite sides, they centered themselves within the corridor. An imbalance of echo flow patterns, created by manipulating the pole-spacing on opposite corridor walls, caused bats to veer away from the side of densely-spaced poles and toward the side of sparsely-spaced poles (Warnecke et al., 2016). However, it is unclear whether bats adjusted their flight paths to use echo flow information, or to steer away from the more echoic, densely spaced corridor side. In this study, we manipulated pole echo intensity in addition to pole spacing. To understand how the bat might process echo flow, we also recorded local field potentials (LFP) from the bat inferior colliculus (IC) while broadcasting acoustic stimuli mimicking dynamic echo cascades arriving at the bat's ears as it flew through the corridor. Our analysis compares expected and actual peak times of the LFPs in response to stimuli representing echoes from the experimental corridor walls and provides insight to the bat's flight adaptations under different echo flow conditions.

**Disclosures: M. Warnecke:** None. **B. Falk:** None. **S. Macias Herrera:** None. **C.F. Moss:** None.

**Poster**

**599. Neuroethology: Navigation and Locomotion**

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ONR N00014-12-1-0339

**Title:** Adaptive echolocation behavior modulates sensory, sensorimotor and premotor neural activity in free flying bats

**Authors: *N. B. KOTHARI**, M. J. WOHLGEMUTH, C. F. MOSS
Johns Hopkins Univ., Baltimore, MD

**Abstract:** The superior colliculus (SC) is a laminated midbrain structure implicated in species-specific orienting behaviors. The superficial layers of the SC receive retinal input, intermediate layers show multisensory and sensorimotor properties, and deep layers exhibit pre-motor activity. Sensory information from the superficial and intermediate layers is combined to generate motor commands for orienting behaviors. Most previous research on the functional classes of SC neurons in mammals and birds, however, has almost exclusively been conducted in head fixed or anesthetized preparations using artificial stimuli, which most animals would never encounter in their natural environments. Such artificial experimental paradigms allow the experimenter to separate sensory events from motor events, which facilitates the classification of neural activity into sensory, sensorimotor and pre-motor activity. However, in nature, animals are constantly moving, and differentiating between sensory, sensorimotor and premotor neural activity is a difficult and yet unsolved problem in freely behaving animals. Here we exploit the echolocating bat's active sonar system to characterize SC activity through a unique methodology that combines both empirical and theoretical approaches. With a lightweight telemetry device, we transmitted and recorded neural signals picked up by a 16-channel silicon probe implanted in the SC of the free-flying, echolocating bat. Using the physics of sound we constructed an echo model, which allowed us to reconstruct the instantaneous acoustic stimulus space as the bat flew in a large room equipped with 13 high-speed video cameras and a 16-channel microphone array placed along the four walls. Combining neural recording with the echo model revealed that sensory, sensorimotor and motor activity in the SC is modulated by adaptive echolocation and flight behaviors in bats engaged in an ethologically relevant task.

**Disclosures: N.B. Kothari:** None. **M.J. Wohlgemuth:** None. **C.F. Moss:** None.

**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.01/LL32

**Topic:** F.04. Stress and the Brain

**Support:** I+D COMISION SECTORIAL DE INVESTIGACION CIENTIFICA 187

**Title:** Factors assocciated to symptoms of depression and stress during pregnacy

**Authors: *N. SANDBERG**[1], B. BERTONI[2], G. REY[3], D. MUSETTI[4], G. GRANDI[4], A. S. FLEMING[5], D. E. OLAZÁBAL[1]
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**Abstract:** Several studies around the world show a high incidence of depression and stress during pregnancy. These emotional disturbances are associated with negative effects on the outcome of pregnancy and fetal development, such as pre term birth and low birth weight. Oxytocin (OXT) is a peptide involved in the mediation of stress responses and also recently related to depression. In the present study, we investigated which factors better predict prenatal depression and stress in a heterogeneous population of pregnant women in Uruguay. Two hundred pregnant women were randomly invited to participate in the study, provided blood samples and completed several questionnaires and scales that evaluated early experiences, demographic information, current family context, depression and stress symptoms, among other information. Several polymorphisms of the OXT and OXT (OXTR) receptors were also investigated. We found a high incidence of symptoms of depression (26%) and stress (30%) in the Uruguayan population. Emotional abuse of the pregnant woman in her family of origin, couple conflicts, and lack of social support during pregnancy were the best predictors of depression and stress during pregnancy. We will discuss the contribution of genetic variants for the OXT and the OXTR receptor. Our study suggests that screening of early experiences in pregnant women and their social and family context during pregnancy may contribute to the early detection of emotional disturbances and the mitigation of the adverse effects that depression and stress have on mothers and fetuses.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.02/LL33

**Topic:** F.04. Stress and the Brain

**Support:** The research funding of Shanghai Jiao Tong University (YG2016ZD06)

The research funding of Shanghai Jiao Tong University (14JCRZ05）

**Title:** Systematical investigation of plasma metabolomics alteration among experienced meditators of Tibet Buddhism monks

**Authors: T. XUE**, T. J. XU, H. LI, *D. H. CUI
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**Abstract: Abstract:** Based in Buddhism philosophy, the biological mechanism underlying the promising benefit of mindfulness mediation on physiological well-being has increased interest since its growing emphasis on clinical trials, yet is still unclear. To advance our knowledge on this traditional mediation, this study first systematically investigated the altered plasma metabolomics profile in Tibetan Buddhist monks from two sects of meditation (20 Nyingma monks and 20 Gelug monks), who have practiced the historic tradition from 5 to 35 years, compared to their local meditative naïve Tibetan (20 each). Plasma were collected at their mountain retreat in Tibet. A total of 63 and 72 differential metabolites were identified and annotated in Nyingma and Gelug meditative monks, respectively, compared to their local Tibetan, 13 of which were shared between two compared groups, which was primarily associated with glucose metabolism, indicating an overlapped character of the two meditative trainings. Principal component analysis (PCA) further revealed that, regardless of meditation types, a significant discrimination of metabolites between monks and its local Tibetan was observed, suggesting markedly altered metabolic activity triggered by long-term meditation. However, different types of meditation led to distinguishable pathway changes. In terms of Nyingma monks and its local Tibetan, the pathway of peroxisome proliferator regulation was primarily altered, suggesting an affected cell activity associated with immunity through Nyingma meditation. However, nicotine metabolism and circadian rhythm were more likely to be downregulated during Gelug meditation compared to its local Tibetan, indicating an altered neural activity, which could contributed the spiritual and peaceful mind of monks. Overall, our data revealed distinctive metabolomic features displayed in Tibet Buddhism meditators and controls as well as different meditation sects, offering an initial contribution towards increased understanding of the biological processes underlying meditation.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

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**Program#/Poster#:** 600.03/MM1

**Topic:** F.04. Stress and the Brain

**Support:** NIA P50 AG05146

NIMH R01 MH102392

**Title:** Emotional and pleasure circuit alterations associated with fragmented and unpredictable early-life sensory signals

**Authors: *S. J. GRANGER**[1], M. E. MONTCHAL[2], B. G. VEGETABILE[2], E. HADDAD[2], A. OBENAUS[4], D. KEATOR[3], A. SOLODKIN[3], S. L. SMALL[3], H. S. STERN[3], C. A. SANDMAN[3], E. P. DAVIS[3], L. M. GLYNN[5], T. Z. BARAM[3], M. A. YASSA[3]
[1]Neurobio. and Behavior, Univ. of California, Irvine, Coto De Caza, CA; [2]Neurobio. and Behavior, [3]Univ. of California, Irvine, Irvine, CA; [4]Loma Linda Univ., Loma Linda, CA; [5]Chapman Univ., Orange, CA

**Abstract:** Unpredictability and fragmentation of sensory signals (FRAG) during the perinatal period is emerging as a novel, important contributor to aberrant maturation of several neural circuits related to both cognition and emotional processing, with important emotional and cognitive consequences. It is possible that FRAG impacts synaptic growth and pruning, leading to compromised structural circuitry involved with cognition and emotional processing that then predisposes adolescents to increased risk for anhedonia. Evidence for this scenario is now found in rodent models, where pups subjected to FRAG developed unique functional changes including anhedonia and enhanced amygdala reactivity. In this study, we investigated differences in structural and functional connectivity in children (range 8-11 years old) as a result of differences in patterns of sensory signals from their mothers early in life, focusing on the fragmentation and unpredictability of these signals. Using diffusion tensor imaging, resting-state functional MRI, and a unique naturalistic observation paradigm, we characterized the interaction between fragmented care (quantified as entropy rate) and functional and structural changes in amygdala (AMY), medial prefrontal cortex (mPFC), nAcc, and anterior cingulate cortex (ACC). We found that axial diffusivity (a measure known to increase with brain maturation) **is negatively correlated with increases in entropy specifically in fiber tracts between the right nACC and right mPFC**. This suggests that high unpredictability in early-life sensory signals is associated with deficits in white matter in circuits associated with pleasure/reward processing. In addition, in a longitudinal analysis of functional connectivity in resting state fMRI data**, correlated activity in the AMY-ACC circuit increased significantly and this change (higher connectivity) correlated with entropy rate**. This result suggests that high FRAG is associated with functional changes in areas involved in emotional processing during childhood. Further study is needed to test if this early aberrant development in pleasure/ reward and emotional circuitries predicts adolescent risk for anhedonia.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.04/MM2

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant MH096093

Harvey Family Endowment

**Title:** Differential epigenetic patterns in children with documented trauma

**Authors: *B. S. MULLIGAN**[1], E. L. BEARER[2]
[1]Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [2]Dept. of Pathology, UNM Sch. of Med., Albuquerque, NM

**Abstract:** Pediatric trauma is prevalent, a leading cause of death in children in the USA (accounting for 34-39% of all deaths in ages 1 to 14 years), and is associated with development of post-traumatic stress disorder (PTSD). Why adverse childhood experiences (ACEs), such as traumatic injury, have life long impact on both mental and physical health is not understood. Our hypothesis is that ACEs trigger alterations in stress hormones and epigenetic changes (DNA methylation, DNAme) that persist over the lifespan. To test this we investigated cortisol levels from hair samples and DNA methylation from saliva collected from children, ages 4-8, referred for trauma-focused cognitive behavior therapy (TF-CBT). These children were followed at four time points, over the 6 to 8 months of therapy. We measured cortisol levels in 3cm of hair and DNA methylation at 850,000 sites in saliva at entry and one month after therapy. All but one of the children displayed altered cortisol levels: some were elevated and dropped towards normal at conclusion; one child was normal and rose significantly during TF-CBT. We found a global DNAme difference between the child whose cortisol rose and the other children. Since anxiety is known to cause dry mouth in children, we explored whether this global change may be due to cell composition of the saliva by obtaining methylation patterns for keratinocytes and whole blood and performing a reference-based cell deconvolution in RnBeads. We found that the global DNAme patterns were attributable to cell type composition of the saliva: children with ACE-related changes had saliva samples composed of 68.653% (+/- 3.343%) keratinocytes (K), with the remaining being whole blood. Those that did not report trauma showed 36.452%(+/- 5.735%) K composition. This variation in saliva cell composition of traumatized children was replicated with children from a different study, who also displayed high K composition (69.854% +/- 6.534%). Significant epigenetic differences between children who recently had trauma and those that did not remained after cell composition correction. These changes were primarily in genes involved in immune response and regulation of DNA methylation, while some were altered for genes over-expressed in the brain. To what extent duration and intensity of trauma effect these

changes, or what mechanism may drive an individual's "resiliency" is unclear; we intend to explore these questions further by investigating the relative impact of injury on the methylome.

**Disclosures: B.S. Mulligan:** None. **E.L. Bearer:** None.

**Poster**

**600. Early-Life Stress: Clinical Studies**

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**Program#/Poster#:** 600.05/MM3

**Topic:** F.04. Stress and the Brain

**Support:** Research Council of Norway FRIMED program (project number 204935/F20)

Samarbeidsorganet between NTNU and Helse Midt-Norge (project number 46056907)

**Title:** Region-specific sensitivity to long-term effects of preterm birth with very low birth weight: Longitudinal structural MRI including the MoBa study

**Authors: *K. SRIPADA**[1], K. J. BJULAND[2], A. E. SØLSNES[1], A. K. HABERG[1,3], K. GRUNEWALDT[1,3], G. C. C. LØHAUGEN[2], L. M. RIMOL[1], J. SKRANES[1,2]
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**Abstract: Background**: Preterm-born (gestational age ≤ 37 weeks) children with very low birth weight (VLBW, birth weight < 1500 g) face higher risks for periventricular white matter injury and lasting adverse effects on motor skills, cognitive performance, behavior, and quality of life. Longitudinal structural MRI assesses which brain structures appear most vulnerable to long-term effects and serves as a reference point for functional differences. We investigated possible changes in the cortex and subcortical structures longitudinally to assess whether and to what extent the neurodevelopmental trajectories differ in these groups, as well as development of executive function, in early school age. **Design/Methods**: Preterm-born VLBW subjects (n=46) born between 2003-2007 were recruited based on admittance to the St. Olav's University Hospital NICU in Trondheim, Norway. Term-born control subjects from the Trondheim region (n=134) were recruited from the Norwegian Mother and Child Cohort Study (MoBa). MRI and cognitive data were collected at mean age 8.0 years (range: 4.9-11.1) and 9.1 years (range: 6.1-12.0). 123 participants had two 1.5 T MRI scans; 52 with one scan were also included. FreeSurfer version 5.3.0 was used for morphometry. Cognitive skills were assessed with age-appropriate IQ tests, NEPSY, and Wechsler Memory Scale-III. **Results**: Cortical surface area in all 4 lobes, and to a lesser extent cortical thickness, were impacted in the VLBW population, yet no group x time effects in cortex were seen, indicating similar growth patterns between the groups. Subcortical structures were generally smaller in the VLBW group but with some catch-

up growth of hippocampus bilaterally, brainstem, left amygdala, left cerebellar white matter, and left thalamus over approximately 16 months. VLBW children had an average IQ 1 SD below term-born peers (95 vs 107) and lower scores on WMS-III Spatial Span, but statistically similar performance on the NEPSY Visual Attention and Statue tests. **Conclusion**: Pre-term born VLBW participants showed similar developmental trajectories to their term-born peers in this middle- to late-childhood window, with some catch-up development of hippocampus bilaterally, brainstem, left amygdala, left cerebellar white matter, and left thalamus visible in the VLBW group. While cortical surface area, and to a lesser extent cortical thickness, are impacted in the VLBW population, the growth curve for cortical development during this period appears more influenced by age rather than compounding effects of preterm birth with VLBW, which may owe to early childhood medical and educational interventions.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.06/MM4

**Topic:** F.04. Stress and the Brain

**Support:** Heffter Research Institute

RiverStyx Foundation

NYU CTSA grant UL1 TR000038

**Title:** Mechanisms underlying psilocybin-induced change in anxiety, depression, and spirituality in cancer patients

**Authors: *S. E. MENNENGA**, L. T. OWENS, T. MALONE, M. P. BOGENSCHUTZ, S. ROSS
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**Abstract:** End-of-life distress, including clinically significant anxiety and depression, is common in patients diagnosed with cancer, and is associated with poor psychiatric and medical outcomes. A double-blind, placebo-controlled, crossover trial was conducted at the New York University School of Medicine to evaluate the therapeutic potential of psilocybin-assisted psychotherapy to treat cancer-related anxiety and depression. Twenty-nine patients with cancer-related anxiety and depression were randomly assigned to receive treatment with psilocybin (0.3 mg/kg) followed by niacin (control condition) separated by seven weeks, or niacin followed by psilocybin (0.3 mg/kg), both in conjunction with preparatory, supportive, and integrative

psychotherapy. Psilocybin treatment produced immediate, substantial, and sustained improvements in anxiety, depression, and spiritual wellbeing. At the 6.5-month follow- up, approximately 60–80% of participants continued with clinically significant reductions in depression or anxiety, and both treatment groups exhibited sustained benefits in spiritual wellbeing, existential distress, and quality of life. To understand the mechanism(s) underlying these immediate and lasting changes in anxiety, depression, and spirituality, we investigated relationships between subjective aspects of the mystical experience for each participant's active (psilocybin-induced) treatment session, and short-term and persisting changes in anxiety, depression, and spirituality. We found that short- and long-term increases in spiritual wellbeing were related to higher scores on the Mystical factor of the Mystical Experience Questionnaire (MEQ; r= 0.50-0.75), whereas anxiety and depression reductions were related to higher ratings of Transcendence of Time and Space, Ineffability, and Positive Mood (r= 0.40-0.50). The importance of these and other recent findings for understanding the neural networks and cellular mechanisms that likely contribute to psilocybin-induced experiences of transcendence of time and space, positive mood, ineffability, mystical states, and lasting cognitive and behavioral change will be presented.

**Disclosures: S.E. Mennenga:** None. **L.T. Owens:** None. **T. Malone:** None. **M.P. Bogenschutz:** None. **S. Ross:** None.

**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.07/MM5

**Topic:** F.04. Stress and the Brain

**Support:** FRQ-S

ERA-NET NEURON

AFSP Grant SRG-0-088-1

CIHR Grant MOP-111022

**Title:** Convergent epigenetic, transcriptional and morphological evidence associate a history of child abuse with impaired myelination in the anterior cingulate cortex

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**Abstract: Background.** Child abuse has devastating and long-lasting consequences on individuals, considerably increasing the lifetime risk of negative mental health outcomes such as depression and suicide. Yet, the neurobiological processes underlying this increase in vulnerability remain poorly understood. Here, we investigated the hypothesis that epigenetic, transcriptomic and cellular adaptations in the anterior cingulate cortex may associate with a history of child abuse. **Method.** Post-mortem brain samples from a total of N=78 human subjects and from a rodent model of the impact of early-life environment (N=24) were analysed. Groups were constituted of depressed individuals who died by suicide, with (N=27) or without (N=25) a history of severe child abuse, as well as of psychiatrically healthy controls (N=26). Genome-wide DNA methylation and gene expression were investigated using Reduced Representation Bisulfite Sequencing and RNA-Sequencing, respectively. Cell-type specific validation of differentially methylated loci was performed following fluorescence-activated cell sorting of oligodendrocyte and neuronal nuclei. Differential gene expression was validated using Nanostring technology. Finally, oligodendrocytes and myelinated axons were analysed using stereology and Coherent Anti-stokes Raman Scattering microscopy, allowing for high-throughput and high-resolution imaging of individual myelinated fibers in the cingulate cortex. **Results.** A history of child abuse associated with cell-type specific changes in DNA methylation of oligodendrocyte genes and a global impairment of the myelin-related transcriptional program. These effects specifically occurred as a function of child abuse, as they were absent in depressed suicides with no history of early life adversity, and strongly correlated with myelin gene expression changes observed in the animal model. Furthermore, a selective and significant reduction in the thickness of myelin sheaths around small-diameter axons was observed in individuals with history of child abuse. **Conclusion.** This study indicates that child abuse, in part through epigenetic reprogramming of oligodendrocytes, may lastingly disrupt cortical myelination, a fundamental feature of cerebral connectivity.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.08/MM6

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant R01-MH098348

**Title:** Investigating the effects of violence exposure, physical abuse, and sexual abuse on brain activity following exposure to psychosocial stress

**Authors: *J. PURCELL**[1], A. M. GOODMAN[1], N. G. HARNETT[1], S. MRUG[1], M. N. ELLIOTT[2], S. TORTOLERO EMERY[3], M. A. SCHUSTER[4], D. C. KNIGHT[1]
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**Abstract:** Violence exposure (e.g., witnessing or experiencing an assault) is a prevalent issue that affects approximately 25% of adolescents in the United States (McLaughlin et al., 2016). In particular, physical and sexual abuse are associated with poor physical (Irish et al., 2010) and psychological (Chen et al., 2010) outcomes. Given the prevalence of childhood violence exposure and its deleterious effects, it is important to understand how these experiences affect neural activity in response to future stressful experiences. Therefore, the current study aimed to examine the effects of violence exposure, physical abuse, and sexual abuse on blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) signal in a community sample. Changes in BOLD signal associated with cumulative violence, physical abuse, and sexual abuse were expected in the hippocampus, amygdala, and prefrontal cortex. A community sample of adolescents and emerging adults were interviewed prospectively (ages 11, 13, 16, & 20 years) regarding their exposure to violence and experiences of physical abuse and sexual abuse. Following the last assessment, participants underwent a separate fMRI session during which they completed a psychosocial stress task (i.e. the Montreal Imaging Stress Task; Dedovic et al., 2005). Functional images were collected using a 3T Siemens Allegra MRI scanner and analyses were completed using the AFNI software package (Cox, 1996). Linear mixed effects models revealed differences in activation within the hippocampus and the prefrontal cortex in response to psychosocial stress for participants who had experienced either physical or sexual abuse. Cumulative violence exposure alone did not predict differences in activation in response to psychosocial stress in this study. Results suggest that physical or sexual abuse in childhood is associated with altered prefrontal cortex and hippocampal activation in response to future stressors. Understanding the relationships between these childhood stressors and future stressful experiences may offer new insights into the genesis and maintenance of psychopathology associated with childhood violence exposure.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.09/MM7

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant RO1-MH098348

**Title:** The influence of environment during adolescence on white matter structure

**Authors: *K. BELL**[1], N. G. HARNETT[1], A. M. GOODMAN[1], S. MRUG[1], M. A. SCHUSTER[2], M. N. ELLIOTT[3], S. R. TORTOLERO[4], D. C. KNIGHT[1]
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**Abstract:** The white matter tracts of the cingulum bundle, uncinate fasciculus, and stria terminalis/fornix, provide communication pathways between brain structures that are important for emotion learning, expression, and regulation. Decreased white matter microstructure within these fiber tracts has been associated with emotional dysfunction including depression and anxiety. Exposure to neighborhood disadvantage during childhood and adolescence is a risk factor for depression and anxiety symptoms (Santiago, Wadsworth, & Stump, 2009). Although prior research has examined the relationship between household socioeconomic status (SES) and white matter structure, the effects of neighborhood influences on white matter microstructure has received limited attention. Determining the relationships between neighborhood disadvantage and white matter microstructure of the brain may offer new insight into mechanisms by which adverse life experiences impact developing neural systems. The current study investigated the effect of adolescent exposure to varying degrees of neighborhood disadvantage on white matter tracts of the cingulum bundle, uncinate fasciculus, and stria terminalis/fornix in young adults. Using geocoded addresses for each participant at age 11, multiple indicators of neighborhood disadvantage from the U.S. Census were combined. Diffusion tensor imaging (DTI) data were collected from these individuals in young adulthood (N=169; Mean age 19.6years; SD = 1.17). The results demonstrate that the white matter structure in the cingulum bundle, uncinate fasciculus, and stria terminalis/fornix varied with neighborhood disadvantage, such that greater neighborhood disadvantage was associated with reduced QA in each of these white matter tracts. These findings suggest that the neighborhood a child lives in may play an important role in the microstructure of white matter connections of the brain; these neural effects may explain the impact of neighborhood disadvantage on emotional and behavioral outcomes.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.10/MM8

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant MH098348

**Title:** Self-reported stress, violence exposure, and neural activity

**Authors: *E. DAVIS**, A. GOODMAN, T. OREM, M. WHEELOCK, N. HARNETT, S. MRUG, D. KNIGHT
Univ. of Alabama At Birmingham, Birmingham, AL

**Abstract:** There is limited prior research on the effect of violence exposure on the neural function that underlies the emotional response to stress. However, violence exposure during adolescence can have an impact on stress reactivity later in life. Therefore, the present study investigated the relationship between prospectively measured adolescent violence exposure (average age 14) and the neural response to psychosocial stress as young adults (average age 19). In the present study, 224 participants that had been previously assessed for adolescent violence exposure completed the Montreal Imaging Stress Task (MIST). The MIST is a psychosocial stress task designed to investigate brain reactivity to stress. Our results showed that participants with higher rates of violence exposure, compared with participants with lower rates of violence exposure, rated the stress task as less stressful ($p < .01$). In addition, participants with high rates of violence exposure showed decreased differential neural activity in response to the MIST within the hippocampus and multiple regions of the prefrontal cortex ($p < .01$). The present findings suggest that violence exposure in adolescence reduces stress reactivity as young adults, which may be facilitated by decreased neural activity in the prefrontal cortex and hippocampus.

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**Poster**

**600. Early-Life Stress: Clinical Studies**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 600.11/MM9

**Topic:** H.03. Schizophrenia

**Title:** Effects of childhood trauma experiences on cognitive ability and emotion processing in schizophrenia

**Authors: *S. WON**, S. LEE, H. YUN, S. WOO, B. JIN
Psychiatry, Kyungpook Natl. Univ. Hosp., Daegu, Korea, Republic of

**Abstract: Background:** Deficits in cognitive ability and emotion perception have been frequently reported in schizophrenia patients. However, it is still unclear the effects of childhood maltreatment experiences on cognitive ability and emotion perception in schizophrenia. **Methods:** In this study, 83 schizophrenia patients and 54 controls were recruited. Cognitive abilities were measured by several tasks including the Wisconsin Card Sorting Test (WCST), the Continuous Performance Test (CPT), and Auditory Verbal Learning Test (AVLT). Also, we developed affective Go/No-Go and emotion perception task using E-prime software. **Results:** Experiences of emotional neglect ($p = 0.01$), emotional abuse ($p < 0.001$), and sexual abuse ($p = 0.003$) are greater in schizophrenia than in control. In results of cognitive tasks and affective Go/No-Go task, control group showed greater performances in several tasks including AVLT, CPT, WCST, and affective Go/No-Go task (all ps $< 0.05$). In emotion perception task, schizophrenia group had lower accuracy rate on contempt and sad faces perception compared to control group (all ps $< 0.05$). Delayed response time on surprise and anger faces was related to childhood maltreatment experiences (all ps $< 0.05$). Also, interaction effect was significant in response time of sad face perception ($p < 0.001$). In addition, schizophrenia with high childhood maltreatment experiences showed greater depressive symptoms compared to schizophrenia with low childhood maltreatment experiences. **Discussion:** Childhood maltreatment experiences can modulate the ability of emotion perception in schizophrenia. These alterations in emotion processing can be linked to emotional distress, such as depressive symptoms, in schizophrenia patients.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.01/MM10

**Topic:** F.04. Stress and the Brain

**Support:** NIH/NIAAA R37 AA007789 and RO1 A022460 to JW

NeuroDevNet (Canadian NCE) to JW

Canadian Foundation on Fetal Alcohol Research to JW & CR

NIH/NIAAA F31 AA023151 to PJH

**Title:** Adolescent development of social behavior and oxytocin receptor system following prenatal alcohol exposure and early life adversity

**Authors: *P. J. HOLMAN**, L. ELLIS, C. RAINEKI, J. WEINBERG
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**Abstract:** Social behavior deficits associated with prenatal alcohol exposure (PAE) emerge early in development and persist across the lifespan, often becoming more pronounced as the individual approaches adolescence. Importantly, early-life adversity (ELA) can also dramatically shape social behavior development, and individuals with PAE are more likely to experience early-life adversity. The central oxytocinergic (OT) system has been implicated as a key regulator of social behavior, and its disruption of may underlie deficits in social behavior observed following PAE and/or ELA. Here, we combine animal models of PAE and early-life adversity to investigate the potential role of the OT system in mediating the unique and/or synergistic effects of PAE and ELA on social behavior function in adolescent male and female rats. Offspring of PAE, Pair-Fed, and *ad libitum* Control dams were evaluated first on a social motivation task followed by a social discrimination task in early (P28-35) or late (P39-P45) adolescence. In a separate cohort, we assessed both social (social discrimination) and non-social (object recognition) learning and memory using a well-established rat model of PAE in conjunction with a model of ELA to begin to dissect the unique and/or synergistic contributions of each insult. Specifically, from P8-12, half the dams were provided with insufficient nest bedding, which increased abusive-like maternal behaviors such as rough handling of and stepping on pups and reduced arch-backed nursing. Brains were removed and assayed using OT receptor binding and c-fos mRNA to measure neural activation (PFC, lateral septum, amygdala, hypothalamus). Results from social motivation testing showed that all animals spent significantly more time in the social chamber, suggesting that social motivation is not inhibited by PAE. Social discrimination data indicate sexually dimorphic effects of PAE, such that only males were unable to discriminate between a familiar and novel social stimulus. Data from our combined PAE and ELA models demonstrated that at P30, PAE impaired social discrimination in males regardless of rearing conditions, while ELA impaired social discrimination in PAE and control females. Analysis of brains revealed no differences in amygdala OT receptor binding in P30 females; however, early-life adversity decreased OT receptor binding in the lateral part of central amygdala in P30 control but not PAE males. Taken together, these results suggest that PAE alters social behavior development in a sexually dimorphic way, and that these behavioral changes, at least in males, are associated with altered development of the OT system during adolescence.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.02/MM11

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant U54HD083092

NIH Grant U01 CA170930-01A1

**Title:** Neonatal colonization with Bifidobacterium species elicits sex-specific effects on memory, sociability, and anxiety-like behaviors of adult gnotobiotic mice

**Authors: *B. K. LUK**[1], S. VEERARAGAVAN[2], M. ENGEVIK[1], A. MAJOR[3], J. VERSALOVIC[3]
[1]Baylor Col. of Medicine/Texas Children's Hospit, Houston, TX; [2]Mol. and Human Genet., Baylor Col. of Med., Houston, TX; [3]Pathology, Texas Children's Hosp., Houston, TX

**Abstract: Background**: Postnatal colonization of the human gastrointestinal tract with *Bifidobacterium* species overlaps in time with a critical period of neural circuit development and organization in the brain. Given that these bacterial species are predominant members of the human infant microbiota, CNS modulation by *Bifidobacterium* early in life may have pervasive and lasting effects on brain function and behavior. We hypothesized that neonatal colonization with a consortium of "infant-type" *Bifidobacterium* species would result in long-term effects on adult behavioral phenotypes. **Methods:** In order to examine the effects of early *Bifidobacterium* colonization, germ-free Swiss Webster mice were treated with a mixture of "infant-type" *Bifidobacterium* species, including *B. bifidum, B. longum* ss. *infantis, B. breve*, and *B. dentium* (~$1x10^9$ CFUs/treatment). The treatment began at postnatal day 1 (P1) and was repeated every other day from P1-P20. This treatment resulted in immediate and stable colonization of the pups over the course of the experiment. Control groups of mice received either sterile saline gavages (germ-free controls), or were colonized with a complex murine microbiota (conventionalized controls). All mice were raised in identical gnotobiotic isolator units and were handled in the same manner. At 6-7 weeks of age, the mice were removed from the isolators and underwent a battery of behavior testing to assess a variety of motor and non-motor behaviors. **Results:** Colonization of female mice with the consortium of *Bifidobacterium* mimicked the effects of colonization with a complex murine microbiota, restoring normal anxiety-like behaviors and improving recognition memory in adult mice. However, *Bifidobacterium* colonization was not sufficient to rescue the hyperactive phenotype, or the sociability deficit observed in female germ-free mice. Colonization of male mice with *Bifidobacterium* also normalized the anxiolytic phenotype of germ-free mice, and improved motor performance and recognition memory, though no differences were observed in locomotor activity or sociability between groups of males.

**Conclusion:** Together, these data indicate that postnatal colonization with a small consortium of select *Bifidobacterium* species is sufficient to recapitulate the results observed when mice are colonized with a complex microbiota in a sex- and behavior-dependent manner.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.03/MM12

**Topic:** F.04. Stress and the Brain

**Support:** IA206516 to CJMR from DGAPA-UNAM

**Title:** Resilience or vulnerability? A sum of life experiences

**Authors: V. PIÑA-DIAZ**, E. HERNANDEZ-REYES, M. VALLE-NAVA, J. YAÑEZ-VARGAS, *C. J. MONTES RODRIGUEZ
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**Abstract:** Resilience is the capability of coping behaviors under stressful/adverse situations that allows organisms to keep psychological and physiological health, while nonadaptive behaviors are related to vulnerable phenotypes. Most of the resilience studies consists in exposing animals to one stress model (i.e. Social Defeat, Unpredictable Chronic Mild Stress, UCMS) and, the evaluation of several behaviour tests (i.e. social interaction, anxiety, motivation) after the stressful experience. However, resilience in humans seems to be different, coping behaviors depends of several factors and early development play a crucial role (Dannlowski et al., 2012). We studied the effect of adverse early postnatal development (Maternal Separation (MS) from PD2-14), the effect of stressful conditions in the adult (UCMS vs Medium Social Stress, MSS) or the sum of these conditions (MS+stress) on the behavioural output. We observed that each stress model promotes a specific behavioral output, the MSS promotes high social interaction, decreases anxiety and motivation; UCMS also increase social interaction but promotes anxiety and decrease motivation; MS decrease social interaction, promotes anxiety and decrease motivation. The effects of UCMS are potentiated by MS on motivation but counterbalanced on social interaction and anxiety, while the MS on the MSS has an additive effect, increasing even more the social interaction and decreasing anxiety. Our results show that resilience or vulnerability is the result of experiences throughout lifespan more than of a unique aversive/traumatic experience. Also, we support that early life experience is crucial for developing coping behaviors.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.04/MM13

**Topic:** F.04. Stress and the Brain

**Support:** NIGMS-P20GM103643

**Title:** Acute and inflammatory neonatal pain disrupts later-life fear conditioning and sensory function: The role of CRF

**Authors:** ***M. A. BURMAN**[1], S. DAVIS[2], E. HOLMQVIST[2], E. HARRIS[1], V. EATON[1], A. STEINIS[1], M. RICE[1]
[1]Psychology, Univ. of New England, Biddeford, ME; [2]Univ. of New Engalnd, Biddeford, ME

**Abstract:** In humans, early-life trauma has been linked to an increased prevalence of psychological disorders, including anxiety and depression, as well as sensory disorders such as chronic pain. Time in the neonatal intensive care unit (NICU), often with repeated painful skin-breaking procedures without the benefit of analgesics, is correlated with increased risk for psychological and sensory dysfunction. Our lab has been using a rodent model of neonatal pain during postnatal days (PNDs) 1-7 to explore its effect on subsequent fear conditioning and sensory function at the equivalents of early childhood (PND 24), adolescence (PND 45) or adulthood (PND 66). Our previous data have shown that repeated hindpaw pricks during the neonatal period produce age-dependent changes in tactile sensitivity as well as alterations in fear conditioning. Our current studies have explored the neurobiological role of corticotropin releasing factor (CRF) and differing pain modalities. In *Experiment 1* rats were subjected to the neonatal pain manipulation, and on PND 6, tissue samples from the amygdala and hypothalamus were collected and analyzed using RT-qPCR to measure genetic markers for CRF. Early results suggest that neonatal pain produces increased CRF expression in the amygdala respective to their non-pain counterparts in a sex-dependent manner. In *Experiment 2*, rats were treated with the CRF-R1 antagonist, antalarmin hydrochloride or vehicle, and subjected to the neonatal manipulation. Preliminary results suggest that blockade of CRF can attenuate the subsequent alterations in fear response and sensory function. Finally, in *Experiment 3* rather than the hindpaw pricking procedure (an acute pain model), rats received an injection of either vehicle or λ-carrageenan into the hindpaw on PNDs 1 and 4 and subsequently tested using our fear conditioning and sensory testing protocol. Preliminary results suggest an impairment of fear conditioning that is sex and age dependent, as well as an apparent thermal hyposensitvity. These

data show lasting emotional and sensory consequences of neonatal pain and implicate the CRF system in modulating these effects.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

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**Program#/Poster#:** 601.05/MM14

**Topic:** F.04. Stress and the Brain

**Support:** CV Starr Foundation

**Title:** Early life adversity: Neuroplasticity and anxiety-related neuronal oscillations in the brain

**Authors:** ***S. MURTHY**, D. E. HERMAN, P. LARA-MEJIA, G. OBIOFUMA, E. GOULD
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**Abstract:** Adverse experiences during early life lead to significant effects on brain function and behavior. Early adverse experiences produce not only neurobiological and behavioral changes, but also increase susceptibility to anxiety and mood disorders during adulthood (Heim C et al., 2001). Rodent models of early life adversity have been well-characterized in the rat, and have been used to study both the behavioral and neurobiological effects of early life stress. A rat model of early life adversity involving the maternal separation stress paradigm showed increased anxiety and decreased neurogenesis in the hippocampus in adulthood (Mirescu et al., 2004). In mice, maternal separation does not produce consistent increases in anxiety, numbers of new neurons or numbers of several inhibitory interneuron subtypes, so we used an alternate model of early life adversity, the maternal separation stress with early weaning model (MSEW) (George et al., 2010), to study and characterize changes in behavior and neuroplasticity. We found an increase in anxiety in MSEW mice in adulthood but no change in the numbers of new neurons in the hippocampus, suggesting other cellular processes likely underlie MSEW-induced anxiety increases. The ventral hippocampus and the medial prefrontal cortex have been associated with anxiety-like behaviors (Padilla-Coreano et al., 2016), and lesions of either area have shown to reduce anxiety-like behavior (Bannerman DM et al., 2003; Sullivan R et al., 2002). Theta frequency (4-12 Hz) range neuronal oscillations have been implicated in anxiety-like behavior, and increases in theta power in both regions as well as an increase in theta frequency synchrony between the two regions have been demonstrated during high anxiety (Adhikari et al., 2010). These findings raise the possibility that increased anxiety with early adversity may arise from changes in neuronal oscillations in these brain regions. However, the electrophysiological profile of adult rodents following early life adversity has been understudied. Here we have used the

MSEW model in mice, to study and characterize changes in neuroplasticity as well as theta range neuronal oscillations within the brain during anxiety-like behaviors in adulthood.

**Disclosures: S. Murthy:** None. **D.E. Herman:** None. **P. Lara-Mejia:** None. **G. Obiofuma:** None. **E. Gould:** None.

**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.06/MM15

**Topic:** F.04. Stress and the Brain

**Support:** NIMH P50MH096890

Hope for Depression Research Foundation

NIDA

**Title:** Transcriptomic alterations across reward circuitry by early life stress in male and female mice

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**Abstract:** Abuse, neglect, and other forms of early life stress (ELS) significantly increase risk for psychiatric disorders including depression. Individuals with a history of ELS also have worse forms of depression including earlier onset and more frequent episodes, and are less responsive to traditional antidepressant medications. We recently developed an ELS paradigm in male and female C57/Bl6 mice wherein stress in a specific postnatal window from P10-17 increased the likelihood that additional stress in adulthood results in depression-like behavior, without altering behavior prior to additional stress. We performed RNA-sequencing in adult male and female ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), and ventral hippocampus (vHIP) to investigate transcriptomic changes that reflect long-lasting ELS-induced adaptations in the brain's reward circuitry central to depression-like behaviors. Here we present detailed analyses of the transcriptomic adaptations resulting from ELS, and after a "second hit" of stress in adulthood. Multifactor bioinformatic analysis reveals that ELS has a greater impact on NAc transcription among male mice, while recent adult stress dysregulates more genes in male VTA, PFC, and vHIP. Among females, ELS had a greater impact on transcription than sub-threshold adult stress in all brain regions examined. Using a threshold-free rank-rank hypergeometric overlap analysis, we show that ELS transcriptionally "primes" the brain in a

similar way as adult stress, even prior to presentation of depression-like behaviors. Finally, we are making comparisons with transcriptional profiles from mice responsive or non-responsive to antidepressant treatments to predict treatment efficacy in ELS-exposed mice, work suggesting that individuals with a history of ELS have unique transcriptional brain signatures that necessitate unique pharmacological treatment.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.07/MM16

**Topic:** F.04. Stress and the Brain

**Support:** The Robertson Therapeutic Development Fund

Hope for Depression Research Foundation

**Title:** An epigenetic biomarker for depression and trait of childhood trauma with sex-specific effects

**Authors: *N. L. RASGON**[1], B. BIGIO[2], S. YOUNG[4], M. KAUTZ[5], A. COCHRAN[6], J. BEASLEY[4], D. MILLINGTON[4], J. KOCSIS[6], J. MURROUGH[5], F. LEE[6], B. S. MCEWEN[3], C. NASCA[7]
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**Abstract:** Development of diagnostics and more effective treatments for major depressive disorders (MDD) can be guided by mechanistic insights from animal studies. These are urgent medical and public health need given that MDD is projected to be the second leading cause worldwide to the medical and economic disease burden by 2020. There is a broad literature from our and other groups supporting rapid antidepressant effects of the epigenetic modulator of glutamatergic function with insulin-sensitizing properties, acetyl-L-carnitine (LAC), across animal models. A common trait of such animal models with depressive and metabolic-like dysfunction was an endogenous reduction in LAC levels in the plasma and in mood regulatory brain regions, such as the hippocampus and prefrontal cortex. Therefore, based upon several converging preclinical evidence, we started by investigating the association between LAC and MDD in humans with a targeted-oriented and mechanistic-driven approach. Plasma distribution

of LAC and the internal control free-carnitine were determined in samples from 45 healthy controls (HC) and 71 patients suffering with MDD using LC-MS/MS. HC and MDD patients did not differ on any demographic and clinical characteristics, except that, as expected, MDD patients had significantly higher depression severity scores as assessed with the two psychiatric scales, HAM-17 and MADRS (Ham-17: HC=0.6+/-1.1, MDD=20.2+/-3.3, $p<0.0001$; MADRS: HC=1.4+/-2.4, MDD=32.9+/-4.7, $p<0.0001$). Our recent data show that LAC is significantly lower in MDD patients compared to HC. Reduced LAC levels in MDD patients are associated with greater depression severity and earlier onset of depression, and a treatment resistant course of illness. Consistent with lower LAC in more severe forms of MDD, our data also show an association between reduced LAC levels and childhood trauma in patients with a treatment resistant course of illness. In all, while a growing literature on LAC supplementation provides converging evidence for its rapid antidepressant-like effects, our current human findings show that LAC can serve as a marker to delineate a novel biologically-defined MDD subtype that could benefit by treatment with LAC. Given our previously reported link between LAC and insulin resistance (IR) in animal models with depressive vulnerability, more work is warrant to test feasibility of utilizing LAC supplementation alone or in conjunction with therapies with other medications (e.g.: insulin-sensitizing agents, GLP agonists etc), in treatment of MDD.

**Disclosures:** **N.L. Rasgon:** None. **B. Bigio:** None. **S. Young:** None. **M. Kautz:** None. **A. Cochran:** None. **J. Beasley:** None. **D. Millington:** None. **J. Kocsis:** None. **J. Murrough:** None. **F. Lee:** None. **B.S. McEwen:** None. **C. Nasca:** None.

**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.08/MM17

**Topic:** F.04. Stress and the Brain

**Support:** American Foundation for Suicide Prevention Fellowship

Hope for Depression Research Foundation

NIH UL1 TR000043

**Title:** Maternal care influences adulthood acetylcarnitine levels and trajectory of the 3D's in individuals at genetic risk

**Authors: *B. BIGIO**[1], C. NASCA[1], F. LEE[2], D. ZELLI[1], T. LAU[1], A. FERRIS[3], P. DEANGELIS[1], S. HARVEY[3], J. LAI[4], A. KALIDINDI[5], N. L. RASGON[6], B. MCEWEN[1]
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New York, NY; [3]Barnard Col., New York, NY; [4]Columbia Univ., New York, NY; [5]Emory, Atlanta, GA; [6]Stanford Univ. Sch. of Med., Palo Alto, CA

**Abstract:** Early life experiences related to parental care as well as childhood trauma play a powerful role in later mental and physical health. Recent work showed that childhood abuse is associated with reduced levels of the epigenetic modulator of glutamatergic functions with insulin-sensitizing properties acetyl-L-carnitine (LAC) in patients with depression. Here, we implemented a novel mating model, using wild-type or heterozygous BDNF val66met (hets) dams and controlling for paternal care, to assess the effects of maternal care on the brain and the body in individuals at genetic risk for the development of the depression, diabetes, progressing to dementia (3D's). Our data show that wild-type and hets dams differ in the amount of maternal care provided to offspring assessed by in and out of the nest caring and self-maintenance behaviors. We then assessed the impact of such different maternal care on male hets offspring across two different adulthood ages. Behavioral, molecular and metabolic characterizations of adult offspring show that the 3D's manifests in individuals at genetic risk (i.e.: hets) that receive low nurturing only. Indeed, low nurtured hets, as opposed to high nurtured hets, show depressive-like traits, including abnormal social behaviors and psychomotor retardation-like symptoms. These behavioral differences are concomitant with a peripheral metabolic dysregulation, including insulin-resistance (IR), and reduced levels of LAC in low nurtured hets as opposed to high nurtured hets. Molecularly, these features of depressive- and IR-like states along with reduced levels of LAC, were found to be associated with striking different transcriptomic profiles in the hippocampal ventral dentate gyrus (vDG). Among the most significant differentially expressed genes, RNAseq analyses revealed the inhibitor of glutamate release mGlu2 that, as confirmed by qPCR and protein analyses, is reduced in the vDG of low vs high nurtured hets in line with previous work from our and other groups supporting a role for mGlu2 in stress responses. Finally, more aged hets that received low nurturing showed cognitive impairments in memory tasks at the Y-maze, suggestive of dementia-like symptoms, which were not apparent before. Such cognitive impairments were not observed in high nurtured hets at either age. Cross-fostering is ongoing to causally prove that high maternal care counteracts a genetic predisposition to development of the 3D's. In all, these findings suggest that maternal care has huge influences on brain and body health and point to positive early life experience as mean to "block" manifestation of the 3D's in individuals at genetic risk.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.09/MM18

**Topic:** F.04. Stress and the Brain

**Support:** Hope for Depression Research Foundation

American Foundation for Suicide Prevention

**Title:** A novel mechanistic role for mGlu2 in a vDG-MeA circuit underling sex-dimorphic social behavior: Importance of early life experiences

**Authors: *C. NASCA**[1], *C. NASCA[1], F. LEE[2], D. A. ZELLI[3], B. BIGIO[4], T. LAU[1], P. DEANGELIS[1], O. ISSLER[5], H. M. CATES[6], E. J. NESTLER[7], B. S. MCEWEN[4]
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**Abstract:** Social behavior is a complex behavioral domain that is impaired in several psychiatric disorders, including depression (MDD) - a life-threatening disorder in urgent need of improved treatments and with occurrence by sex that is twice as high in women as in men. Although studies with pharmacological agents that unselectively modulate group-II metabotropic glutamate receptors, namely mGlu2 and mGlu3 receptors, have shown a role for group-II mGlu receptors in mood regulation, a circuit-level understanding of the specific activation of these receptors in mood regulation, including social interaction, is lacking. Therefore, we developed a herpex-simplex viral construct to selectively overexpress mGlu2 receptors (HSV-mGlu2-OE) in the hippocampal ventral dentate gyrus (vDG) and studied the effects of the specific HSV-mGlu2-OE on in vivo mammalian social interactions, and on structural plasticity of the vDG and a sexually dimorphic brain region, the medial amygdala, which is interconnected to the ventral hippocampus. We find that both male and female BDNF val66met mice (hets) that received low nurture[1] early in life - and are known to be resistant to common antidepressant SSRIs and yet are responsive to the novel rapid acting antidepressant, acetyl-L-carnitine (LAC) - show a sex-common reduction in mGlu2 expression in the vDG. Instead, low nurtured male and female hets show sex-dimorphic structural plasticity of MeA stellate neurons and social interactions that are impaired in male hets only. Specific HSV-mGlu2-OE in the vDG corrects social interactions and MeA structural plasticity in male hets with behavioral outcomes similar to those of wild-type males. Our circuit-level mechanistic findings reveal a previously unknown role for mGlu2 receptors in a highly integrated brain circuit underlying sex-differences in social behavior and are reminiscent of previous work showing a role for the MeA in social withdrawal in depressive-like illness and the ability of an epigenetic modulator of the glutamatergic system, LAC, to remediate retraction of dendrites of MeA stellate neurons and ameliorate dysfunctional social behavior that results from exposure to chronic restraint stress in males. Our findings also highlight the importance of early life experience in the development of depressive-like symptoms. More studies are needed to understand the role of sex hormones and to further characterize such vDG-MeA circuit underlying sex-differences and commonalities in response to

early life experiences. Identification of specific molecular targets at a circuit-level subserving complex behaviors, such as social interactions, can lead to improved treatment options

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.10/MM19

**Topic:** F.04. Stress and the Brain

**Support:** NI Grant AA17531

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**Title:** Adolescent social isolation increases excitatory synaptic activity in the rat nucleus accumbens core

**Authors: *S. EWIN**, A. G. ALMONTE, E. S. CARTER, J. L. WEINER
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**Abstract:** Our lab has established a rodent model of adolescent social isolation (aSI) which engenders robust and enduring increases in behaviors linked to alcohol addiction vulnerability, including increases in anxiety-like behaviors, ethanol intake and preference. We have recently shown that the basolateral amygdala (BLA), a brain region heavily implicated in the pathophysiology of anxiety and addiction, is hyper-excitable following aSI. The BLA sends glutamatergic projections to other addiction-related brain regions including the ventral hippocampus (vHC) and nucleus accumbens (NAc). We have recently found that the vHC exhibits robust increases in excitatory synaptic activity following aSI, which supports the idea that aSI promotes increased excitability in downstream targets of the BLA. In these studies, we have started characterizing aSI-mediated neurobiological adaptations in the NAc. Despite the well-known role of the BLA-NAc projection in mediating motivational aspects of drug reinforcement, no studies to date have examined how aSI-associated adaptations within this circuit may contribute to the addiction vulnerable phenotype promoted by this model. Our first studies employed an unbiased approach, examining the overall effects of aSI on NAc excitability and plasticity. Initial findings suggest that, as observed in the vHC, aSI leads to an increase in

synaptic excitability in the NAc. Unexpectedly, aSI did not lead to alterations in long term depression in this brain region. Further work is being done, using optogenetic and chemogenetic approaches, to determine if the BLA-NAc projection is impacted by aSI and whether adaptative changes in this circuit contribute to the escalation in ethanol drinking associated with this model of alcohol addiction vulnerability.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.11/MM20

**Topic:** F.04. Stress and the Brain

**Support:** Start-up funds to SB

**Title:** Behavioral effects of prenatal exposure to sertraline, an SSRI antidepressant, and its discontinuation on the offspring in an animal model of maternal depression

**Authors: J. M. KOTT**, S. M. MOONEY-LEBER, K. BADRIA, J. M. YOUNG, *S. BRUMMELTE
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**Abstract:** Major depressive disorder is classified by the World Health Organization as the leading cause of disability worldwide, with more women being affected than men. Women suffering from depression are frequently prescribed antidepressant medication. However, should a woman become pregnant she must choose whether or not to continue antidepressant treatment, as both maternal depression and antidepressant treatment are known to negatively impact fetal development. This study investigated this question using an animal model of depression to better understand the developmental implications of maternal depression, its treatment with sertraline, a frequently prescribed selective serotonin reuptake inhibitor (SSRI), and the discontinuation of treatment during pregnancy. For this, thirty-six female Sprague-Dawley rats were treated with either a vehicle (oil) or corticosterone (CORT; 40 mg/kg, s.c.) for 21 days to create a depressive-like phenotype. On the 16$^{th}$ day of CORT/oil treatment, rats were given either sertraline (SER; 20 mg/kg, p.o.) or a vehicle (water) daily and then mated with males at the end of the CORT treatment period. To investigate any differential effects of discontinuation of SER treatment, half of the SER-animals discontinued treatment at gestational day (GD) 16, and the other half continued receiving the medication through parturition. Once offspring reached adulthood, they underwent a variety of behavioral testing to determine the effects of each condition on anxiety-like and depressive-like behavior, and stress reactivity. Additionally, brains were collected after sacrifice to investigate levels of neurogenesis. Results indicate sex-dependent effects of both

CORT and SER treatment to the dams and its discontinuation in the behavior of the offspring. Currently, there is a critical need for more research on the effects of these exposures and the discontinuation of antidepressant medication during pregnancy in order to better advise pregnant women on whether or not to discontinue their medication.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** F.04. Stress and the Brain

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NeuroDevNet grant 20R64153

Canadian Foundation on Fetal Alcohol Research (CFFAR)

**Title:** Influence of early-life adversity on immune system function in animals prenatally exposed to alcohol: Implications for mental health

**Authors: *C. RAINEKI**, T. S. BODNAR, P. J. HOLMAN, S. L. BAGLOT, N. LAN, J. WEINBERG
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**Abstract:** Brain development is a dynamic process beginning in prenatal life and extending throughout adolescence. Adversity and/or stress in any of these stages can alter the neurodevelopmental trajectory, putting the individual on a pathway to pathology. Alcohol is a teratogen that negatively alters brain development. However, how the early postnatal environment contributes to the pervasive effects of prenatal alcohol exposure (PAE) is poorly understood. Moreover, PAE often carries increased risk of exposure to adversity/stress during early life. Here, we examined whether PAE differentially increases vulnerability to immune dysregulation in response to early-life adversity, which may further alter brain development and lead to increased risk of psychopathologies. PAE and control litters were exposed either to limited bedding (postnatal day [PN] 8-12) to model early-life adversity or normal bedding, and maternal behavior and pup vocalizations were recorded. Peripheral (serum) and central (amygdala) immune (cytokine and CRP) responses of PAE animals to early-life adversity were evaluated in infancy (PN12). Male and female offspring were tested in early (PN30) and late

(PN45) adolescence using the open field (OF), elevated plus maze (EPM), and forced swim test (FST). Following FST, peripheral and central immune responses to stress (FST) were evaluated. Insufficient bedding increased abusive-like maternal behavior in both groups. Early-life adversity increased vocalization in all animals; however, PAE pups vocalized less than controls. In infancy, adversity reduced serum TNF-α, KC/GRO, and IL-10 levels in control but not PAE animals. PAE increased serum CRP, with even higher levels observed in PN12 pups exposed to adversity. PAE reduced KC/GRO and increased amygdala IL-10 levels. Our behavioral assessments indicated that, in females, PAE alone resulted in anxiety-like behaviors in the OF. This anxiogenic profile of PAE females emerged at PN30 and lasted until PN45. In males, PAE in association with early-life adversity increased anxiety-like behaviors in the EPM, and similar to females, this anxiogenic profile emerged at PN30 and lasted until PN45. The behavioral alterations in early and late adolescence were associated with changes in the peripheral and central immune system. Our results indicate that PAE alters immune system development and both behavioral and immune responses to early-life adversity, which could have subsequent consequences for brain development and later life health. Indeed, the alterations in the immune system could be one of the underlying mechanisms of the anxiogenic profile observed following PAE and/or early-life adversity.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.13/MM22

**Topic:** F.04. Stress and the Brain

**Support:** IA206516 to CJMR and IA201916 to PERO from DGAPA-UNAM.

**Title:** Medium social stress promotes resilient behavior in rats

**Authors: E. D. HERNANDEZ-REYES**[1], V. PIÑA-DIAZ[1], M. D. VALLE-NAVA[1], *P. E. RUEDA-OROZCO[2], C. J. MONTES-RODRIGUEZ[1]
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**Abstract:** Several works have reported that social defeat promotes social stress, decreasing social interaction and motivation. Social defeat is a high social aversive model that promotes a depressive phenotype, we studied a medium social stress model (10 min/10 days) in Wistar male rats. In this model, animals were not defeated every day (0-5 in 10 days) and several social interaction events occurred. We called this model medium social stress (MSS) since they had

some aggressive encounters (45.5/10d) but almost none finished in social defeat. We observed that MSS increased resilient behaviors such as high social interaction scores and low anxiety evaluated by the open field test; interestingly, a decrease in motivated behaviors was also observed. The MSS model is closer to the bullying situation where the aggressor send positive and negative signs to the defender, we are reporting that this kind of interaction results in a high social interaction and low anxiety, both behaviors are considered resilient behaviors. It may be possible that low social stress promotes resilience as it is observed in humans (Cyrulnik, 2013) or in other protocols with low stress (Franklin et al, 2011). However, the high social interaction score after social aversive interaction can also be readed as non adaptative, this idea is also supported by the decrease in motivation; suggesting these animals cannot read the context correctly or they are complacent as a strategy to avoid social aggression. We believe that resilience should be read according to the context demands and personal experiences. More behavior evaluation need to be done to understanding the final behavioral outcome of MSS.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.14/NN1

**Topic:** F.04. Stress and the Brain

**Support:** TRDRP 25FT-0007

**Title:** Drd3 signaling in the lateral septum mediates early life stress-induced social dysfunction

**Authors: *S. SHIN**[1], H. PRIBIAG[2], V. LILASCHAROEN[3], D. KNOWLAND[4], X.-Y. WANG[1], B. LIM[3]
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**Abstract:** Early life stress (ELS) in the form of child abuse/neglect is associated with an increased risk of developing social dysfunction in adulthood. However, little is known about the underlying specific neural substrate as well as neuromodulatory signaling that governs ELS-induced social dysfunction. Here, we show that ELS-induced downregulation of dopamine receptor 3 (Drd3) signaling and its corresponding effects on neural activity in the lateral septum (LS) are both necessary and sufficient to cause social abnormalities in adult mice. Drd3-expressing-LS (Drd3 LS) neurons show blunted neuronal activity in response to social stimuli in animals exposed to ELS, while the optogenetic activation of Drd3 LS neurons rescues ELS-induced social impairments. Furthermore, pharmacological treatment with the Drd3 agonist

normalizes the social dysfunctions of ELS mice. Thus, we identify Drd3 signaling in the LS as a critical mediator and potential therapeutic target of social abnormalities caused by ELS.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

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**Topic:** F.04. Stress and the Brain

**Support:** MacArthur Foundation

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P50MH078105

UCLA Staglin IMHRO Center

**Title:** Prefrontal cortex BDNF levels and anxiety reversal in females after early life stress

**Authors: *H. S. KNOBLOCH**[1], E. J. KIM[1], L. GABARD-DURNAM[2], N. HODGSON[1], J. L. FUDGE[3], N. TOTTENHAM[2], F. S. LEE[4], J. L. CAMERON[5], T. K. HENSCH[1]
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**Abstract:** Early life stress (ELS) predisposes a variety of psychiatric conditions, such as increased anxiety in females. A potential molecular basis for these lasting consequences remains unknown. Brain-derived neurotrophic factor (BDNF) is an activity-dependent growth factor regulated by experience that is important for timing brain development in sensory cortices. Here, we investigated if it also plays a role in the prefrontal cortex (PFC), a part of the limbic brain involved in the modulation of social behavior across species. We investigated a robust mouse model of fragmented care due to reduced availability of nesting material during the first postnatal week (P2-P9). Levels of BDNF and stress-related molecules were assessed by immunohistochemistry, in situ hybridization, RT-qPCR and HPLC. We found PFC levels of BDNF to be reduced in ELS females. Similar reduction of BDNF was observed in PFC of female rhesus monkeys raised for the first month of life without their mother. A sexually dimorphic BDNF reduction and elevated anxiety was replicated in the PFC of normally raised female knock-in mice carrying one allele of the common human BDNF Val66Met polymorphism. We

next explored the potential to rescue anxious outcomes in female ELS mice by a developmentally timed intervention. Environmental enrichment was confirmed to increase BDNF levels in the mouse PFC. Music exposure after ELS prevented the expected anxiety phenotype in adult female mice. Likewise, human female subjects who had experienced early adversity also exhibited strong anxiolytic response to childhood music when placed under acute stress as adults. Altering BDNF signaling by behavioral or drug therapies in the developing PFC might be a strategic target to mitigate the pre-destined psychiatric outcomes following early adversity.

**Disclosures: H.S. Knobloch:** None. **E.J. Kim:** None. **L. Gabard-Durnam:** None. **N. Hodgson:** None. **J.L. Fudge:** None. **N. Tottenham:** None. **F.S. Lee:** None. **J.L. Cameron:** None. **T.K. Hensch:** None.

**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.16/NN3

**Topic:** F.04. Stress and the Brain

**Support:** K08 MH014743-01A1

NARSAD Young Investigator Award from the Brain & Behavior Research Foundation

**Title:** Maternal social regulation of infant's fear in the selectively-bred anxious rat phenotype

**Authors: *A. M. WHITE**[1,2,3], J. HIDER[1,3], J. BOULANGER BERTOLUS[1,3], D. CHANG[1,3], R. M. SULLIVAN[4], H. AKIL[1,2,3], J. DEBIEC[1,2,3]
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**Abstract:** During the early stages of life in altricial species, the caregiver is a potent regulator of the infant's physiological and emotional state. While pups can learn to avoid an odor paired with a mild foot shock from the 10th day of life (postnatal day - PND - 10), this learning is abolished when the odor-shock pairing is performed in the presence of a non-fearful dam. This effect is supported by the maternal presence blocking the release of the stress hormone corticosterone (CORT), an effect termed maternal buffering of fear. Whereas social buffering of fear occurs throughout life, maternal fear buffering is its most powerful example, since maternal presence may totally suppress the stress response and prevent fear conditioning in preweaning pups until around PND 15. We have previously shown the early emergence of classical fear conditioning at PND 4 and elevated CORT responses in Sprague-Dawley (SD) rat pups from a selectively-bred

anxious rat phenotype (ANX) relative to outbred SD rats. Here, we examine the maternal buffering of fear in ANX and wild-type rat pups at early infancy (PND 4), weaning (PND 21), and one week after weaning (PND 28). Pups received 11 pairings of an odor conditioned stimulus (CS) and a 1s 0.4 mA tail shock in the presence of an anesthetized dam or in the absence of an anesthetized dam. Control groups included ANX pups which received 11 odor presentations in the absence of shock and age-matched wild-type SD rats. CORT levels were measured before and after fear conditioning. Pups were exposed to the CS 3 times during a freezing test at PND 11, 22, or 29 in order to assess fear memory. Behavior was video recorded and pups' freezing in response to the CS was scored. Maternal presence was able to block fear conditioning in ANX pups conditioned at PND 4 ($p = 0.68$), PND 21 ($p = 0.19$), and PND 28 ($p = 0.21$); the pups that received odor shock pairings did not freeze significantly more than pups that received odor in the absence of shock. Surprisingly, maternal presence did not block a rise in corticosterone in ANX pups that received odor-shock pairings at PND 4 relative to pups that received exposure to the odor alone ($p = 0.004$). These findings suggest that although ANX pups can acquire fear conditioning and CORT responses to stressors at an earlier age than wild-type SD rats, the presence of the dam continues to serve as a potent regulator of emotional state at older ages than expected. Additionally, it suggests that CORT may not be the sole mechanism through which maternal buffering operates, and opens new avenues for exploring the underlying neurobiology of this phenomenon.

**Disclosures:** **A.M. White:** None. **J. Hider:** None. **J. Boulanger Bertolus:** None. **D. Chang:** None. **R.M. Sullivan:** None. **H. Akil:** None. **J. Debiec:** None.

**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.17/NN4

**Topic:** F.04. Stress and the Brain

**Support:** Groff Foundation

**Title:** Epigenetic effects of prenatal and juvenile stressors on adult behaviors in rats

**Authors: L. B. STEELE**[1], *B. ZIMMERBERG[2]
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**Abstract:** Stressors during gestation or the juvenile period of development can lead to enduring alterations in affective behaviors. This experiment asked whether there were any interactive effects of experiencing both stressors dependent on genetic variance. Subjects were selectively-bred High and Low Vocalizing lines with line differences in anxiety that persist into adulthood. We hypothesized that maternal and juvenile stress might interact with these line differences.

Maternal restraint stress occurred on gestational days 16-20 and juvenile isolation from postnatal days 35-60. Adults were tested on a social choice test wherein a rat could choose to have proximity to (without touching) a same sex conspecific, compared to proximity to a toy rodent. Anxiety and arousal behaviors were also assessed in this T-maze test. Results indicate that both stressors had gene X environment effects. Line X Prenatal Stress X Juvenile Isolation analyses were significant for social behavior (prenatal stress combined with juvenile isolation to increase social behavior in Lows while Highs only increased social behavior in response to isolation); anxiety behavior (prenatal stress increased risk aversion in double-housed Highs and isolated Lows), and arousal (double-housed Highs reared less than Lows, all prenatally stressed reared less than controls, but isolation increased rearing in Lows and decreased it in Highs). These results will be discussed in relation to the "Double-Hit" Hypothesis in neuroimmunology.

**Disclosures: L.B. Steele:** None. **B. Zimmerberg:** None.

**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.18/NN5

**Topic:** F.04. Stress and the Brain

**Support:** PCOM Division of Research

**Title:** Assessing long-term behavioral changes following seizures and stress in larval zebrafish

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**Abstract:** There is increasing recognition that novel methods are needed to investigate seizure-induced development of epilepsy and behavioral deficits, especially in early life. Early-life seizures may disrupt brain development and lead to deficits such as intellectual disability and autistic-like behaviors (Dodrill, 2002; Gant et al., 2009). Current therapies offer only symptomatic treatment and are not disease modifying, in part because how seizures lead to long-term deficits remains unclear. Though promising signaling pathways have been identified in rodents, many remain difficult to interpret. Thus, we aim to create a more simplified model. A larval zebrafish seizure model is established (Baraban et al., 2005), but long-term behavioral and cognitive consequences are not fully characterized, and thus how well the model aligns with early-life seizures in humans cannot yet be ascertained. To begin assessing behavioral changes after seizures in larval zebrafish, we exposed fish to the chemoconvulsant pentylenetetrazole (PTZ, 8mM) at day post fertilization (dpf) 7. At dpf 28, we assessed behavior after 1 min and 1 hour acclimation to the test tank. Our pilot data shows significant decreases after 1 min

acclimation in fish exposed to PTZ compared to control fish in social behavior (shoaling; p=0.015), total swim distance (p=0.028), and mean swim velocity (p=0.040), and increased freezing (p=0.007). After a 1 hour acclimation time, total swim distance (p=0.006) and mean swim velocity (p=0.002) remained significantly decreased in PTZ fish. Interestingly, zebrafish that experienced the same early-life handling stress as the PTZ group but no exposure to PTZ (and thus no seizures), did not display changes in swim distance, velocity, or freezing compared to unhandled controls. However, they showed hyperactivity and a longer lasting deficit in social behavior than the PTZ group, with significantly less shoaling than unhandled controls at both 1 min (p=0.02) and 1 hour (p=0.03). Taken together, these data imply that seizures alter zebrafish behavior for weeks, potentially with an increase in anxiety (freezing). Further, early life-stress alone may lead to long-lasting social and behavioral deficits that are separable from seizure-induced deficits. Additional experiments are required to determine the implications of the behavioral changes. We are optimistic that using zebrafish to study long-term effects of early-life seizures and early-life stress will allow us to overcome many of the constraints and complications that arise in rodent research, and will open a window for high-throughput molecular and cellular network analysis in a reduced, simplified model.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.19/NN6

**Topic:** F.04. Stress and the Brain

**Support:** Einhorn Family Charitable Trust

Mary Dexter Stephenson and the Fleur Fairman Family

NCRR/NIH UL1RR024156

**Title:** Colostrum oxytocin in the newborn gut modulates cellular stress and inflammation in the brain

**Authors: B. Y. KLEIN, 10032**[1], H. TAMIR[2], R. J. LUDWIG[3], S. B. GLICKSTEIN[4], *M. G. WELCH[5]
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**Abstract:** We previously showed that colostrum oxytocin (OT) modulates cellular stress response, inflammation, and autophagy markers in newborn rat gut villi. Little is known about whether there are analogous responses in the brain. Here, we tested whether brain regions rich in oxytocin receptors (OTR) mirror the effects previously observed in gut villi. As with previous experiments, we measured signaling protein markers associated with endoplasmic reticulum stress, including BiP (an ER chaperone), eIF2a (translation initiation factor), and pPKR (eIF2a kinase), as well as two inflammation-signaling proteins; NF-κB and its inhibitor IκB. We measured these markers in newborn brain nuclei presumably stressed by nutrient insufficiency (viz, after birth and 1-2 hours before the start of nursing) (unprimed), and 1-2 hours after the start of nursing (primed). Brain tissue from unprimed and primed animals was harvested from six brain regions: solitary tract nucleus (STN), paraventricular nucleus (PVN), supra-optic nucleus (SOP), cortex (CTX), striatum nuclei (STR), and medial preoptic nucleus (MPO). Expression of BiP/GRP78, and p-eIF2a were upregulated in unprimed and downregulated in primed NTS tissue, whereas NF-κB was high and stable in both conditions. Expression of BiP and NF-κB was the same in the other tested regions in the both unprimed and primed conditions. The integrated stress response factor eIf2a was phosphorylated by dsRNA dependent kinase (pPKR) in the SON, CTX, STR and MPO. However, eIf2a was phosphorylated by another kinase, general control nonsuppressed2 kinase (GCN2), in the NTS, and to a lessor extent in PVN. These results indicate that PVN and SON utilize a different phosphorylation mechanism from the other regions, one that may be immune to the impact of nutrient insufficiency. Collectively, our data shows that brain responses to nutrient insufficiency stress may be subsequently offset by signaling from colostrum-primed gut. Our data also suggest that the newborn gut stress modulating mechanism we previously observed in enterocytes is mirrored in brain regions rich in OTR.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.20/NN7

**Topic:** F.04. Stress and the Brain

**Support:** Israel Science Foundation (ISF 738/11)

National Institute for Psycobiology in Israel (NIPI NO.7-2011-12)

The Open University of Israel research fund

**Title:** The effects of antidepressant treatment on prenatal stress induced behavioral and biochemical abnormalities in mouse offspring

**Authors: *R. DORON**[1], N. SIMON[2], O. SIMHON[2], Y. SIMCHON TENENBAUM[3], M. REHAVI[4]
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**Abstract:** Depression and anxiety during pregnancy reach high prevalence of 20%. Stressful events experienced by pregnant women might negatively impact fetal development and cause mood and anxiety disorders later in life. Pre- and post-partum usage of antidepressants such as serotonin reuptake inhibitors (SSRI) has been recently increased, however their influence on newborn development is still unclear. Here, we aimed to investigate the effect of antidepressant treatment given to nursing mothers on the anxiety-like behavior and brain-derived neurotrophic factor (BDNF) of their prenatally stressed offspring. Two antidepressants, which also have anxiolytic features, used in this study are the SSRI escitalopram and the Shan-zha herb (also known as Hawthorn). The current study was designed to mimic the situation in which infants are nursed by mothers treated with antidepressants for post-partum depression/anxiety began at pregnancy. Pregnant mouse dams were randomly assigned to either control or restraint stress during the last week of pregnancy. Following parturition, dams were treated with either escitalopram, Shan-zha, saline or remained non-treated for 21 days while nursing. Offspring could receive treatment only via lactation. Subsequently, anxiety-like behavior of dams and offspring (postnatal day 21) was evaluated using the elevated plus maze. Escitalopram levels in the serum were assessed by radioactive binding assay of unoccupied serotonin transporter on the platelets. In addition, hippocampal BDNF levels in the dams and offspring were assessed using enzyme-linked immunosorbent assay. Our results indicate that prenatal stress induced anxiety-like behavior in the dams and their offspring which was normalized by both escitalopram and Shan-zha treatments. Escitalopram was evident in the serum of the dams but not in the serum of the offspring, implying it did not reach the brains of the offspring. Therefore, we suggest that the behavioral effect seen in the offspring was mediated by indirect effect of escitalopram on mothers' behavior rather than direct effect of escitalopram on the offspring brain. Since Shan-zha's mechanism of action is unknown, we could not determine whether it was transferred to the offspring blood via lactation. The results of the present study underscore the importance of preventing the deleterious effect of prenatal stress on newborn behavior by treating the mother with antidepressants.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.21/NN8

**Topic:** F.04. Stress and the Brain

**Support:** R01MH100241

**Title:** Measuring stress response in the Collaborative Cross

**Authors: P. KUMAR**[1], S. SCHOENROCK[3], J. FARRINGTON[2], F. PARDO-MANUEL DE VILLENA[2], *L. M. TARANTINO[4]
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**Abstract:** Stress is both a trigger and a symptom of many psychiatric diseases but little is known about the shared genetic and environmental influences that act on the hypothalamic pituitary adrenal (HPA) axis and increase the risk and severity of these devastating diseases. Animal models can be used to study the behavioral, physiological, circuit-based and molecular consequences resulting from an altered HPA axis and provide insights for translational studies in human patients.
We measured HPA axis activity in the recently established Collaborative Cross (CC). The CC combines the genomes of 8 inbred mouse strains that represent the three major subspecies of *Mus musculus*. This inbred population contains unique allele combinations that have not yet been studied in existing mouse resources. As such, the CC has the ability to extend phenotypic diversity in a way that has not been observed previously.
We examined basal corticosterone (CORT) and CORT following an acute restraint stress and two hours post restraint in male and female mice from 13 CC strains. We identified significant strain and sex differences in CORT levels at all time points. As expected, several strains showed vastly higher basal and stress-induced CORT levels than has been previously observed in other inbred mice and selected populations.
These data represent the first characterization of HPA axis activity in the CC. These initial studies will form the basis for examining the effects of a dysregulated HPA axis on behavior, physiology and brain circuitry and allow us to gain more insight, at the systems genetics level, into the underlying role of stress on affective disorders.

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**Poster**

**601. Early-Life Stress: Anxiety, Motivation, and Depression**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 601.22/NN9

**Topic:** F.04. Stress and the Brain

**Support:** NIH Grant P50MH096889

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George E. Hewitt Foundation for Medical Research Postdoctoral Fellowship

**Title:** Anhedonia following early-life stress involves aberrant interaction of pleasure/reward circuits and anxiety/fear circuits and is reversed by partial silencing of amygdala corticotropin-releasing hormone

**Authors: *J. L. BOLTON**[1,2], J. MOLET[1], L. REGEV[1], Y. CHEN[1], N. RISMANCHI[1], E. HADDAD[2], D. Z. YANG[1], A. OBENAUS[2], T. Z. BARAM[1,2]
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**Abstract: Background:** Anhedonia, the diminished ability to experience pleasure, is an important biological entity associated with both depression and schizophrenia. Anhedonia often arises already in adolescence, but its origins and mechanisms are poorly understood. We have previously identified anhedonia, manifest as decreased sucrose preference and reduction in social play, in adolescent male rats that experienced early-life chronic stress. Here we probed the molecular, cellular and circuit mechanisms underlying this serious emotional problem.
**Methods:** We examined functional brain circuits and neuronal populations activated by social play in adolescent rats experiencing early-life stress (CES), compared with controls. Structural connectivity between stress- and reward-related networks was probed using high-resolution diffusion tensor imaging (DTI). We intervened with the aberrant activation patterns observed in CES rats via viral-directed reduction of corticotropin-releasing hormone (CRH) expression in the central nucleus of the amygdala (ACe), and tested for reversal of anhedonia in individual rats.
**Results:** Sucrose preference was reduced in adolescent CES rats, indicating anhedonia. Social play, an independent measure of anhedonia, activated brain regions involved in pleasure and reward in both control and CES groups. Notably, in CES rats, social play also provoked activation of CRH-expressing neurons in ACe, typically involved in anxiety and fear, indicating aberrant functional connectivity. DTI revealed aberrant structural connectivity of bilateral amygdalae to prefrontal cortex in CES rats. Administration of CRH-shRNA, but not control shRNA, into ACe reversed the CES-induced anhedonia in individual rats, without influencing

other emotional measures.
**Conclusions:** Together, these findings provide the first evidence for aberrant interactions of stress-related and reward networks after CES, and for a mechanistic role of CRH-expressing amygdala neurons in emotional deficits that portend major neuropsychiatric disorders.

**Disclosures: J.L. Bolton:** None. **J. Molet:** None. **L. Regev:** None. **Y. Chen:** None. **N. Rismanchi:** None. **E. Haddad:** None. **D.Z. Yang:** None. **A. Obenaus:** None. **T.Z. Baram:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.01/NN10

**Topic:** F.04. Stress and the Brain

**Support:** PIRE-NSF-OISE-1545803

**Title:** Isolation stress during adolescence increases conditioned place preference to cocaine in female rats

**Authors: *C. J. RIVERO**[1], J. A. FREIRE[1], C. DELGADO[2], I. G. SANTIAGO[3], A. C. SEGARRA[1]
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**Abstract:** Adolescence is a developmental period characterized by neuronal growth. Environmental factors such as maternal neglect, social stress and drugs of abuse have a direct impact on future behavioral patterns. Because rats are highly social animals, we decided to investigate if isolation stress during adolescence increases conditioned place preference (CPP) to drugs of abuse. Since D2 receptors play a role in cocaine sensitization in adult animals and because adolescents and adults differ in dopaminergic connectivity and D2 receptor populations, we investigated if D2 receptors are altered during these conditions. Female rats were weaned at postnatal day 23 and housed in singly or in pairs. At day 38 rats were tested for open field behavior and elevated plus maze and at day 40 they tested for CPP to cocaine (15mg/kg). Rats were then euthanized, brains collected and stored at -80 C. Our data show that rats that were singly housed showed greater conditioning to cocaine compared to grouped housed rats. When studying the brains of these animals we found that single housed rats showed a decrease in the number of D2 immunoreactive cells in the nucleus accumbens, lateral septum and prefrontal cortex compared to grouped housed rats. However, cocaine treatment abolished the decrease in D2 immunoreactivity induced by isolation stress. These data show that isolation stress increases the rewarding properties of cocaine, and suggest that this effect may be mediated by altering D2

receptor population in brain areas associated with motivation and reward. It also shows that the social environment during adolescence exerts long-lasting effects on motivational and reward pathways.

**Disclosures: C.J. Rivero:** None. **J.A. Freire:** None. **C. Delgado:** None. **I.G. Santiago:** None. **A.C. Segarra:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.02/NN11

**Topic:** F.04. Stress and the Brain

**Title:** Effects of chronic corticosterone exposure on neuroendocrine function of adolescent and adult male mice

**Authors: Z. SHAHANOOR**, M. R. BAKER, R. SULTANA, *R. D. ROMEO
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**Abstract:** Adolescence is associated with the maturation of the hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine axis mediating the hormonal stress response. Adolescence is also a developmental stage marked by a variety of stress-related vulnerabilities, including psychological and physiological dysfunctions. Many of these vulnerabilities are accompanied by a disrupted HPA axis. A mouse model of disrupted HPA function has been developed using oral chronic corticosterone (CORT) adminstration, which result in various physiological and neurobehavioral abnormalities, including changes in stress reactivity and anxiety-like behaviors. However, this model has only been established in adults. In an effort to further compliment and extend this model, we tested how disrupting the HPA axis in adolescent and adult mice would influence hormonal and neurobiological stress reactivity. Specifically, we exposed adolescent and adult male C57BL/6 mice to 4 weeks of oral corticosterone exposure (25 μg/ml in 1% ethanol) or tap water vehicle (tap water with 1% ethanol) and tested their plasma corticosterone and neural activity patterns, as indexed by FOS immunohistochemistry, before, during, and after a 30 min session of restraint stress. Our data indicate that chronic CORT treatment during adolescence or adulthood leads to hormonal hypo-responsiveness, such that stress failed to elevate plasma CORT levels in treated mice. Despite this reduced hormonal response, we found significant neural activation in both adult- and adolescent-treated mice in the paraventricular nucleus of the hypothalamus (PVN), a brain region necessary for the initiation of the hormonal stress response. Therefore, these data indicate a dissociation between stress-induced peripheral and central stress responses following chronic CORT exposure during either adolescence or adulthood. Moreover, we found that stress-induced neural activation in the PVN was highest in

animals treated during adolescence, indicating an age-dependent effect of chronic CORT treatment on later PVN stress reactivity. Together these experiments extend this mouse model of chronic HPA disruption on neurobiological outcomes to include adolescent animals and highlight unique effects of HPA disruption during adolescence. Given the substantial vulnerabilities to HPA dysfunctions during adolescence this model may prove useful in better understanding these vulnerabilities.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.03/NN12

**Topic:** F.04. Stress and the Brain

**Support:** NIH grant P50AA017823

**Title:** Adolescent Sprague-Dawley rats display a blunted neuroimmune response evoked by an acute stress challenge

**Authors: *D. LOVELOCK**, M. ORLOFSKY, T. DEAK
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**Abstract:** Adolescence represents a critical developmental period during which CNS sites critical to emotion-regulation are impacted by major life experiences, including stress challenges. Studies using cross-sectional designs to compare adolescent and adult stress reactivity have revealed delayed recovery of the corticosterone (CORT) response to several stress challenges. Adolescents also display a blunted cytokine response in the CNS when challenged with acute alcohol exposure or lipopolysaccharide. Since acute stress challenges also increase expression of cytokines, the primary goal of the present experiment was to examine age-related differences in expression of several key pro-inflammatory cytokines in stress-sensitive CNS sites as well as in the spleen. We hypothesized that adolescents would show an impaired cytokine response relative to adults, as well as a delayed corticosterone recovery. To test this, adolescent (P29-31) and adult (P73-78) male Sprague-Dawley rats were exposed to acute footshock (1 mA, 90 second variable ITI, 5 sec each) for 1 hour (40 shocks), 2 hours (80 shocks), or for 2 hours with a 2-hour recovery period in order to establish the time course of cytokine and neuroendocrine changes evoked by acute footshock exposure. Expression of pro-inflammatory cytokines interleukin (IL)-1β, IL-6, TNF-α, as well as the immediate early gene c-Fos, were measured in the paraventricular nucleus of the hypothalamus (PVN), the medial amygdala (MeA), and the ventral hippocampus (vHPC) using real time RT-PCR. In addition, plasma concentrations of CORT and progesterone (PROG) were measured as humoral indices of the stress response. As predicted,

adolescents displayed a significantly reduced IL-1β response compared to adults in the PVN. In the vHPC, IL-1β expression levels peaked at 4 h in adults, and this effect was also blunted in adolescents. In adults, TNF-α levels tended to decrease during stress exposure and recovered by 4 h in all brain regions examined, whereas in adolescents recovery was not complete by 4 h in the PVN and TNF-α did not change at all in the MeA. Induction of c-Fos was also blunted across all brain regions in adolescents relative to adults. Acute stress led to increased plasma CORT and PROG in both adolescents and adults, yet delayed recovery was observed in PROG, but not CORT. Overall, these findings indicate that adolescents have a blunted neuroimmune response to acute stress challenges, and may have implications for developmental vulnerability to subsequent stress-related pathologies later in life.

**Disclosures: D. Lovelock:** None. **M. Orlofsky:** None. **T. Deak:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.04/NN13

**Topic:** F.04. Stress and the Brain

**Support:** FONDECYT Grant N° 1141276

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**Title:** endocannabinoid modulators and PUFAs regulate decision-making and attention in adolescent stressed rats

**Authors: *A. DAGNINO-SUBIABRE**, M. OVANDO, P. CASTRO
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**Abstract:** Endocannabinoids and polyunsaturated fatty acids (PUFAs) play a key role in cognitive functions, for example learning and memory. However, their effects on complex cognitive functions such as decision-making and attention during adolescence remain unknown. The aim of this study was to determine whether both endocannabinoid modulators and PUFAs affect decision-making and auditory attention in adolescent stressed rats. Male Sprague-Dawley rats were trained in a two-alternative choice task (2-ACT), a behavioral paradigm to study complex cognitive functions in rats. Trained animals that reached a performance over 80% of correct trials in the 2-ACT were randomly assigned to control and stress experimental groups. To analyze the effects of restraint stress (3 h/d, PND: 42-48), trained rats of both experimental groups were subjected to 50 2-ACT trials one day before and one day after the stress period. A difference score was determined by subtracting the time spent in the inter-trial interval (decision-making) and the number of correct trials (auditory attention) after those before the stress

protocol. In the course of the stress period, animals were treated with endocannabinoid modulators [AM251 ($CB_1$ antagonist) or URB597 (FAAH inhibitor)] or supplemented with PUFAs [n-3 PUFAs (fish oil) or n-6 PUFAs (primrose oil)]. In the animals of the control experimental group, n-6 PUFAs supplementation itself impaired decision-making and auditory attention, while endocannabinoid modulators and n-3 PUFAs improved them. These results suggest that chronic stress and n-6 PUFA increases the endocannabinoid system activity, which in turn impairs decision-making and auditory attention. Conversely, endocannabinoid modulators and n-3 PUFAs would decline the effects of chronic stress on endocannabinoid system.

**Disclosures: A. Dagnino-Subiabre:** None. **M. Ovando:** None. **P. Castro:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.05/NN14

**Topic:** F.04. Stress and the Brain

**Support:** NR014886

**Title:** Chronic adolescent stress impairs memory but not learning in adult female rats

**Authors: *M. M. HYER**[1], M. BEKHBAT[2], G. N. NEIGH[1]
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**Abstract:** Chronic adolescent stress (CAS) has been implicated in increased incidence of cognitive and emotional deficits in adulthood. The hippocampus, a brain region that mediates learning, memory, and emotion, is rich in hormone receptors, providing a site of action for hormones to directly alter behavior. Adolescents are particularly vulnerable to perturbations as neural systems that regulate these behaviors are developing - including the hippocampus. While the effects of CAS on learning and memory in adult male rats are relatively well characterized, parallel effects in females are less clear. We tested the hypothesis that female Wistar rats exposed to CAS would show deficits in learning and memory performance in adulthood. Females exposed to CAS or control conditions were trained on the Barnes Maze task. Both groups showed parallel, improved performance on the task over 10 trials indicating that learning was not altered by CAS in female rats ($p>0.05$). When tested 48 hours after the final training trial, a significant effect of CAS on memory performance was evident. Females exposed to CAS showed an increased latency to initially locate the position of the goal box ($p<0.05$). Additionally, CAS females revisited the location of the goal box less often during the probe trial ($p<0.05$). Interestingly, CAS also resulted in differing search strategies. CAS females exhibited a more randomized search pattern while control females engaged in a serial search strategy

($p<0.05$). The memory deficit in the CAS females persisted throughout three probe trials across a 4 week period and was still evident following ovariectomy ($p<0.05$) - suggesting that ovarian steroids were not driving these effects. Taken together, these findings suggest that CAS has a significant and long lasting impact on memory performance in adult female rats. Additionally, CAS appears to alter their search strategy when females are engaged in a spatial memory task. The work from this study highlights the need to identify factors that may contribute to poor memory performance in women that may be related to overall cognitive deficits following CAS. Implications from these findings suggest that identifying early life stress in females may allow for intervention to attenuate poor cognitive performance and improve memory strategies in females. Further investigation into possible underlying neural and genetic correlates is ongoing.

**Disclosures:** **M.M. Hyer:** None. **M. Bekhbat:** None. **G.N. Neigh:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.06/NN15

**Topic:** F.04. Stress and the Brain

**Support:** NIH NINR NR014886

T32-GM008602

**Title:** Adult effects of chronic adolescent stress on glucocorticoid receptor regulation

**Authors: *S. A. ROWSON**[1], M. BEKHBAT[2], S. D. KELLY[3], G. N. NEIGH[4,3]
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**Abstract:** Women are more likely than men to develop stress-related mood disorders such as major depression. Furthermore, adolescents may be particularly susceptible to the harmful effects of chronic exposure to stressors. A rat model has been useful in studying the long-term effects of chronic adolescent stress exposure, and previous studies have found that female, but not male rats exposed to chronic adolescent stress exhibit enhanced depressive-like behaviors in adolescence that persist to adulthood. However, the molecular consequences of chronic adolescent stress exposure in adulthood are not known. These studies examine the impact of chronic adolescent stress on adult regulation of the glucocorticoid receptor. The glucocorticoid receptor mediates negative feedback on the hypothalamic pituitary adrenal (HPA) axis, a major component of the neuroendocrine system that mediates the response to stress. Male and female rats were exposed to a mixed-modality chronic adolescent stress paradigm consisting of isolation, restraint, and social defeat or non-stress control conditions during adolescence (PND

35-49). In adulthood (PND 94), rats were exposed to a novel acute forced-swim stressor, and brain tissue was collected 15, 30, or 120 minutes following acute stress exposure. A separate control group was collected at baseline without acute stress exposure. The hippocampus was dissected and quantitative PCR was used to assess gene expression of *Fkbp5*, the negative regulator of glucocorticoid receptor nuclear translocation. Glucocorticoid receptor localization was assessed by western blot in nuclear and cytoplasmic protein fractions. Adult female rats exposed to chronic adolescent stress exhibit increased *Fkbp5* gene expression in the hippocampus at baseline compared to non-stressed controls ($p < 0.05$). Additionally, female rats exposed to chronic adolescent stress exhibit reduced hippocampal nuclear GR 30-minutes following acute stress exposure, suggesting impaired nuclear translocation activity of the receptor. Adult males do not exhibit altered *Fkbp5* expression or GR localization in chronic adolescent stressed rats compared to non-stressed controls ($p > 0.05$). These studies provide novel evidence that chronic adolescent stress impacts the molecular regulation of the glucocorticoid receptor with long-term implications into adulthood. Furthermore, these studies highlight the sex-specific nature of the consequences of adolescent stress exposure on the molecular level and the potential for a role of the glucocorticoid receptor in mediating the sex-specific behavioral effects of chronic adolescent stress exposure.

**Disclosures:** **S.A. Rowson:** None. **M. Bekhbat:** None. **S.D. Kelly:** None. **G.N. Neigh:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.07/NN16

**Topic:** F.04. Stress and the Brain

**Support:** NR014886

**Title:** Adolescent stress leads to enduring enrichment of inflammatory pathways in the hippocampus without peripheral immune consequences

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**Abstract:** Chronic adolescent stress (CAS) has previously been shown to prime the hippocampal inflammatory response in adult male rats; however, the underlying mechanism and origin are undefined. Here we tested the hypothesis that CAS exaggerates induction of the pro-inflammatory NF-kappa-B pathway in adult rat hippocampus without compromising the

peripheral immune response. Male and female adolescent rats underwent a mixed-modality CAS paradigm or received no stress. Five weeks following the last stressor all rats received a single, systemic injection of either a low dose of lipopolysaccharide (LPS) or vehicle to unmask possible priming effects of CAS. Total RNA from the hippocampus was used to perform RNA-Seq and enriched transcriptional pathways were identified using gene set enrichment analysis. NF-kappa-B emerged as the most enriched pathway in CAS males and females compared to non-stressed controls following LPS. Targeted qPCR experiments further confirmed that CAS exaggerated the expression of I-kappa-B ($F(1,105)=4.209$, $p=0.043$), p65 ($F(1,105)=5.262$, $p=0.024$), and p52 ($F(1,105)=8.186$, $p=0.005$). To explore the underlying mechanisms of CAS-potentiated neuroinflammation, we tested whether CAS impaired glucocorticoid-mediated anti-inflammatory gene expression. Interestingly, CAS led to a marginal increase in the induction of the anti-inflammatory gene DUSP1 ($F(1,105)=3.253$, $p=0.074$), indicating partial anti-inflammatory compensation in CAS rats. CAS impacted neither LPS-induced NF-kappa-B activity in the spleen ($F(1,57)=0.256$, $p>0.05$) nor plasma IL-6 ($F(1,58)=1.083$, $p>0.05$) and TNF-alpha ($F(1,43)=0.60$, $p>0.05$), suggesting that the central effects of CAS on the NF-kappa-B pathway are independent of changes to the peripheral immune response. Collectively, our results indicate that chronic stress experienced during adolescence leads to long-lasting changes to the hippocampal genomic profile, and that the inflammatory consequences of CAS are specific to the brain.

**Disclosures:** **M. Bekhbat:** None. **S.A. Rowson:** None. **S.D. Kelly:** None. **G.K. Tharp:** None. **M.G. Tansey:** None. **G.N. Neigh:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

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**Topic:** F.04. Stress and the Brain

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**Title:** Adolescent social stress differentially impacts affect-related behaviors and nicotine responses in C57BL/6J and BALB/cJ mice

**Authors: *M. J. CARUSO**[1], D. E. REISS[1], J. L. THOMAS[1], J. I. CAULFIELD[1], N. A. CROWLEY[2], S. A. CAVIGELLI[1], H. M. KAMENS[1]
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**Abstract:** Affective disorders and nicotine use are significant contributors to global morbidity and mortality as independent and comorbid diseases. Exposure to adolescent stress, potentially via stress-induced hypothalamic-pituitary-adrenal axis dysregulation, can exacerbate both. Little is known about the factors that promote susceptibility to comorbidity of these disorders. We examined the relationship between stress-induced changes to affect-related behaviors and nicotine responses. Male and female mice (BALB/cJ & C57BL/6J) were exposed to either chronic variable social stress (CVSS) or control conditions during adolescence (PND 25-59). In adulthood, anxiety/depression-related behaviors were measured using the elevated plus-maze (EPM) and social interaction test. Nicotine responses were assessed via acute effects on body temperature, corticosterone production, locomotor activity, and voluntary oral nicotine intake. We also characterized spontaneous inhibitory and excitatory postsynaptic currents (sIPSC/sEPSC) in the prefrontal cortex (PFC), basolateral amygdala, and nucleus accumbens core (NAc), because these stress-susceptible regions regulate affect- and drug-related behaviors. Relative to controls, CVSS males exhibited reduced EPM open arm activity and increased social avoidance (male C57BL/6J). Alternatively, CVSS increased nicotine-induced locomotion during late-adolescence and adult nicotine-induced corticosterone production in BALB/cJ males, relative to controls. CVSS decreased adult voluntary nicotine consumption in BALB/cJ males, relative to controls. In C57BL/6J males, CVSS increased sIPSC frequency in the mPFC, relative to controls. The amplitude and frequency of sEPSCs were also decreased by CVSS in the mPFC and NAc, respectively. Females were unaffected by CVSS. Results indicate that CVSS differentially impacts affect-related behavior/physiology or nicotine responses in C57BL/6J and BALB/cJ males. Thus, changes in affect-related behavior/physiology and nicotine responses following adolescent social stress are sex- and genotype-dependent.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

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**Topic:** F.04. Stress and the Brain

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National Center of Competence in Research (NCCR) “SYNAPSY - The Synaptic Bases of Mental Diseases” from the Swiss National Science Foundation (n° 51NF40-158776)

**Title:** Peri-pubertal period is a sensitive time for stress exposure in wild-type and mice with a genetic redox dysregulation

**Authors: M. R. SCHNIDER**, P. STEULLET, *J. CABUNGCAL, K. Q. DO
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**Abstract:** Accumulating evidence suggests that combination of genetic and early-life risk factors favours development of pathologies associated with psychiatric disorders such as schizophrenia. Furthermore, the nature of pathological phenotypes could depend on the timing of the environmental insult during postnatal development and the specific genetic vulnerability. Here, we explored these issues using WT mice and mice with a genetic redox dysregulation due to low GSH levels (Gclm KO). Our results show that a mild sub-chronic stress during pre-weaning period (PND10-20) did not induce overall stress effects in adulthood whereas the same stress protocol applied during peri-puberty (PND 30-40) altered startle response to an acoustic stimulus and induced spatial short-term memory deficit. Further we found a spatial recognition memory deficit that was caused by both factors (stress, redox dysregulation). These suggested that the peri-pubertal period is a sensitive time period for psychosocial stress. When exposed during peri-puberty to a more severe stress protocol (social defeat stress), mice exhibited in adulthood anxiety-like behavior, reduced sociability, anomalies in sensory gating capacity (pre-pulse inhibition of acoustic startle) and increased sensitivity to NMDA-receptor antagonist dizocilpine. In contrast, social defeat stress during late adolescence (PND 50-60) led to a hyper-locomotor phenotype (open field) but did not affect any other behaviors. We found however that social defeat had for the most part similar effects in WT and KO mice with the exception of affective behavior. This suggests a modulatory role of the GSH system on anxiety-like behavior. We then investigated the effect of a peri-pubertal social defeat stress on antioxidant systems in the anterior cingulate cortex (ACC) and hippocampus. In both regions, the positive correlation between GSH peroxidase (GPx) and GSH reductase (GR) activities was disrupted in socially defeated mice. Interestingly, a loss of correlation between GPx and GR activities was also found in the blood of schizophrenic patients that suffered from early-life trauma. In the hippocampus only, social defeat also decreased thioredoxin levels but increased expression of Nrf2, a key transcription factor in the antioxidant defense. These stress-induced alterations were found in both genotypes. This suggests that peri-pubertal psychosocial stress affects redox systems particularly in the hippocampus. In summary, we found that the type of long-term stress-induced behavioral alterations depend on the intensity and exact timing of the stress, with the peri-pubertal period being a particularly vulnerable time.

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**Poster**

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**Topic:** F.04. Stress and the Brain

**Support:** Brain & Behavior Foundation NARSAD Young Investigator Grant

Ohio University CRSCA Student Enhancement Award

**Title:** Perinatal fluoxetine and maternal stress differentially affect serotonin and synaptic densities in juvenile and adult male and female offspring

**Authors: *M. GEMMEL**[1], E. BÖGI[2], S. DE LACALLE[3], S. TANDA[1], N. KOKRAS[4], C. DALLA[4], J. L. PAWLUSKI[1,5]
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**Abstract:** Women are susceptible to developing perinatal depression if they suffer from a history of depressive-episodes, anxiety, or prolonged stress. Selective serotonin reuptake inhibitors (SSRIs) are the most common antidepressant for perinatal maternal depression, with 10% of pregnant women being prescribed these medications. SSRIs cross the placental and blood-brain barrier, potentially affecting offspring neurodevelopment. While clinical work suggests an effect of prenatal SSRIs on infant neural development via peripheral biomarkers, it is unclear if there are long-term effects of perinatal SSRI exposure on the developing brain. The aim of this study was to determine how fluoxetine exposure, a popular SSRI used during the perinatal period, affects the serotonergic system and synaptic plasticity in the prefrontal cortex (PFC) of rat offspring. To model aspects of maternal depression, prior to breeding, Sprague-Dawley rat dams were subjected to chronic unpredictable stress. Perinatal treatment with fluoxetine (10mg/kg/day) or vehicle occurred from gestation day 10 to postnatal day 21. The following four groups of male and female offspring, in the juvenile or adult period, were used: 1. Control+Vehicle, 2. Control+Fluoxetine, 3. Pre-gestational Maternal Stress+Vehicle, 4. Pre-gestational Maternal Stress+Fluoxetine. Brains of offspring were used for monoamine analysis (juveniles) in the PFC and measures of synaptic plasticity (juveniles and adults) in the cingulate cortex (mPFC). Perinatal fluoxetine increased juvenile serotonin levels in the PFC. However, pre-gestational maternal stress, regardless of perinatal fluoxetine, reduced post-synaptic density, via PSD-95 immunoreactivity, in the mPFC of juvenile and adult male offspring, but had no effect on pre-synaptic density as measured by synaptophysin immunoreactivity. There were also no long-term effects of maternal stress or perinatal fluoxetine on synaptophysin or PDS-95

densities in the mPFC of female offspring. These results point to an enduring, sex-dependent effect of maternal stress on plasticity in the PFC. Investigating the impact of perinatal SSRIs and maternal stress on neurodevelopment will further our understanding of the benefits and risks of these medications to treat maternal affective disorders.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.11/NN20

**Topic:** F.04. Stress and the Brain

**Support:** NIH

**Title:** Hyperactivation of HPA axis during adolescent critical period induces aberrant spine pruning in medial PFC and depressive behavior mediated by Rho-PAK signaling

**Authors: *K. AN**, S. K. BARODIA, J. R. MOORE, T. CASH-PADGETT, H. JAARO-PELED, M. NIWA, A. SAWA
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**Abstract:** Adolescence is the time period when individuals expose themselves to much more social communication, which in turn provides them higher level of stressors. Although spine change has been understood by intracellular mechanisms triggered by neural activation, it is still unclear whether stressors may also participate in the physiological and possibly pathophysiological synaptic pruning in adolescence. In the present study, we first identify that aberrant synaptic pruning in the layer 5 pyramidal neurons of the medial PFC during adolescent critical period using a DISC1 transgenic mouse, which showing depressive behavior phenotype. In this model, we demonstrate increased glucocorticoid levels during early adolescent period activates the expression of RhoU mediated by GR and/or MR signaling and affected PAK activation. Blocking the downstream effector of RhoU, *i.e.*, PAK with FRAX 486 prohibited the arousing of depressive behavior as well as synaptic pruning. The effect of RhoU-mediated PAK activation on E/I balance in the pyramidal neuron and *in vivo* γ-oscillation will be explored.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** F.04. Stress and the Brain

**Support:** NSF Grant #1545803

**Title:** Isolation during adolescence increases CPP to cocaine in male rats

**Authors: *R. J. TORRES**[1], I. G. SANTIAGO[2], J. A. FREIRE[2], C. J. RIVERO[2], A. C. SEGARRA[2]
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**Abstract:** Adolescence and early adulthood are the age period of highest drug abuse. Dopaminergic (DA) output from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), prefrontal cortex (PFC) and lateral septum (LS) are essential components the reward circuitry and differ between adolescent and adult rats. In this study we investigated if isolation during adolescence alters the rewarding properties of cocaine in male rats. Since Dopamine D2-receptors (D2DR) are inhibitory receptors associated with decreased reinforcement to the rewarding effects of drugs of abuse, D2 receptors in the NAc, LS and PFC were assessed as well. Following weaning (day 23), male rats were single (isolation) or group-housed with siblings of the same sex. At day 34 they were tested in an open field (OF) and subsequently in an elevated plus maze (EPM). From days 35-48 they were tested for Conditioned Place Preference (CPP) to cocaine (15 mg/kg).
We found that isolated male rats spent more time in the margins of an open field and also in the closed arms of an EPM than grouped housed males, indicative of greater anxiety. They also showed greater conditioning to cocaine than their grouped-housed counterparts. We also observed that isolation decreased D2 immunoreactivity in the NAc, LS and PFC, a difference that was not observed in isolated rats that were treated with cocaine. These data indicate that a social stressor such as isolation during adolescence, can induce profound and long lasting changes in emotional and addictive behaviors. This work was partially supported by NSF PIRE program #1545803.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.13/NN22

**Topic:** F.04. Stress and the Brain

**Support:** CONACyT

**Title:** Social isolation during adolescence induces sex-dependent differences on the activity of the hypothalamic-pituitary-thyroid axis, and its response to cold exposure

**Authors: D. RODRÍGUEZ-SARMIENTO**, *E. L. JAIMES, P. JOSEPH-BRAVO
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**Abstract:** Social stressors in rats such as perinatal maternal separation or social isolation can have long-lasting effects on their neuroendocrine development and response to environmental stressors later in life. Response to social isolation depends on the animal's age, sex, duration of the stressor, and to external factors (environmental events). As the hypothalamic-pituitary-thyroid (HPT) axis activity is susceptible to various forms of stress, we studied if post-weaning social isolation altered the HPT axis function and its response to an environmental stressor, such as cold, and if changes were dependent on the animal´s sex. The initial response to acute exposure to cold begins with the increased expression of *Trh* in the paraventricular nucleus (PVN) and its hypothalamic release. This, in turn, stimulates the release of TSH from the anterior pituitary gland and thyroid hormones from the thyroid gland into the circulation. Male and female Wistar rats were socially isolated at weaning on postnatal day (PND) 23 and through adolescence. Starting adulthood (PND 60), half of the male and female rats were left undisturbed at room temperature (RT) or exposed to a cold environment (4°C) for one hour, then immediately sacrificed. mRNA levels of *Trh* in the PVN, *Trh-r1*, *Tsh-β*, *Dio2* in adenohypophysis; and expression of genes associated with brown adipose tissue (BAT) thermogenesis (*β3-AR*, *Pgc1-α*, *Dio2* and *Ucp-1*) were semi-quantified by RT-PCR. Serum hormones were quantified by radioimmunoassay or ELISA. Social isolation induced a significant increase of serum corticosterone concentration in females but not male rats. Basal HPT axis activity was not affected by social isolation in female rats, and serum TSH concentration increased in response to cold exposure as expected, compared to animals kept at RT. In male rats, isolation induced a hypothyroidism, concentration of serum TSH increased and that of T4 decreased compared to naïve animals. In response to cold exposure, *Trh* mRNA levels increased in isolated male rats, but not that of serum TSH. No significant changes were found in gene expression on either anterior pituitary or BAT of isolated cold exposed male or female rats. In conclusion, social isolation induces differences in the HPT axis function depending on the

animal´s sex. Male isolated rats become hypothyroid, partially blunting the activation of the HPT axis in response to cold exposure.

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**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.14/NN23

**Topic:** F.04. Stress and the Brain

**Support:** FAPESP

**Title:** Different physical exercise programs during pregnancy attenuates the negative effects of PTZ-induced seizures in offspring from mothers submitted to prenatal stress

**Authors: *G. M. LOPIM**[1], E. A. DA SILVA[3], D. V. CAMPOS[2], A. A. DE ALMEIDA[3], J. FERNANDES[2], R. C. GUTIERRE[2], R. M. ARIDA[2]
[2]Physiol., [1]Univ. Federal De Sao Paulo, Sao Paulo, Brazil; [3]Physiol., Univ. Federal de São Paulo, Sao Paulo, Brazil

**Abstract: 1. Purpose:** Prenatal environmental factors such as stress in pregnant rats exert a profound influence on development of nervous system and can affect susceptibility to epilepsy. On the other hand, environmental stimuli such as maternal physical exercise may favor brain development in the offspring. Thus, physical exercise contributes to reduce seizure susceptibility before brain insult and seizure frequency in the chronic epilepsy condition. Although studies have demonstrated the deleterious influence of stress during pregnancy on offspring behavioral and seizure susceptibility, very little is known about how to minimize these negative effects. This work aimed to verify whether physical exercise during pregnancy of mothers submitted to prenatal stress minimizes offspring seizure susceptibility in the beginning of postnatal development. **2. Methods:** Pregnant rats were divided into the following groups: control (n=4); forced exercise (treadmill) (n=3); stress (restraint) (n=3); forced exercise/stress (n=3); voluntary exercise (n=3); voluntary exercise/stress (n=3). Male pups were divided into the following groups: control (n=17); forced exercise (n=14); stress (n=14); forced exercise/stress (n=17); voluntary exercise (n=16); voluntary exercise/stress (n=17). The threshold for first motor manifestation after a unique dose of pentylenetetrazole (PTZ) (45mg/kg) was analyzed at 25 postnatal days. **3. Results:** A reduction in the threshold for first motor manifestation was observed in the stress group compared to control group ($p<0.001$). Both forced and voluntary exercise/stress groups increased the threshold for first motor manifestation compared to stress group ($p<0.005$). Increased threshold for first motor manifestation was also noted in physical exercise (forced and voluntary) groups compared to forced ($p<0.001$) and voluntary ($p<0.000$)

exercise/stress groups. **4. Conclusion:** Our results demonstrate that both forced and voluntary exercise during gestation attenuates the negative effects of PTZ-induced in offspring from mothers submitted to prenatal stress.

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**Poster**

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**Title:** Mouse strain differences in SSRI sensitivity correlates with serotonin transporter binding and function

**Authors: *Z. JIN**[1], Y. LI[2]
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**Abstract:** Selective serotonin reuptake inhibitors(SSRIs) bind 5-HT transporters, leading to the accumulation of 5-HT and amelioration of depression. Although different mouse strain showed different sensitivity to SSRIs in mouse models of depression, the reason for these strain differences remains unclear.Therefore, in the present study, we examined immobility time and locomotor activity in two mouse strains, namely, C57BL/6J and DBA/2J mice, and the effects of the SSRIs fluoxetine, paroxetine, and citalopram in these mice. Furthermore, we analysed 5-HT transporter binding and reuptake inhibition in both strains to explore their relationship to the immobility and locomotor activity effects of the three SSRIs in these two mouse strains.We found that the SSRI citalopram dose dependently reduced immobility time in both the FST and TST in DBA/2J but not C57BL/6J mouse strains, whereas fluoxetine showed opposite results. Paroxetine reduced immobility time similarly in both strains. The affinity of citalopram for the 5-HT transporter in DBA/2J mice was 700-fold higher than that for in C57BL/6J mice, whereas the affinity of fluoxetine in C57BL/6J mice was 100-fold higher than that in the DBA/2J mouse.Furthermore, High citalopram concentrations were required to [$^3$H]5-HT uptake in C57BL/6J but not DBA/2J mouse cortical synaptosomes, whereas fluoxetine also showed opposite results. The effects of paroxetine on 5-HT transporter binding and on the synaptosomal 5-HT uptakeParoxetine showed similarly in both strains. These results suggest that immobility duration depends on 5-HT transporter binding levels, leading to apparent strain differences in

immobility time in the FST and TST. Furthermore, differences in 5-HT transporter binding may cause variations in SSRI responses on behaviors.

**Disclosures: Z. Jin:** None. **Y. li:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.16/NN25

**Topic:** F.04. Stress and the Brain

**Support:** ARRS J7-7226

ARRS P4-0053

**Title:** Sham injection stress of pregnant mice affects behavior of adult male offspring

**Authors: *G. MAJDIC**[1], *G. MAJDIC[1], M. OGRIZEK[2], K. KOZINC KLENOVSEK[2], N. GRGUREVIC[2]
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**Abstract:** Numerous studies in recent years have shown that prenatal or postnatal stress could affect brain development and cause long term consequences on brain function. In developmental studies, pregnant mice are often injected with different substances to study their effects on developing fetuses. In the present study, we therefore investigated whether the prenatal sham injection of pregnant mice could affect the development of the fetal brain. Pregnant mice were divided into 4 groups. Three groups were sham injected (skin pierced with a 20G needle in the back region as for subcutaneous injections); the first group on days 13, 14 and 15 p.c., the second group on days 17 and 18 p.c. and the third group on days 13, 14, 15, 17 and 18 p.c. Control group was not injected. In male offspring of injected mice, intermale aggressive behavior was evaluated. Additionally, we followed the offspring bodyweight, and at sacrifice measured their testosterone levels in blood and processed their brain for immunocytochemistry. Male mice from the second and the third experimental groups (injections from E17-E18 and from E13-E18) had significantly reduced intermale aggressive behavior in comparison to the males from control group and the first group (injected on days E13-E15). Furthermore, testosterone levels in the second and the third groups were significantly reduced, although testis weights and seminal vesicle weights were not affected by prenatal injections. Body weight in male offspring was significantly higher in the first (E13-E15) and in the third (E13-E18) group in comparison to the second group and control mice. Immunocytochemical staining in two sexually dimorphic areas, calbindin positive group in the preoptic area and vasopressin expressing fibers in the lateral

septum did not differ between groups. These results suggest that prenatal injections of pregnant mice are serious enough stress that could cause behavioral consequences in adult offspring, and should be taken into account when planning and executing experiments with mice.

**Disclosures: G. Majdic:** None. **G. Majdic:** None. **M. Ogrizek:** None. **K. Kozinc Klenovsek:** None. **N. Grgurevic:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.17/NN26

**Topic:** F.04. Stress and the Brain

**Support:** NIDA Grant R00 DA033372

**Title:** Adolescent stress exposure impairs short-term synaptic plasticity in the Nucleus Accumbens

**Authors: *A. U. DEUTSCHMANN**, A. Q. FOSNOCHT, L. A. BRIAND
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**Abstract:** Adolescent social stress puts individuals at increased risk for multiple psychiatric diseases, including substance use disorders. However, little is known about how adolescent social isolation alters the brain to make an individual more vulnerable to addiction. The nucleus accumbens plays a central role in the development and expression of addictive behaviors and is influenced by stress exposure. The current study utilized an adolescent isolation stress model that we have shown to increase motivation for cocaine in adulthood in order to examine the effects of stress on accumbal physiology. Here we show that adolescent social isolation stress leads to a persistent disruption in short-term synaptic plasticity in the nucleus accumbens. As adults, animals that experienced adolescent social isolation exhibit a decrease in paired pulse facilitation and an increase in sEPSC amplitude and frequency. A disruption in paired pulse facilitation often indicates an increase release probability. However, adolescent isolation stress impairs paired pulse facilitation without altering initial release probability, suggesting alterations in the readily releasable pool. Future studies are underway to test this hypothesis. Taken together, we have demonstrated that adolescent isolation stress alters presynaptic function in the nucleus accumbens. The nucleus accumbens receives glutamatergic input from multiple brain regions including the amygdala, hippocampus, and prefrontal cortex. Work is underway to examine the individual contributions of these inputs to the effects of adolescent social stress on accumbal physiology.

**Disclosures: A.U. Deutschmann:** None. **A.Q. Fosnocht:** None. **L.A. Briand:** None.

**Poster**

**602. Adolescent Stress: Neurological and Neurobehavioral Outcomes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 602.18/NN27

**Topic:** F.04. Stress and the Brain

**Support:** R00 DA033372

Temple University Creative Arts, Research And Scholarship (CARAS) Program

**Title:** The role of glutamate receptor trafficking in vulnerability to a social defeat stress

**Authors: *K. E. LUCERNE**, A. Q. FOSNOCHT, L. A. BRIAND, J. LENZ, A. S. ELLIS
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**Abstract:** Individuals who experience social stressors such as social rejection or isolation are more likely to develop psychiatric disorders such as substance use disorder, anxiety disorders, or depression. However, not all individuals who experience these social stressors go on to develop psychiatric disorders. Understanding the mechanisms by which individuals are more or less vulnerable to social stress could provide avenues for prevention. Exposure to stress leads to neuroadaptations in glutamatergic transmission and AMPA receptor trafficking. However, very little is known about the role that this trafficking plays in vulnerability to stress. The current studies utilized two different mutant mouse models with disruptions in AMPAR trafficking proteins. We first utilized a mouse with a point mutation in the GluA2 AMPA subunit that prevents PKC-dependent phosphorylation and internalization. We found that disrupting activity-dependent GluA2 internalization lead to an increased susceptibility to social defeat. Next, we examined whether mice with a constitutive knockout of PKMzeta, a protein involved in insertion of AMPARs, exhibited alterations in susceptibility to social defeat. Preventing the insertion of AMPARs into the membrane led to a decreased susceptibility to social defeat. Taken together this suggests that increased AMPAR expression leads to increased vulnerability to social defeat stress. Future work will examine how this affects the ability of social stress to potentiate cocaine-taking behavior.

**Disclosures: K.E. Lucerne:** A. Employment/Salary (full or part-time):; Temple University. **A.Q. Fosnocht:** A. Employment/Salary (full or part-time):; Temple University. **L.A. Briand:** A. Employment/Salary (full or part-time):; Temple University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; R00 DA033372. **J. Lenz:** A. Employment/Salary (full or part-time):; Temple University. **A.S. Ellis:** A. Employment/Salary (full or part-time):; Temple University.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.01/NN28

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** Natural Sciences and Engineering Research Council of Canada (NSERC)

Fondation de recherche en chiropratique du Québec (FRCQ)

Fonds de recherche du Québec- Santé (FRQS)

Chaire de recherche en neurophysiologie de la douleur

**Title:** Spinal cord neurovascular coupling is not affected by isoflurane anesthesia: Evidence from decerebrated rats

**Authors:** *T. PAQUETTE[1], ***T. PAQUETTE**[1], *T. PAQUETTE[3], H. LEBLOND[1], M. PICHÉ[2]
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**Abstract:** Neurovascular coupling is a physiological process involving a local hemodynamic response associated with surrounding neuronal activity. Functional magnetic resonance imaging (fMRI) uses this relation to infer neuronal activity by measuring magnetic signals related to changes in deoxyhemoglobin concentration. In a previous study, isoflurane-anesthetized rats showed tight spinal cord neurovascular coupling that was unaffected by large fluctuations in systemic blood pressure. However, the influence of isoflurane on spinal neurovascular coupling is not established. The objective of this study is to examine the effect of anesthesia on spinal neurovascular coupling in anesthetized decerebrated and non-anesthetized decerebrated rats, during nociceptive processing.
All experimental procedures were approved by the Université du Québec à Trois-Rivières animal care committee, and were in accordance with the guidelines of the Canadian Council on Animal Care. Six male Wistar rats were anesthetized using isoflurane (1.2-1.5 %). After a craniectomy, rats were decerebrated mechanically. Local field potentials (LFP) and spinal cord blood flow were recorded simultaneously in the lumbosacral enlargement, where activity was evoked by electrical stimulation of the sciatic nerve. The mean arterial pressure was recorded continuously with a pressure transducer connected to a cannula inserted in the right carotid artery. Constant current electrical stimulation was applied on the sciatic nerve at 8 stimulus intensities ranging between 0.1 and 9.6 mA. Physiological responses to electrical stimulation were recorded in

decerebrated rats with or without isoflurane anesthesia.
In decerebrated rats under anesthesia, mean arterial pressure changes evoked by sciatic stimulation were not significantly different compared with those of decerebrated rats without anesthesia (p=0.064). Moreover, anesthesia has no effect on the LFP (p=0.53) and spinal cord blood flow responses evoked by electrical stimulation (p=0.57). The neurovascular coupling remained tight and comparable between rat with or without anesthesia (p=0.39). Furthermore, stimulus intensity did not alter neurovascular coupling, which remained comparable for all intensities between 0.15 and 9.6 mA compared with the 0.1 mA intensity.
These results support the use of isoflurane in neurovascular studies. Also, results suggest that fMRI is an adequate method to quantify the neuronal activity based on the spinal hemodynamic changes in anesthetized or non-anesthetized subject.

**Disclosures:** **T. Paquette:** None. **H. Leblond:** None. **M. Piché:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.02/DP12/NN29 (Dynamic Poster)

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** The study was funded by grants from the Simons Foundation (SFARI 314688 and 400101, A.G.).

**Title:** Brainwide mapping of spontaneous network dynamics in the mouse brain

**Authors: D. GUTIERREZ BARRAGAN**[1], S. PANZERI[1], *A. GOZZI[2,1]
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**Abstract:** Spontaneous brain activity is characterized by recurrent coupling and decoupling of local and remote neuronal substrates across different spatio-temporal scales. Resting-state functional magnetic resonance imaging (rsfMRI) has been recently employed in humans to non-invasively map brainwide network dynamics via recordings of spontaneous blood oxygen level-dependent (BOLD) signal.
Such investigations have shown that spontaneous network activity undergoes spatio-temporal reconfiguration recapitulating a finite number of brain functional states, which can be related to higher-order cognitive processes.
By mapping the repertoire of spontaneous BOLD co-activation among brain regions, here we show that analogous transitions occur in rsfMRI datasets of the mouse brain. We used clustering analysis to sort and selectively average rsfMRI activity timeframes into distinct and recurrent patterns of non-stationary co-activation and co-deactivation that can be mapped with voxel

resolution.
We describe a reduced number of reproducible brainwide patterns encompassing the co-occurrence of previously described intrinsic connectivity networks of the mouse brain, including integrative high-order cortical regions (default-mode, salience) as well as motor sensory, hippocampal and sub-cortical networks. Notably, inverse co-occurrence of default-mode and lateral cortical networks activity is a prominent feature of many of the identified states, suggesting a competing relationship between these two macroscale neural systems, recapitulating a cardinal feature of human network organization. We also show that the identified network states are characterized by smooth transitions from one state to another, as well as gradual assembly and disassembly.
Collectively, our findings corroborate the presence of dynamic reconfiguration of spontaneous network activity as a fundamental, evolutionary-conserved principle of mammalian cortical activity, and identify a set of recurrent brain states that characterize brainwide network dynamics in the mouse. This approach paves the way to targeted investigations of the elusive neural drivers of spontaneous network dynamics via interventional approaches in rodent models.

**Disclosures: D. Gutierrez Barragan:** None. **S. Panzeri:** None. **A. Gozzi:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.03/NN30

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** RO1MH089003

RO1NS085200

**Title:** Novel restraint device in awake rat imaging

**Authors: *S. R. CRAMER**[1], Y. MA[1], N. ZHANG[2,3]
[1]Biomed. Engin., [3]The Huck Inst. of Life Sci., [2]The Pennsylvania State Univ., State College, PA

**Abstract:** Functional imaging in awake animals has become an important tool for measuring whole-brain functional connectivity. Unfortunately, the imaging environment is stressful, and the loud noises emanating from the magnetic resonance imaging (MRI) gradients are not conducive to motionless subjects. To achieve better imaging performance, recent studies have focused on improving head restraint, but little attention has been given to the restraint of the body. In the absence of proper body restraint, motion artifacts arising from head movement persist. Moreover, appropriate design of the body restraint may significantly reduce pain, stress, and

fear. Otherwise, the over activity of brain regions responsible for these behavioral manifestations may cause incorrect interpretations of data. In this study, we developed an integrated full body restraint device that optimizes both head and body restraint. In addition to minimizing total subject motion, this device focused on reducing stress levels associated with imaging. Blood corticosterone levels were measured before, during, and after a novel acclimation procedure that involved the full body restraint device. Other physiological indexes were monitored and recorded during acclimation including respiration and heart rate. To further validate this new restraining setup, single and multi-echo fMRI data were acquired in awake rats during resting state and visual stimulations.

**Disclosures: S.R. Cramer:** None. **Y. Ma:** None. **N. Zhang:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.04/NN31

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NINDS Grant R01NS085200

NIMH Grant R01MH098003

**Title:** Myelination covariance of human cerebral cortex

**Authors: *Z. MA**, N. ZHANG
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**Abstract:** Brain morphometric covariance of gray matter density or cortical thickness has been widely used for studying brain connectivity. These structural covariance approaches were based on the morphometry of mixed brain compartments, whereas the covariance of any specific component such as cortical myelin has yet to be explored. In the present study, we extended this structural covariance concept by adopting T1w/T2w ratio myelin content for cross-population covariance measurement. Using T1w/T2w ratio myelin maps from the Human Connectome Project, we systematically investigated cross-subject myelination covariance of the human cerebral cortex and identified highly reproducible myelination covariance patterns. We constructed a whole-brain myelination covariance network and characterized its topological properties. We further evaluated the correspondence between myelination covariance and resting-state functional connectivity and revealed their unique network-specific relationship. This study has promoted the understanding of the brain organization from myelination covariance and also demonstrated the structural covariance-function relationship of the human cerebral cortex.

**Disclosures:** **Z. Ma:** None. **N. Zhang:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.05/NN32

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** DFG Wa2673/4-1

German SFB 779/A06

**Title:** Brain-derived neurotrophic factor (BDNF) as a potential biomarker for resting-state network remodeling after ketamine infusion

**Authors: *M. WOELFER**[1,2,3], M. LI[1,2], L. COLIC[1,2], V. LESSMANN[4], T. BRIGADSKI[4,5], M. WALTER[1,2,6]
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**Abstract:** Introduction Ketamine, an NMDAR antagonist, produces fast antidepressant response with a maximum efficacy 24h after infusion [1]. Animal studies showed that ketamine generates higher synaptic plasticity via induction of Brain-derived neurotrophic factor (BDNF) [2]. Patients with depression have lower blood level of BDNF and show an increase after ketamine infusion [4]. Besides, depressed patients have hyperconnectivity within the Default mode network (DMN) [5]. Ketamine decreases the Functional Connectivity (FC) in healthy subjects between posterior cingulate cortex (PCC) and medial prefrontal cortex (mPFC), two main regions within DMN [6], revealing possible mechanisms of the antidepressant effect.
Methods In a double-blind study, 81 healthy subjects received infusion of either ketamine (0.5 mg/kg) or saline. In each subject, resting state fMR images (TR= 2.8s, voxel size= $2 \times 2 \times 2$ $mm^3$, 7T ) and plasma blood samples were acquired at baseline and 24h post-infusion. Ketamine (n=32) and placebo (n=30) groups were preprocessed using scripts developed in the functional connectome 1000 project. Dorsal PCC (dPCC) was chosen as a priori seed region [6] and FC maps were calculated. The mean FC value was extracted from clusters showing significant change after infusion. Extracted FC values were correlated with the plasma BDNF at 24h (n=45).
Results 24h after infusion, ketamine treated subjects showed higher BDNF level compared to placebo (p=0.015). A decrease in FC between the dPCC and both dorsolateral PFC (p=0.005 FWE) and two cluster in mPFC (p=0.001 & p=0.030 FWE) was observed 24h after infusion.

Subjects with higher BDNF level after ketamine show significantly higher FC disconnection between dorsal PCC and mPFC (r= 0.64, p=0.006).
Conclusions Ketamine decreases FC within the DMN and increases BDNF 24h after ketamine. Higher BDNF level correlates with stronger FC disconnection between anterior (mPFC) and posterior (dPCC) part of DMN. This finding highlights the role of BDNF as a potential biomarker for network remodeling in the brain.
1 Zarate et al. (2006) A Randomized Trial of an N-methyl-D- aspartate Antagonist in Treatment-Resistant Major Depression. 2Duman et al. (2012) Signaling pathways underlying the rapid antidepressant actions of ketamine. 3Haile (2013) Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. 4Kaiser et al. (2015). Large-scale network dysfunction in major depressive disorder. 5 Scheidegger et al. (2012). Ketamine decreases resting state functional network connectivity in healthy subjects: implications for antidepressant drug action.

**Disclosures:** **M. Woelfer:** A. Employment/Salary (full or part-time):; Ms. Woelfer is supported by scholarship from the Otto-von-Guericke-University Magdeburg. 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.; This study was supported by German Research Foundation (SFB 779/A06 and DFG Wa2673/4-1), Centre for Behavioural and Brain Sciences (CBBS NN05) and Leibniz Association to Prof. Walter. **M. Li:** None. **L. Colic:** A. Employment/Salary (full or part-time):; received a scholarship from German Research Foundation (SFB 779, 2013-2016). **V. Lessmann:** None. **T. Brigadski:** None. **M. Walter:** A. Employment/Salary (full or part-time):; Supported by German Research Foundation (SFB 779/A06 and DFG Wa2673/4-1), Centre for Behavioural and Brain Sciences (CBBS NN05), Supported by Leibniz Association (Pakt für Forschung und Innovation).

**Poster**

## 603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.06/NN33

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Title:** Brainwide mapping of endogenous serotonergic transmission via chemogenetic-fMRI

**Authors: *M. PASQUALETTI**[1,2], A. GIORGI[1], S. MIGLIARINI[1], M. GRITTI[3], A. GALBUSERA[2], G. MADDALONI[1], M. A. DE LUCA[4], R. TONINI[3], A. GOZZI[2]
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**Abstract:** Serotonergic transmission affects behaviours and neuro-physiological functions via the orchestrated recruitment of distributed neural systems. It is however unclear whether serotonin's modulatory effect entails a global regulation of brainwide neural activity, or is relayed and encoded by a set of primary functional substrates. Here we combine DREADD-based chemogenetics and mouse fMRI, an approach we term "chemo-fMRI", to causally probe the brainwide substrates modulated by phasic serotonergic activity. We describe the generation of a conditional knock-in mouse line that, crossed with serotonin-specific cre-recombinase mice, allowed us to remotely stimulate serotonergic neurons during fMRI scans. We show that chemogenetic stimulation of the serotonin system does not affect global brain activity, but results in region-specific activation of a set of primary target regions encompassing parieto-cortical, hippocampal, and midbrain structures, as well as ventro-striatal components of the mesolimbic reward systems. Many of the activated regions also exhibit increased c-Fos immunostaining upon chemogenetic stimulation in freely-behaving mice, corroborating a neural origin for the observed functional signals. These results identify a set of regional substrates that act as primary functional targets of endogenous serotonergic stimulation, and establish causation between phasic activation of serotonergic neurons and regional fMRI signals. They further highlight a functional cross-talk between serotonin and mesolimbic dopamine systems hence providing a novel framework for understanding serotonin dependent functions and interpreting data obtained from human fMRI studies of serotonin modulating agents.

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**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.07/OO1

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH grant

**Title:** Cerebral oxygenation and blood flow distributions along the capillary path in awake mice

**Authors: *B. LI**[1], I. SENCAN[1], T. ESIPOVA[2], K. KILIÇ[3], M. MOEINI[5], M. YASEEN[1], B. FU[1], S. KURA[1], F. LESAGE[5], S. VINOGRADOV[2], A. DEVOR[4], D. BOAS[1], S. SAKADZIC[1]
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**Abstract:** Cortical capillary blood flow and oxygenation are highly heterogeneous. Mapping the absolute capillary blood flow and oxygenation along the capillary path is a key step towards understanding how oxygen is transported and delivered in a complex microvascular network to enable adequate tissue oxygenation. In this work, we applied two-photon microscopic imaging of intravascular oxygen partial pressure ($PO_2$) to measure both oxygen concentration and red blood cell (RBC) flux in cortical arterioles, capillaries, and venules. The $PO_2$ measurements with high signal-to-noise ratio were enabled by a novel oxygen-sensitive phosphorescence probe, PtG-2P. Imaging was performed in awake, head-restrained C57BL/6 mice (n=15), through a chronic sealed cranial window centered over the E1 whisker barrel.
We obtained a detailed mapping of the resting state cortical microvascular $PO_2$ in all arterioles and venules, and both $PO_2$ and RBC flux in most capillaries down to 600 μm depth from the cortical surface (n=6,544 capillaries across all mice). Capillary RBC speed and density were also extracted and all measurements were co-registered with the microvascular angiograms. We characterized the distributions of capillary $PO_2$ and flow as a function of branching order and cortical depth. The results show strong positive correlation between oxygenation and flow in the capillary segments, with an increased correlation in downstream capillaries. We have also observed homogenization of both oxygenation and flow in deeper cortical layers, which may imply a mechanism to improve oxygen delivery without increasing global blood flow in the area with increased metabolism.

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**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.08/OO2

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH Grant 1P20 RR021938

NIH Grant AA019462

**Title:** Effects of chronic olanzapine exposure on functional network connectivity in the anesthetized rat

**Authors: *C. I. RODRIGUEZ**[1], J. P. RICE[2], F. T. CANDELARIA-COOK[3], Y. YANG[3], R. PURVIS[3], C. ABBOTT[4], J. BUSTILLO[4], N. PERRONE-BIZZOZERO[5], V. CALHOUN[6], D. A. HAMILTON[1]

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**Abstract:** While understanding the neural correlates of schizophrenia represents a major challenge for neuroscientists, the quantification of functional network connectivity (FNC) measured from functional neuroimaging data obtained from schizophrenia patients is emerging as a promising approach towards a more complete understanding of the disorder. Despite these advances in research approaches, the interpretation of FNC data is complicated by the widespread history of exposure to antipsychotic medications in schizophrenia patients. As a result, the potential contributions of antipsychotics to FNC in schizophrenia patients remain to be fully explored. Because ethical considerations render the evaluation of the effects of chronic exposure to antipsychotics in healthy controls impossible, the present study evaluated the effects of chronic olanzapine exposure on FNC in an animal model. Adult male rats were given daily injections of olanzapine (2mg/kg) or vehicle for 30 days. Resting state BOLD signal data were obtained in separate 10 minute echoplanar imaging (EPI) sequences conducted prior to drug treatment and 24h following the completion of the drug treatment protocol utilizing a 4.7T Bruker Biospin magnetic resonance imaging scanner. Group independent component analysis (gICA) was then performed on the data to identify individual components or local connectivity networks. Finally, cross-correlations between individual component timecourses were calculated to estimate FNC and compared across time points and drug treatment conditions. Olanzapine exposure was associated with alterations in FNC for components localized to the hippocampus, striatum, retrosplenial cortex, cingulate cortex and cerebellum. Additionally, olanzapine decreased spectral power within the 0.08Hz-0.12Hz range in a broad set of components. Arterial spin labeling (ASL) data obtained during each session suggest a large, but non-significant, reduction in blood flow as a result of olanzapine exposure that may partially account for alterations in FNC and component time courses. Comparison of pre-treatment data with data obtained 30 days later also revealed widespread alterations in FNC as a function of the temporal delay between the first and second scanning session regardless of drug treatment condition. These observations indicate that chronic exposure to low-dose olanzapine in the rat results in altered FNC and component timecourses in a distributed set of brain regions implicated in schizophrenia.

**Disclosures: C.I. Rodriguez:** None. **J.P. Rice:** None. **F.T. Candelaria-Cook:** None. **Y. Yang:** None. **R. Purvis:** None. **C. Abbott:** None. **J. Bustillo:** None. **N. Perrone-Bizzozero:** None. **V. Calhoun:** None. **D.A. Hamilton:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.09/OO3

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH Grant NS091230

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**Title:** Quantitative measurement of cortical microvascular oxygenation responses during functional hyperemia in awake mice

**Authors: *I. SENCAN**[1], T. ESIPOVA[2], K. KILIÇ[3], B. LI[1], M. DESJARDINS[3], M. A. YASEEN[1], H. WANG[1], R. JASWAL[1], S. KURA[1], B. FU[1], D. A. BOAS[1], A. DEVOR[3,1], S. VINOGRADOV[2], S. SAKADZIC[1]
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**Abstract:** We characterized cortical microvascular $pO_2$ and blood flow changes in response to whisker and shoulder stimulation in awake mice. Measurements were performed by combining two photon microscopy (TPM) phosphorescence lifetime imaging of the cortical oxygenation, and optical coherence tomography (OCT) imaging of the cerebral blood flow. In order to perform fast spatio-temporally resolved measurements of $pO_2$, we used a newly-developed oxygen-sensitive probe PtG-2P, which has significantly higher brightness than the established two-photon-enhanced oxygen sensor PtP-C343. We characterized the performance of the new probe in vivo and mapped the amplitudes and shapes (e.g. initial dip, overshoot, and post stimulus undershoot) of the $pO_2$ changes as a function of the vessel type (e.g., arterioles, capillaries, and venules) and a distance from the activation center. Our measurements in the awake mice are not affected by the confounding factors of anesthesia on the animal physiology, including the level of cerebral metabolism and the amplitude and speed of neuronal and vascular responses. These results will help to understand changes in cortical oxygenation and blood flow at the microvascular scales, and will help us improving quantitative interpretation of fMRI signals.

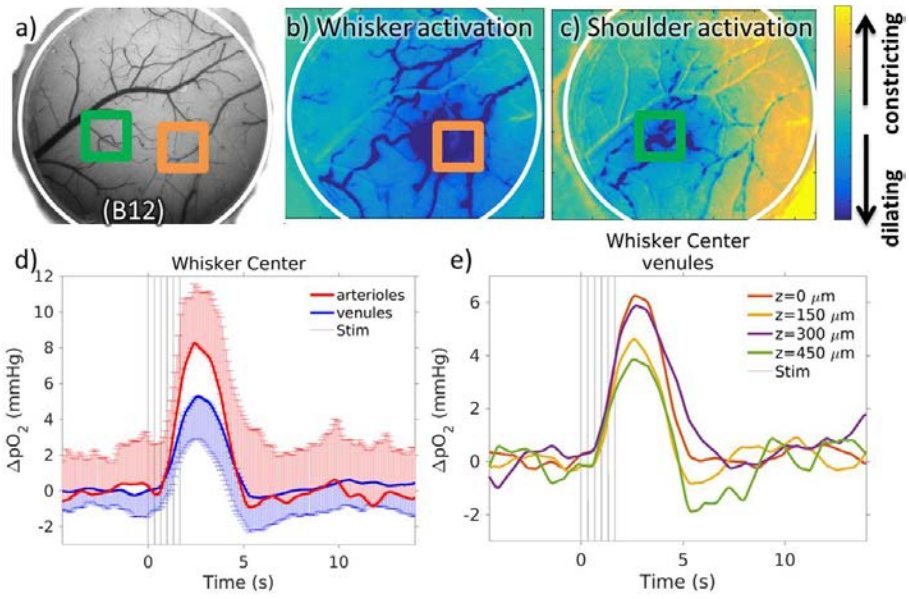


Figure 1. Optical Intrinsic Signal Imaging (OISI) and intravascular Two Photon Microscopy (TPM) measurements during functional activation with whisker and shoulder stimulation in awake mice; a) Single CCD frame over field of view (FOV) of 2.5x3.2 mm; Cortical activation center mapping using OISI with b) whisker and c) shoulder stimulation. Green and orange boxes mark FOVs for TPM (600x600 µm); d) Average $pO_2$ changes in arterioles (standard deviation plotted upward) and venules (standard deviation plotted downward) with whisker stimulation at the activation center from 11 animals; e) venous $pO_2$ responses at different depths from pial surface

**Disclosures: I. Sencan:** None. **T. Esipova:** None. **K. Kiliç:** None. **B. Li:** None. **M. Desjardins:** None. **M.A. Yaseen:** None. **H. Wang:** None. **R. Jaswal:** None. **S. Kura:** None. **B. Fu:** None. **D.A. Boas:** None. **A. Devor:** None. **S. Vinogradov:** None. **S. Sakadzic:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.10/OO4

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIMH IRP

**Title:** A high-resolution population MRI template and automated processing tools for standardized analysis and visualization of the macaque brain

**Authors: *C. SPONHEIM**[1], J. SEIDLITZ[2], B. JUNG[1], A. MESSINGER[1], L. G. UNGERLEIDER[1]
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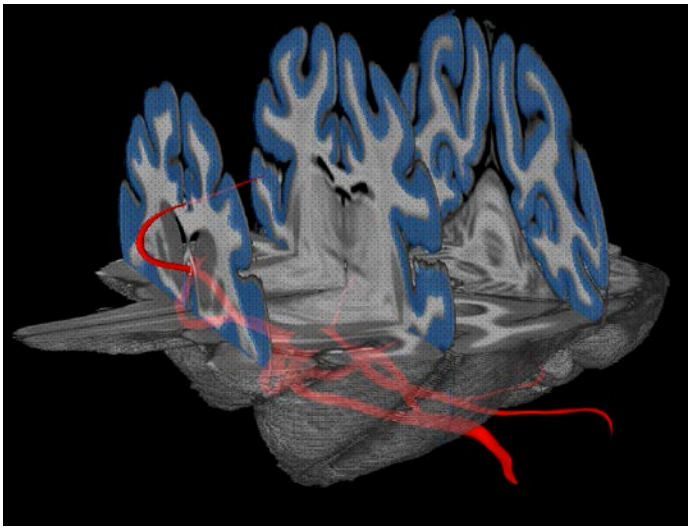
**Abstract:** Anatomical MRI templates are commonly used in human neuroimaging to facilitate data analysis and comparison across subjects and studies. Template use is rare in non-human primate neuroimaging, in part because previous in vivo templates of the monkey brain were not detailed enough to reliably identify anatomical structures. To address this need, we made a high-resolution in vivo MRI template of the macaque brain by averaging T1-weighted scans from 31 adult subjects using linear and nonlinear registration methods. We call this anatomically representative template the National Institute of Mental Health Macaque Template, or NMT for short (Seidlitz et al., 2017).
The NMT is freely available and includes digitial maps to differentiate the brain from the rest of the head and to parcellate tissue into gray matter, white matter, cerebrospinal fluid, and - for the first time - the major arterial blood vessels. Cortical thickness and the volume of major structures have been characterized. We provide several cortical surface reconstructions for visualization of individual or group data (e.g. functional MRI maps). We supply nonlinear transformations to (and from) other templates (e.g. D99 and F99) so that digital atlases associated with these templates can be applied to the NMT. For example, one could determine what brain areas coincide with a functional activation or target a brain area for electrode placement, making a lesion, or pharmacological manipulation.
Finally, we provide a set of processing tools that use our characterization of the NMT as a starting point to automatically and objectively register and characterize the T1-weighted scans of individual subjects. These scripts replace the time-consuming manual processes of brain extraction, tissue segmentation, and morphometric feature estimation for each individual subjects. The NMT package thus establishes a common platform for data visualization, precise single-subject data analysis, comparing findings across subjects and studies, and combining data acquired using both different methodologies.



**Disclosures:** **C. Sponheim:** None. **J. Seidlitz:** None. **B. Jung:** None. **A. Messinger:** None. **L.G. Ungerleider:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.11/OO5

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** BPI "ROMANE" program

Association France Alzheimer

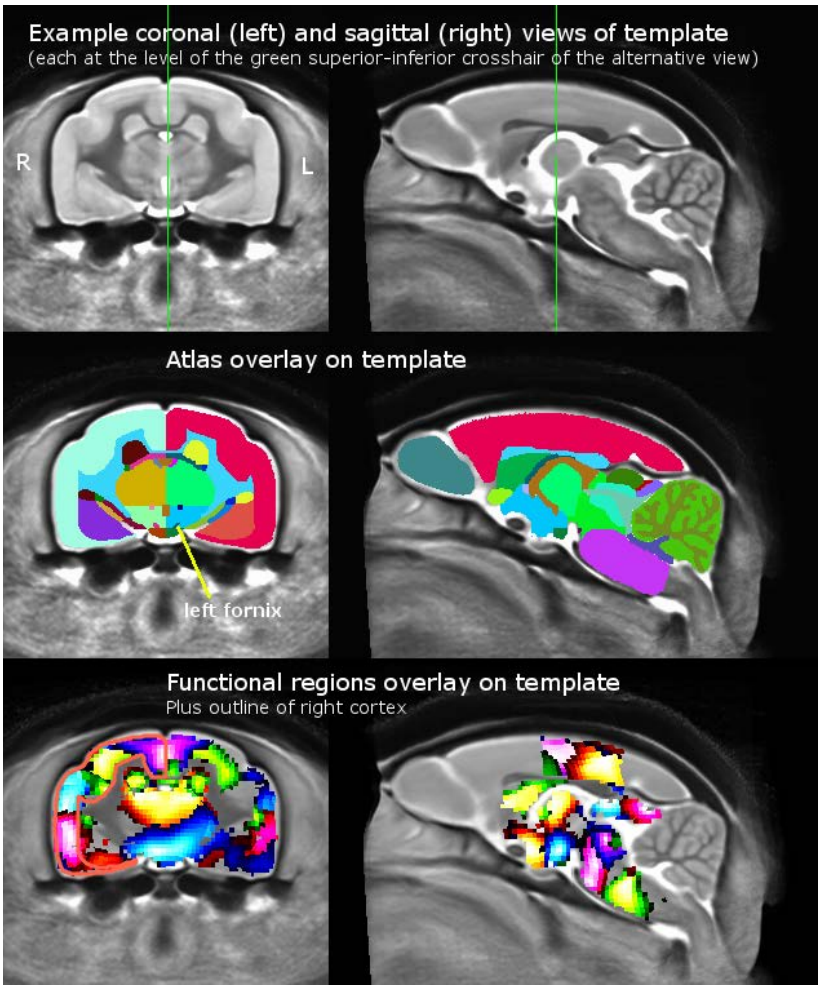
Fondation Plan Alzheimer

**Title:** An MRI anatomical atlas and resting state activity-based functional parcellation of the brain of the mouse lemur primate (Microcebus murinus)

**Authors: *N. A. NADKARNI**[1], S. BOUGACHA[1,2], C. GARIN[1], M. DHENAIN[1], J.-L. PICQ[1,3]
[1]Neurodegenerative Dis. Lab., MIRCen, CEA, Fontenay aux Roses Cedex, France; [2]U1077, INSERM, Caen, France; [3]Lab. de psychopathologie et de neuropsychologie, Univ. Paris 8, St Denis, France

**Abstract:** The gray mouse lemur (*Microcebus murinus*), a small non-human primate, is attracting increased attention as a model for studying aging and age-related diseases in the brain. Its rodent-like size (typical length 12cm, 60-120g weight), rapid maturity (puberty at 6-8 months) and decade-long lifespan brings rodent-like practicality to a primate model. MRI is now widely exploited in animal models for the unique longitudinal anatomical and functional information it provides. Automation and standardization of analysis require images to be registered to a standard space template. We have developed the first one for the mouse lemur brain, including an 80-region anatomical atlas, and also begun a functional delineation using data from resting state functional MRI (rsfMRI). Template data sets were acquired in 34 animals, 15-60 months old, at 7T using a T2-weighted sequence, resolution 115×115×230µm. Aided by brain identification using the IIBI RATS skull stripper, images were registered and averaged with AFNI through linear then non-linear stages to produce a final template. Segmentation of structures was carried out by hand. RsfMRI data was acquired in 15 other animals, 10-40 months old, at 11.7T using a T2*-weighted sequence, resolution 208×313×1000µm, 450 volumes at TR=1s, plus a 200µm isotropic anatomical scan to aid registration (carried out in AFNI to the above template). Regional intrinsic functional organization was characterized using Nilearn by applying a fast dictionary learning decomposition on the registered concatenated rsfMRI 4D

data. As shown in the figure, template image contrast was of high quality, allowing all major brain regions to be segmented, including small (fornix) and intricate (cerebellar white matter arborescence). Functional parcellation produced an aligned but differing set of regions, especially cortical subdivisions, demonstrating its complementarity. This original combination of anatomical and functional MRI delineation is a powerful new tool for brain-wide studies of morphology and function in the increasingly-important mouse lemur model.




**Disclosures:** **N.A. Nadkarni:** None. **S. Bougacha:** None. **C. Garin:** None. **M. Dhenain:** None. **J. Picq:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.12/OO6

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2017R1C1B1007829).

**Title:** Dynamic functional-PET imaging of time-dependent brain activity change by optogenetic stimulation of the motor cortex

**Authors: *D.-H. KWON**, J. CHO, J.-Y. PARK, H.-I. KIM
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**Abstract:** Positron emission tomography (PET) is an excellent tool for providing metabolic information of the radiolabeled biomarkers. [18F]FDG PET allows quantitative measurement of changes in regional brain glucose metabolism following stimulation. However, the PET provides an amount of glucose consumption over the full-time course and lacks the temporal information to catch physiological alterations. We tried to track time-dependent brain activity after motor cortex stimulation to decipher the dynamic neural circuit activated by optogenentic stimulation. In this study, the dynamic PET data were obtained. Rats (n=18) received AAV5-CaMKIIα-ChR2(H134R)-eYFP (1μl) in the motor cortex and virus construct was expressed. Following implanting the optical ferrule in the same area, the 34-minute dynamic PET was performed simultaneously with injection of [18F]FDG (100mCi/100g). Next day, the similar PET scan was performed with 30-minute optogenentic motor cortex stimulation. The entire PET scan was divided into thirty-four 60s-frames. The difference of regional glucose metabolism was examined statistically by comparing each time frame of two separate scans. Furthermore, the statistical tests for static PET integrated with all time frames were also compared to metabolic map derived from the dynamic PET. The data were spatially normalized by using SPM8 with a toolbox, spmratIHEP, and intensity normalization and paired t-tests were done by using AFNI. The brain regions with significant FDG changes in a group with stimulation were yielded based on a P value of less than 0.05 and a cluster-size of greater than 12 contiguous voxels. Plotting the normalized mean activity of the significant regions over the whole time, we could observe the time-dependent fluctuation of long-range signaling. Motor cortex (M1) as higher in metabolism than sham group throughout stimulation time. The striatum responded actively at the beginning of the stimulus and gradually diminished. However, static PET analysis showed no statistical results in the striatum regions. The activities of internal capsule and thalamus were found to be significant in the early (<10min) and late (>20min) stimuli, while the middle frames showed little difference between two groups. Therefore, independent PET analysis at each time point may overcome the problems of general PET image analysis, where statistical significance can be lowered or ignored for a small number of brain regions where the change is small during the entire reaction time. It is possible to compare the signal pattern during a certain time interval with the continuous electrophysiological signal by acquiring more number of time points for analysis.

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**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.13/OO7

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** ERC-2013-AD6; 339513

ANR/NSF 15-NEUC-0003-02

**Title:** Imaging sensory responses in the same animal using two'photon microscopy, BOLD-fMRI and ultrafast ultrasound imaging

**Authors: *D. F. BOIDO**[1], R. L. RUNGTA[1], B.-F. OSMANSKI[1], M. ROCHE[1], T. TSURUGIZAWA[2], D. LE BIHAN[2], L. CIOBANU[2], S. CHARPAK[1]
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**Abstract:** BOLD fMRI signals detect changes in the concentration of deoxyhemoglobin, and as such depends in a complex manner on functional hyperemia, oxygen consumption and blood volume. Consequently, the extent to which these signals report specific cellular activity and local vascular changes remains unknown. Here, we use the mouse olfactory bulb (OB) as a neurovascular model and three imaging techniques- two-photon imaging (GCaMP6f and PO2), ultrafast ultrasound imaging (fUS) and 17T BOLD fMRI to link microscopic neuronal and vascular activation to mesoscopic vascular activation, i.e. changes in Power Doppler and BOLD signals, in response to odor stimulation. Transgenic mice expressing GCaMP6 under control of the Thy1 promoter were implemented with chronic windows compatible with the three imaging techniques and repetitively imaged under anesthesia. We first demonstrated that for each imaging technique, repetitive imaging sessions gave reproducible responses to odor stimulation. In each animal, we then imaged the same olfactory bulb region with the three imaging approaches. We report differences in the threshold and dynamic range of local changes in identified mitral cell activity, capillary red blood cell flow and oxygenation, Power Doppler and BOLD signals in response to odor. This work allows to discard some uncertainties regarding the relationship between local neuronal activation and both microscopic and mesoscopic vascular responses, and characterizes some of the limits inherent to mesoscopic imaging based on blood flow changes (fUS and BOLD fMRI).

**Disclosures:** **D.F. Boido:** None. **R.L. Rungta:** None. **B. Osmanski:** None. **M. Roche:** None. **T. Tsurugizawa:** None. **D. Le Bihan:** None. **L. Ciobanu:** None. **S. Charpak:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

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**Program#/Poster#:** 603.14/OO8

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** Canadian Institute of Health Research Grant MOP 133568

The Alzheimer's Society of Canada Doctoral Award

**Title:** Can three echoes do what one echo cannot in BOLD fMRI of the ageing brain?

**Authors: *S. ATWI**[1,3,4], A. W. S. METCALFE[1,2], A. D. ROBERTSON[1,3], B. J. MACINTOSH[1,3]
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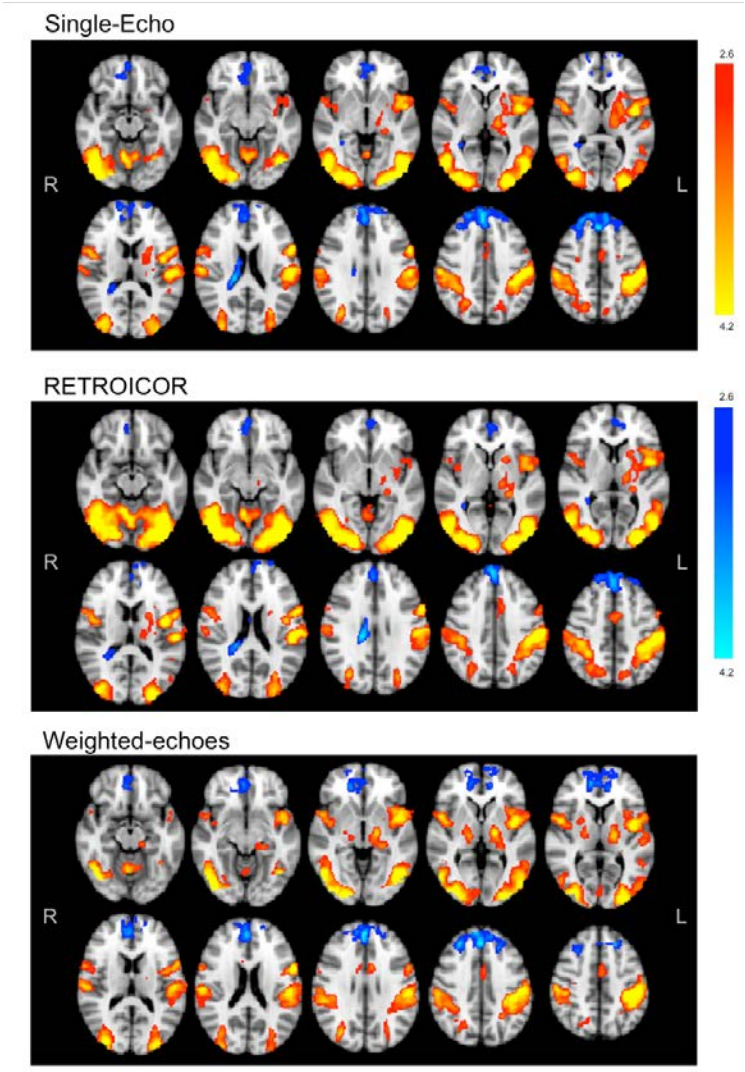
**Abstract:** Blood oxygenation level dependent functional magnetic resonance imaging (BOLD fMRI) is an ideal non-invasive brain mapping technique. Acquisition and methods development make fMRI compelling to study cerebrovascular ageing, while acknowledging that non-neuronal nuisance sources, like increased cardiac-related effects, are more pronounced in older adults. Retrospective temporal and spatial filtering technique, RETROICOR, is effective at removing fMRI artifacts and rapid multiband fMRI also has promise for ageing research. These approaches, however, inadvertently limit the scope of corrections or increase the presence of structured noise. This study presents another approach that has yet to be applied to ageing, specifically multi-echo fMRI in which three consecutive echo acquisitions are combined per volume. This approach may yield added BOLD specificity (Posse et al., 1999). We hypothesized that multi-echo-weighted fMRI will enhance image quality compared to both an uncorrected single-echo, and RETROICOR. Our three participant groups were 16 younger adults, 29 healthy older adults, and 13 older adults with cerebral small vessel disease. In support of our first objective, we found task-related activation Z-statistics were significantly reduced using the weighted multi-echo fMRI compared to the uncorrected single-echo approach ($P<0.001$). Qualitative visual comparison revealed multi-echo-weighted fMRI was effective at reducing 'activation' from areas notorious for their non-neuronal contributions (Figure); however there was no difference in Z-statistics between RETROICOR and multi-echo-weighted approaches ($P=0.6$). Lastly, post-hoc, we observed a trend towards an ageing group effect for the multi-echo fMRI ($P=0.1$) but not RETROICOR ($P=0.8$). This multi-echo fMRI approach appears to improve activation quality akin to other data-scrubbing tools, which may improve spatial specificity of

neurovascular functional mapping. Therefore, multi-echo fMRI may have practical benefits in the study of brain ageing and vascular dysfunction.




**Disclosures: S. Atwi:** None. **A.W.S. Metcalfe:** None. **A.D. Robertson:** None. **B.J. MacIntosh:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.15/OO9

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH Grant NS036722

NIH Grant NS085478

**Title:** Noninvasive quantification of baseline oxygen extraction fraction (OEF) in the human brain using velocity-selective excitation and arterial nulling (VSEAN)

**Authors: *E. Y. LIU**[1,2], J. GUO[1,5], E. C. WONG[1,3,4], R. B. BUXTON[1,2,3]
[1]Ctr. for fMRI, [2]Neurosci. Grad. Program, [3]Radiology, [4]Psychiatry, UCSD, La Jolla, CA; [5]Radiology, Stanford Univ., Stanford, CA

**Abstract:** The local brain oxygen extraction fraction (OEF), the ratio of oxygen metabolized by the brain ($CMRO_2$) to oxygen delivered by cerebral blood flow (CBF), is a fundamentally important indicator of metabolism. With an additional measurement of CBF, absolute $CMRO_2$ can be calculated ($CMRO_2$ = CBF * arterial $O_2$ concentration $C_aO_2$). Quantification of $CMRO_2$ provides a measure of the brain's energy utilization and baseline levels of neuronal activity, and serves as a marker of brain function and viability. Accurate measurements of OEF and $CMRO_2$ are thus significant for defining and understanding brain physiology, especially as perturbations of these baseline markers can be seen in pathologies including stroke and Alzheimer's disease. Raichle et al. (2001) suggested that a baseline state of the brain can be described in terms of OEF, changes to which are also the origin of blood oxygenation level dependent (BOLD) signal changes. Although measuring OEF is challenging, in recent years several MRI methods have been developed based on inhalation of different gas mixtures, on the quantitative measurement of the susceptibility effect of the venous blood, or on the relaxation effects of altered hemoglobin $O_2$-saturation on the transverse relaxation rate ($T_2$) of venous blood (e.g., TRUST and QUIXOTIC). A newer method, velocity-selective excitation and arterial nulling, (VSEAN, Guo et al. 2012) was introduced to overcome some limitations of earlier techniques, providing a more sensitive and noninvasive approach to measure OEF. This method isolates the signal from venous blood which, taken along with a $T_2$-venous oxygen saturation ($Y_v$) calibration curve, yields estimates of venous oxygenation and consequently OEF. We report here a test of VSEAN in 19 subjects scanned during a baseline state: subjects performed a simple one-back memory task by viewing a white screen with single-digit numbers projected at the center. This was used to define the "baseline" to be consistent with other tasks in the session. A dual-echo arterial spin labeling (ASL, PICORE QUIPPS II) acquisition simultaneously recorded CBF and BOLD dynamics, also in the baseline state task. Preliminary results yield values of baseline OEF similar to previously reported measurements of extraction, validating the accuracy of the new methodology. Additionally, absolute $CMRO_2$ values were calculated from the OEF and CBF measurements, assuming typical $C_aO_2$, thus painting a complete picture of the brain's baseline energy state using a toolbox of noninvasive techniques. The tolerable nature of these methods make them good candidates for future application to patients in clinical environments.

**Disclosures: E.Y. Liu:** None. **J. Guo:** None. **E.C. Wong:** None. **R.B. Buxton:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

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**Program#/Poster#:** 603.16/OO10

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** IBS-R015-D1

NRF-2014-H1A2A1-020612

**Title:** Dynamics of neurovascular coupling between excitatory neurons and adjacent vessels at ictal onset and termination revealed by *In vivo* two-photon imaging

**Authors: *H. LIM**[1,2], S.-G. KIM[1,3], M. SUH[1,3]
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**Abstract:** Epileptic seizures involve hyperexcitable and hypersynchronous neuronal activities resulting in high cerebral metabolic demands and hyperemia. Many studies utilized animal seizure models in order to study the dynamics of neurovascular coupling during seizure evolution. However, the neurovascular coupling has not been fully understood yet at a single neuronal cell type and a single vessel level. Here, we investigated the dynamics of the neurovascular coupling between principal neurons and adjacent vessels (arteriole & venule) with *in vivo* two-photon imaging in thy1-GCaMP6f mice. We induced focal ictal events by an intracortical injection of 4-aminopyridine and used fluorescent dextran to visualize cortical vessels during imaging. At ictal onset, calcium signals of excitatory neurons surged and maintained at a heightened level until seizure termination. Arterioles near the seizure focus dilated right after the ictal onset and maintained their maximum dilation until seizure termination. Unlike arterioles, venules did not change in response to the onset or termination, but seemed to maintain a dilated tone. During post-ictal periods, alongside with arteriole constrictions, calcium signals reduced below the baseline. In addition, the pattern of calcium activity seemed to be dependent upon the type of ictal events. In sum, the activities of calcium signal from excitatory neurons revealed a tight coupling with the dynamic of arterioles at ictal onset and termination.

**Disclosures:** **H. Lim:** None. **S. Kim:** None. **M. Suh:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

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**Program#/Poster#:** 603.17/OO11

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH R01 AG023084

NIH R01 AG039452

NIH R01 NS034467

NIH R01 NS100459

**Title:** Breaking bad resolution: Multiphoton techniques for *In vivo* measurement of capillary diameter and blood flow

**Authors: *K. KISLER**[1], M. D. SWEENEY[1], A. J. BRUMM[2], A. R. NELSON[1], B. V. ZLOKOVIC[1]
[1]Dept. of Physiol. and Biophysics, Zilkha Neurogenetic Inst., Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA; [2]Nikon Instruments Inc, Melville, NY

**Abstract:** Given the growing understanding of the importance of healthy vasculature in the maintenance of healthy brain function, there has been increasing focus on measurement of vascular dynamics *in vivo* in animal models. Capillaries, the smallest vessels in the brain, make up about 85% of brain vasculature, yet reports of their dynamic functionality are widely varied. Historically, it has been difficult to measure small vascular changes using optical imaging, capillary diameter measurements in particular. Several common pitfalls likely contribute to these difficulties, including choice of imaging parameters including pixel size and acquisition speed, noise in the data, and post-processing. Similarly, accurate measurements of red blood cell (RBC) velocity from image sequences historically suffer from speed issues. To date, the most successful published techniques rely on high-speed line scans, precluding simultaneous diameter measurements in all but the most specialized multiphoton systems. Here we describe techniques to image and measure small changes in capillary diameter with sub-pixel resolution and to accurately track RBCs and record RBC velocity using a commercially available multiphoton microscope. Furthermore, we show that these two methods are compatible with each other and can be successfully used simultaneously to parse out diameter and velocity information from the same image acquisition.

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**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

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**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** China Youth Talent 1000 Program

China NSF 61671198

Zhejiang NSF LZ15H180001

**Title:** Caffeine caused a widespread increase of resting brain entropy

**Authors: *D. CHANG**, Q. GE, J. ZHANG, Z. WANG
Hangzhou Normal Univ., Zhejiang, China

**Abstract:** Entropy indicates the degree of irregularity of a system, and is an important trait of brain function. Entropy is often considered as a measure of complexity and high entropy suggests high information processing capacity. At rest, human brain is consistently shuffling between different intermediate states around a critical point. This restless effort is believed to facilitate brain function in order to be better prepared for upcoming unexpected events. Such a system-wise trait may also provide a unique window for probing brain both at healthy and diseased condition. We have previously developed a technique to map brain entropy (BEN) using fMRI and shown that BEN is stable across time and differs between controls and patients with various brain disorders. To further establish its usefulness in neuroscience and clinical research, a critical step is to examine its sensitivity and specificity under experimental modulations. To this end, we performed the first BEN variation study under pharmaceutical challenge using caffeine, which is the most widely used psychostimulant. Our hypothesis is that the beneficial effects of caffeine on the brain will induce higher resting BEN. Resting fMRI were collected from sixty caffeine-naïve healthy subjects (30/30 males and females, age: 23 ± 3 years) before and after taking a 200 mg caffeine pill with signed IRB consent forms. BEN and cerebral blood flow (CBF) maps were calculated using BENtbx and ASLtbx respectively. Statistically analysis through paired-t test showed that caffeine induced significant CBF reduction in the whole brain ($p<0.001$, corrected) and BEN increase in a large portion of the cerebral cortex ($p<0.001$ corrected) with the highest increase in lateral prefrontal cortex, the default mode network (DMN), visual cortex, and motor network. Increased BEN was not related to CBF reduction, suggesting a neuronal nature of the observed BEN effects. Increased BEN means increased brain activity irregularity and subsequently greater information processing capacity. Increased resting BEN in the sensorimotor system, DMN, and frontal area is consistent with the

enhanced facilitation effects (such as vigilance and attention) of caffeine. In conclusion, we revealed, for the first time, the neuronal effects of caffeine on BEN using a large sample size, proving truth of our hypothesis. These data also proved the sensitivity of BEN to pharmaceutical modulation, suggesting it as biomarker for monitoring longitudinal brain function variations.

**Disclosures: D. Chang:** None. **Q. Ge:** None. **J. Zhang:** None. **Z. Wang:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.19/OO13

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH BRAIN Initiative R01MH111359

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DFG (MTH)

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Natural Sciences and Engineering Research Council of Canada (MD)

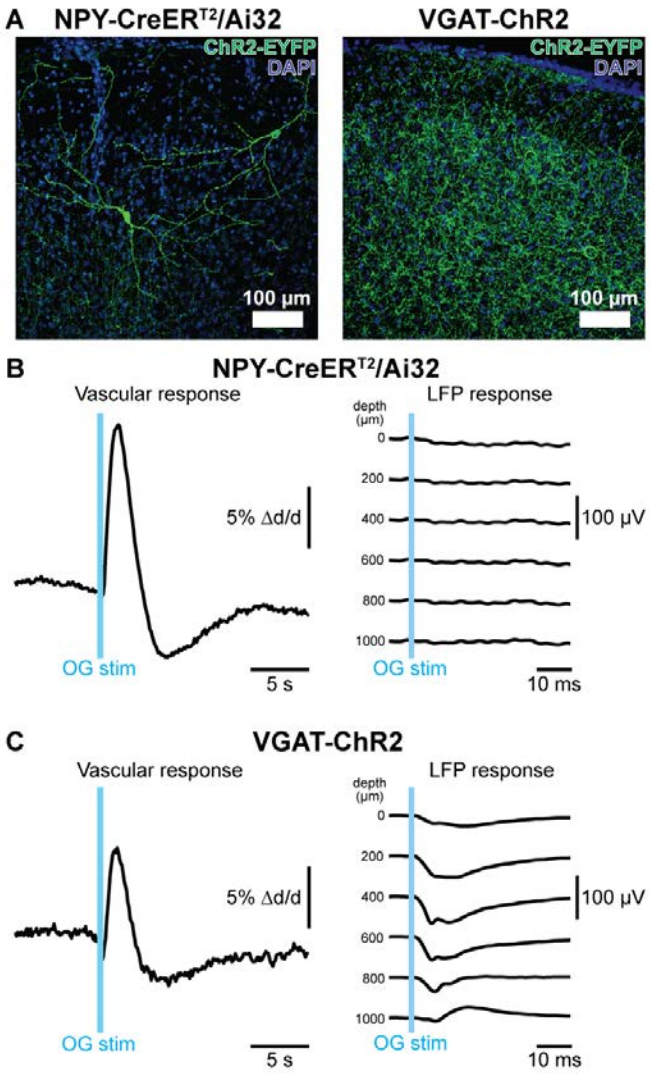
**Title:** Activation of cortical NPY-expressing interneurons produces vascular effects without measurable LFP

**Authors: *M. THUNEMANN**[1], T. V. NESS[4], K. KILIÇ[2], M. DESJARDINS[2], S. BOMPIERRE[2], M. VANDENBERGHE[1,5], Q. CHENG[2], K. WELDY[2], S. DJUROVIC[7,8], O. A. ANDREASSEN[9,5], D. A. BOAS[10], H. HERZOG[11], G. T. EINEVOLL[4,6], A. M. DALE[3], A. DEVOR[3,10]
[1]Radiology, [2]Neurosciences, [3]Neurosciences and Radiology, Univ. of California San Diego, La Jolla, CA; [4]Norwegian Univ. of Life Sci., Aas, Norway; [5]NORMENT, KG Jebsen Ctr. for Psychosis Research, Div. of Mental Hlth. and Addiction, [6]Dept. of Physics, Univ. of Oslo, Oslo, Norway; [7]Dept. of Med. Genet., Oslo Univ. Hosp., Oslo, Norway; [8]NORMENT, KG Jebsen Ctr. for Psychosis Research, Dept. of Clin. Sci., Univ. of Bergen, Bergen, Norway; [9]Oslo Univ. Hosp. - Ulleval, Oslo, Norway; [10]Martinos Ctr. Biomed Imaging, Harvard Med. Sch., Charlestown, MA; [11]Garvan Inst. of Med. Res., Darlinghurst Sydney, Australia

**Abstract:** Neurons produce and release an array of vasoactive messengers, such as neuropeptides, which contribute to neurovascular coupling. We recently showed that

Neuropeptide Y (NPY), known to be released from NPY-positive (NPY+) inhibitory cortical neurons (INs), is responsible for the constriction phase of vascular response to neuronal activation [1]. NPY+ INs are a sparse neuronal population (Fig. 1A) co-expressing NPY with a number of vasodilators. In this work, we sought to identify the vascular and electrophysiological "signatures" of selective activation of NPY+ INs. We used mice expressing channelrhodopsin in NPY+ INs (NPY-CreER$^{T2}$/Ai32) and in all cortical INs (VGAT-ChR2). We imaged diameters of single penetrating arterioles using 2-photon microscopy and measured local field potentials (LFP) with a laminar electrode array. Optogenetic (OG) activation of NPY+ INs produced a clear biphasic vascular response but no measurable LFP (Fig. 1B). In contrast, both vascular and LFP signals were detected in response to OG stimulation of all INs (Fig. 1C). The lack of LFP during activation of NPY+ INs can be explained by their sparsity and modest hyperpolarization of their postsynaptic targets, contributing insignificantly to the extracellular potential. These results provide a demonstration that some types of neuronal activity may produce a readily detectable vascular/hemodynamic response in the absence of detectable electrical signals. This argues against a common notion of low sensitivity of fMRI to neuronal activity compared to "direct" electrophysiological measures. Instead, the sensitivity across different measurement modalities will depend on the exact composition of neuronal ensemble activity and cell-type-specific effects on dilation, $O_2$ consumption, and extracellular potential, underscoring the importance of multimodal integration for the inference of circuit activity [2].

[1] Uhlirova, Kilic, et al. 2016. eLife 5:e14315.
[2] Uhlirova, et al. 2016. Phil Trans R Soc B 371:20150356.




**Disclosures:** **M. Thunemann:** None. **T.V. Ness:** None. **K. Kiliç:** None. **M. Desjardins:** None. **S. Bompierre:** None. **M. Vandenberghe:** None. **Q. Cheng:** None. **K. Weldy:** None. **S. Djurovic:** None. **O.A. Andreassen:** None. **D.A. Boas:** None. **H. Herzog:** None. **G.T. Einevoll:** None. **A.M. Dale:** None. **A. Devor:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.20/OO14

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** R01MH098003

R01NS085200

**Title:** Charactering the rat brain's functional network during the awake and anesthetized states using the multi-echo fMRI

**Authors: *Y. MA**[1], Z. LIANG[3], T. NEUBERGER[1,2], N. ZHANG[1,2]
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**Abstract:** The brain's functional network in awake and anesthetized states has been extensively investigated using the whole-brain fMRI technique. However, an increasing number of recent studies have identified different types of artifacts in fMRI signals due to its high susceptibility to head motions and physiological fluctuations, undermining the fidelity and interpretability of fMRI data. Specifically, head motion is one of the major issues in awake animal imaging, and changes of physiological conditions are significant in anesthesia. Recently the multi-echo imaging technique has been developed and demonstrated to minimize the disruptive effects of those artifacts. In this study, we acquired the multi-echo fMRI data during wakefulness and graded anesthetic depths in rats using a newly designed setup that can further reduce head movement. And a novel preprocessing pipeline was used to achieve automatic alignment of rat's fMRI data, especially for large head motions during awake imaging. Then group-ICA method was used to investigate the major signal components within the optimally combined signals derived from the preprocessed data. All independent components were projected to individual multi-echo data for back reconstruction, and BOLD components and non-BOLD artifacts were identified based on the multi-echo signal model. Results show that BOLD components were dominant in our new awake rat imaging setups, and that spatial patterns of BOLD components showed high similarity to anatomically defined brain regions and intriguing consistency across the awake and different anesthetic depths.

**Disclosures: Y. Ma:** None. **Z. Liang:** None. **T. Neuberger:** None. **N. Zhang:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

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**Program#/Poster#:** 603.21/OO15

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NSF Grant BCS1063774

NIH Grant R01NS095933

**Title:** Characterization of gray matter hemodynamic response function in mild traumatic brain injury

**Authors: *A. TAYLOR**, D. RESS, J. KIM
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**Abstract: Abstract:** The hemodynamic response function (HRF) measured by functional magnetic resonance imaging (fMRI) is the signature of neurovascular coupling in the brain. It is possible that this measured response can be used as a biomarker of diseases that lack structural abnormalities or damage. One such disease is traumatic brain injury (TBI), in which diagnosis is based on subjective behavior and symptoms. We hypothesize that measurement of the HRF can reveal changes in neurovascular coupling in TBI subjects with potential diagnostic utility. **Methods:** We evoke the HRF across most (~75%) of cerebral cortex with a 2-sec audiovisual stimulus with a complex finger-movement task. fMRI with high spatial (2-mm) and temporal (1.25-sec, SMS) resolution is used to measure the HRFs, which are parameterized to yield peak amplitude and time-to-peak (TTP). This procedure was performed on a pool of 8 healthy subjects and 3 patients with a history of severe TBI and unresolved cognitive symptoms (loss of taste, dizziness, etc.). **Results:** Healthy subjects yield a reliable spatial pattern of HRF amplitude (Fig A), while TTP is generally stable across cortex and subjects. For TBI subjects, preliminary data shows variable and different amplitude patterns for each subject. But, remarkably, compared with the healthy, the TBI subjects show a delay in their TTP (p=0.039). **Conclusion:** Functional MRI can be used to characterize neurovascular coupling in both healthy subjects and patients that may not express other structural markers of disease. There is potential clinical utility in HRF measurement including diagnosis and monitoring of mild TBI and other similar pathologies. **Figure:** HRF parameters. **A)** patterns of HRF amplitude for healthy subject; **B)** distribution of HRF time-to peak between healthy subjects; **C)** average (and standard deviation) HRF principal component for healthy subjects (N=8) and TBI patient.

**Disclosures:** **A. Taylor:** None. **D. Ress:** None. **J. Kim:** None.

**Poster**

**603. Technical Developments and Assessing Pharmacological Influences on Neuroimaging Responses**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 603.22/OO16

**Topic:** E.05. Brain-Machine Interface

**Title:** IoT for neuroscience: A communication infrastructure for experiments and clinical exams

**Authors: *S. T. FOLDES**[1,2], A. JACOBSON[1]
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**Abstract: Introduction:** Systems used for the research and clinical evaluations of neural activity are highly specialized and have limited customization beyond their specific purpose. A system that incorporates data from various sources and distributes processing in "real-time" would be more useful and could be used to determine when enough data are collected for clinical exams, provide data quality feedback during collection, and be used for brain-computer interfacing (BCI) to control devices.
**Methods:** A modular communication infrastructure was developed to stream data between computers and be easily customized for many projects. This infrastructure was built upon the well-defined and reliable message protocol MQ Telemetry Transport (MQTT). MQTT has been commonly used in Internet of Things (IoT) applications asynchronous communication between devices with minimal overhead in transmission and setup. In our system, modules use MQTT to “publish” data to the network and “subscribe” to data streams to receive the most recent data when it becomes available. This system is agnostic to data format allowing a variety of devices to communicate asynchronously and without regard to specifics such as sampling rates. Furthermore, the interface was developed for MATLAB to allow for easier prototyping; ideal for neuroscientists and biomedical engineers.
**Results:** The first iteration of the infrastructure was designed for BCI. Specifically, the system was evaluated with 1D control incorporating an acquisition module for EEG, processing module to compute event related desynchronization, and subject interface module to provide feedback of brain activity in real-time. The distributed design allowed this system to be run on various system configurations, including across multiple computers with wired or wireless connections. Transmission times for publishing and retrieving messages of different sizes were evaluated. We found the system required only 0.48±0.45 ms for wired and 3.10±1.20 ms for wireless to publish and retrieve 512 floating point values. This represented an extreme data size that might be seen from ECoG or uECoG recordings with 512 channels.
**Conclusions:** We developed a modular neural processing and stimulus control system that is capable of versatile experiments and exams and evaluated it in a BCI. **We uniquely combine the existing protocols from IoT with MATLAB to produce a system that is robust, generalizable, and can be adopted without specialized programing skill.** We are now applying this system to other applications such as more advanced BCI systems in a patient setting, and for remote, online data analysis of multimodal neurocritical care data.

**Disclosures: S.T. Foldes:** None. **A. Jacobson:** None.

**Poster**

**604. Mapping Central Hypothalamic Pathways**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 604.01/OO17

**Topic:** F.10. Food Intake and Energy Balance

**Support:** NIH GM109817 to AMK

**Title:** Further elaboration of the distribution of agouti-related peptide-immunoreactive axons using a canonical rat brain atlas in the adult male rat: High spatial resolution analysis of rostral forebrain regions

**Authors:** *B. E. PINALES[1], ***B. E. PINALES**[1], J. D. HAHN[2], A. M. KHAN[3]
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**Abstract:** Agouti-related peptide (AgRP) is a neuropeptide intensively studied for its role in feeding control; despite this, its brain expression is only partially determined. Here, we extend our earlier immunocytochemical investigation of AgRP chemoarchitecture in the rat forebrain (SFN 2016, San Diego, #453.13), with additional rostral forebrain analysis of AGRP axon distribution. We identified AgRP-immunoreactive (ir) axons and mapped their distribution digitally to sequential levels of a canonical rat brain atlas (L.W. Swanson, *Brain Maps*, 2004). This was accomplished with referenced Nissl cytoarchitecture, the use of camera lucida drawings, and careful determination of plane of section (Zséli G et al., (2016) *J Comp Neurol* 524:2803). Fixed frozen brain sections of an adult male Sprague-Dawley rat were incubated with a rabbit polyclonal antibody raised against the 83-132 amino acid sequence of human AgRP (Phoenix). Labelling was visualized with 3,3'-diaminobenzidine, and the data were mapped with the aid of darkfield microscopy. AGRP-ir axon distribution was enumerated for semi-quantitative analysis with the use of Axiome C software (created by JDH; SFN 2016, San Diego, #467.01).
The cerebral cortex displayed no AgRP-ir except for very sparse labeling in midline structures, notably the dorsal tenia tecta (TTd). In the striatum, there was low to moderate AgRP-ir in the nucleus accumbens (ACB) and the rostroventral part of the lateral septal nucleus (LSr); surrounding areas were devoid of axons. In the pallidum, there was sparse labelling in the substantia innominata (SI). In contrast, the bed nuclei of the stria terminalis (BST) had high expression of AgRP-ir axons, but low to moderate labeling in some BST subdivisions (oval, juxtacapsular). In the thalamus, the paraventricular thalamic- (PVT) and paratenial (PT) nuclei displayed a low (caudal) to high (rostral) AGRP-ir axon density. In the hypothalamus, dense AgRP-ir axons were observed in the paraventricular- (PVH), periventricular- (PV), arcuate- (ARH) and dorsomedial (DMH) hypothalamic nuclei. Moderately dense AgRP-ir was present in

the anterior hypothalamic- (AHA), medial preoptic- (MPO), and lateral hypothalamic (LHA) areas. Sparse AgRP-ir was found in the anterior hypothalamic- (AHN) and ventromedial hypothalamic (VMH) nuclei, and the retrochiasmatic area (RCH).
Collectively, our data provide high spatial resolution rat brain atlas maps of AgRP-ir distribution and will aid in comparing other chemoarchitecture mapped to the same reference atlas. These data may also allow the precise targeting of interventions in forebrain regions that receive inputs from AgRP-expressing neurons.

**Disclosures: B.E. Pinales:** None. **J.D. Hahn:** None. **A.M. Khan:** None.

**Poster**

**604. Mapping Central Hypothalamic Pathways**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 604.02/OO18

**Topic:** F.10. Food Intake and Energy Balance

**Support:** NIH GM109817 to AMK

UTEP SMART-MIND Fellowship to JAS

NIH 8G12MD007592 (BBRC)

**Title:** Diencephalic and mesencephalic neurons projecting to the ventral tegmental area, with special reference to the lateral hypothalamic area: A quantitative mapping study in the adult rat

**Authors: *E. M. WALKER**[1], B. DE HARO[2], J. A. SCHUELER[3], S. D. GONZALEZ[4], J. ARNAL[4], S. JEON[5], R. H. THOMPSON[7], A. M. KHAN[6]
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**Abstract:** The lateral hypothalamic (LHA) and the ventral tegmental areas (VTA) contain neural substrates that participate in the control of many motivated behaviors, but few studies have detailed their interconnections, and none have mapped precisely the distributions of neurons that project to the VTA from cytoarchitectonically defined LHA subdivisions. We identified retrogradely labeled neurons in specific LHA subdivisions and other portions of the fore-, mid- and rostral hindbrain projecting to the VTA by injecting FluoroGold (FG) into the VTA. For this report, we detail maps of two pairs: 1) FG injected into the rostral and caudal VTA and 2) FG injected into the VTA and rostromedial to the VTA. We also identified retrogradely labeled neurons in the diencephalon that were immunoreactive for the neuropeptides hypocretin 1/orexin A (H/O) or melanin-concentration hormone (MCH). FG+ cell counts in specific LHA subdivisions were compared between cases where FG was deposited directly into the VTA (hits;

n = 4) or adjacent to the VTA (misses; n = 4). There was robust FG signal in the bed nuclei of the stria terminalis, amygdala, habenular nuclei and dorsal raphe nucleus. There were select retrogradely labeled neurons in the LHA and zona incerta that were H/O and MCH positive. A three-way ANOVA [case (hit or miss) *vs* side (ipsilateral or contralateral) *vs* subregion (LHA subdivisions or rest of hypothalamus)] revealed greater numbers of FG+ neurons ipsilateral to the injection site as compared to contralateral ($F_{1, 2972}$=27.691, $p < 0.001$). An ANOVA for FG+ neurons on the ipsilateral side revealed: 1) the number of FG+ neurons that occupy the hit versus miss cases ($p < 0.001$); and 2) the LHA versus non-LHA regions in hypothalamus ($p < 0.001$). There is a significant interaction between the number of FG+ neurons that occupy the LHA sub-regions and the rest of the hypothalamus in both the hit versus miss cases ($p = 0.023$). FG+ neurons were distributed across the 25 LHA sub-regions on the side ipsilateral to the injection site in both groups. The most retrograde labeling was found in the LHA, posterior region in the misses (21%) versus the hits (11.2%). There is also robust labeling in the LHA, dorsal region in both the miss (19.3%) and hit (18.2%) cases. Retrogradely labeled neurons that occupy the LHA anterior group were abundant in both the hit (22.9%) and miss (17.8%) groups. Collectively, our results allow the identification of major neuronal populations within LHA subdivisions that project to the VTA. The mapped distributions of discrete neuronal populations may aid the development of a useful animal model for the treatment of eating and metabolic disorders which involve dysfunction of these areas.

**Disclosures: E.M. Walker:** None. **B. De Haro:** None. **J.A. Schueler:** None. **S.D. Gonzalez:** None. **J. Arnal:** None. **S. Jeon:** None. **R.H. Thompson:** None. **A.M. Khan:** None.

**Poster**

**604. Mapping Central Hypothalamic Pathways**

**Location:** Halls A-C

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**Program#/Poster#:** 604.03/OO19

**Topic:** F.10. Food Intake and Energy Balance

**Support:** NIH Grant GM109817 to AMK

HHMI PERSIST Education Grant to AMK

**Title:** Hypothalamic chemoarchitecture of the adult male rat: Further elaboration of results from a high spatial resolution longitudinal mapping study

**Authors: G. FLORES-ROBLES**[1], K. NEGISHI[4], R. A. PACHECO[1], A. ENRIQUEZ[1], E. ACEVEDO[1], B. AVILA[1], E. DOMINGUEZ[1], E. E. HERNANDEZ[1], A. MEDINA[1], E. MEJIA[1], M. A. NOVOA[1], A. T. PROVENCIO[1], F. D. RENTERIA[1], E. SIFUENTES[1], Y. TELLEZ[1], *A. M. KHAN[2,3]
[1]Biol. Sci. and HHMI PERSIST Program, [2]Dept. of Biol. Sci. and Border Biomed. Res. Ctr.,

[3]HHMI PERSIST - Brain Mapping & Connectomics, Univ. of Texas at El Paso, El Paso, TX; [4]UNIVERSITY OF TEXAS AT EL PASO, El Paso, TX

**Abstract:** Hypothalamic functions, by engaging neuroendocrine and autonomic systems as well as behavioral responses, are necessary for homeostasis. Given its range of functions, it is unsurprising that the last half-century has revealed several complex fiber systems and a host of functionally diverse cell types nested within the hypothalamus. Our goal is to examine the chemoarchitecture of the lateral hypothalamic area (LHA) using standardized cytoarchitecture-based spatial framework. Here we build upon previous reports on LHA chemoarchitecture (SfN San Diego, 2016. Poster #453.07; SfN Chicago, 2016. Poster #616.08) generated from efforts of our HHMI-funded Brain Mapping & Connectomics laboratory course for freshman undergraduates. This year's cohort performed triple label immunohistochemistry to stain for hypocretin/orexin (H/O), melanin-concentrating hormone (MCH) and tyrosine hydroxylase (TH). Distributions of immunoreactive (ir) fibers and cell bodies were mapped to reference atlas plates from Swanson (L.W. Swanson, *Brain Maps: Structure of the Rat Brain, 3rd ed*) with the aid of adjacent Nissl-stained sections. Immunostained sections were imaged using wide-field epifluorescence microscopy. As in previous reports, immunodetected cell bodies for H/O and MCH were prominently found in dorsal and dorsolateral parts of the LHA. Additionally, TH-ir neurons and their fibers were visualized co-spatially with these labels. Specifically, TH-ir neurons were detected in the dopaminergic group and the medial tip of the zona incerta. Ventral to this, TH-ir cells were also found within a dorsal part of the arcuate hypothalamic nucleus. Although these cell populations do not co-localize, there is the possibility of interactions among them. This is being explored using confocal imaging at high-magnification. Collectively, these data contribute to our understanding of mesoscale connections within the LHA, and will greatly aid in determining stereotypical patterns of neuropeptidergic neurons and their axonal projections that are prevalent across multiple subjects.

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**Poster**

**604. Mapping Central Hypothalamic Pathways**

**Location:** Halls A-C

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**Program#/Poster#:** 604.04/OO20

**Topic:** F.10. Food Intake and Energy Balance

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NSERC RGPIN-2017-06272 to MJC

**Title:** Immunohistochemical study and atlas mapping of neuronal populations that co-express tyrosine hydroxylase and the vesicular GABA transporter in the hypothalamus

**Authors: *M. J. CHEE**[1], K. NEGISHI[2], K. S. SCHUMACKER[1], R. M. BUTLER[1], A. M. KHAN[2]
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**Abstract:** As part of a collaborative effort to examine the chemoarchitecture of GABAergic neurons within the mouse forebrain, we examined those GABAergic neurons that express the catecholamine-synthesizing enzyme, tyrosine hydroxylase (TH). We crossed the *vGAT-cre* mouse expressing cre recombinase under the vesicular GABA transporter (vGAT) promoter to a L10-EGFP reporter mouse to label the soma of vGAT neurons, then performed multi-label immunofluorescence cytochemistry against TH (mouse, 1:2000) and GFP (rabbit, 1:1000) using formalin-fixed vGAT-L10-EGFP brain tissue. Furthermore, we used Nissl-stained datasets and regional nomenclature from the Allen Brain Atlas online (P56, coronal) to perform Nissl-based parcellation and plane-of-section analysis in order to contextualize the observed TH/GFP expression patterns. Several hypothalamic regions contained neurons that co-localized GFP- and TH-immunoreactivity (ir). The zona incerta contained the most striking co-labeling of GFP/TH-ir neurons. This region corresponded closely to the A13 dopaminergic cell group (A13). There was also a dense grouping of co-labeled cells slightly ventral to A13, at the medial tip of the ZI, which may correspond to the rostral tip of the posterior hypothalamic nucleus (PH) within the *hypothalamic medial zone*. The *periventricular zone* also contained a moderate density of GFP/TH-ir neurons in the parvicellular division (anterior parvicellular part) of the paraventricular hypothalamic nucleus (PVHap); the intermediate (PVi) part of the periventricular hypothalamic nucleus; and at various rostrocaudal levels within the arcuate hypothalamic nucleus (ARH). In the *periventricular region*, there was a moderate density of EGFP/TH-ir neurons in the preoptic (PVpo) part of the periventricular hypothalamic nucleus, as well as some neurons in the dorsomedial hypothalamic nucleus (DMH) and the medial preoptic area (MPO) just ventral to the anterior commissure. Collectively, these results suggest the existence of novel hypothalamic populations that may signal through the co-release of GABA and catecholamines, such as dopamine. Further functional studies may confirm these structural findings and test the neurochemical signatures of these neurons.

**Disclosures:** **M.J. Chee:** None. **K. Negishi:** None. **K.S. Schumacker:** None. **R.M. Butler:** None. **A.M. Khan:** None.

**Poster**

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**Program#/Poster#:** 604.05/OO21

**Topic:** F.10. Food Intake and Energy Balance

**Support:** NIH Grant GM109817 to AMK

BUILDing SCHOLARS Support to JGP

**Title:** Towards automatic registration of histological data to canonical brain atlases

**Authors: *J. G. PEREZ**[1], O. FUENTES[2], A. M. KHAN[3]
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**Abstract:** Since the early 1900s, the creation of brain atlases for model organisms (e.g., mouse, rat) has revolutionized neuroscience by allowing investigators to precisely target probes to identify structure-function relations in the brain, to localize neural systems elements at multiple scales, and to identify connections between neuronal cell populations. Although several brain atlases are now available online, few researchers map their spatial datasets to them. This is mainly due to the bottleneck of requiring trained domain experts to manually enter spatial data onto digital atlas plates, a very slow, laborious and error-prone process. This major rate-limiting step, i.e., the manual mapping of spatial data, has hampered the development of a unifying spatial model of the brain with registered, internally consistent datasets from diverse studies across multiple scales. We seek ultimately to circumvent the manual mapping step by developing high-throughput image analysis software that automates mapping of spatial datasets to brain atlases at cellular- and tissue-level scales. Such an infrastructure will allow neuroscientists to rapidly and accurately populate the atlases with their newly generated spatial datasets.
As a first step towards this long-term goal, we have developed an image analysis method that given an image of a Nissl-stained tissue section of a rat brain automatically finds the likely location of the region in a rat brain atlas. Our method works by first precomputing local descriptors of regions of interest in each plate of the target atlas. We use the scale-invariant feature transform (SIFT) to identify and characterize these regions; SIFT descriptors have been shown to be robust to changes in scale, orientation, noise, and illumination, which makes them well-suited for this application. Given the Nissl-stained image, we compute its SIFT descriptors and then use a stochastic search algorithm to find the best matching region in the atlas according to the similarity of their SIFT descriptors. As a proof of concept, we have used Nissl plates from the Paxinos & Watson atlas (*The Rat Brain in Stereotaxic Coordinates, 7th edition*, 2014) and have registered them to corresponding plates within the Swanson rat brain atlas (*Brain Maps, 3rd edition*, 2004; larrywswanson.com). Experimental results show the method is accurate and efficient, generating results that can be validated by comparing them to independently derived registration solutions obtained from craniometric data. This system will help facilitate the assignment of histological data sets within canonical reference spaces to accelerate the mapping efforts of trained domain experts.

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**Poster**

**604. Mapping Central Hypothalamic Pathways**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 604.06/OO22

**Topic:** F.10. Food Intake and Energy Balance

**Support:** NIH GM109817 to AMK

BUILDING SCHOLARS Program to SNR

UTEP SMART-MIND Summer Fellowship to TNMT

**Title:** Mapping the chemoarchitecture of the arcuate hypothalamic nucleus in the adult male rat to a canonical brain atlas

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**Abstract:** The arcuate hypothalamic nucleus (ARH) is a primary sensor of metabolic signals critical for regulating nutritive state and basal metabolism. Neural substrates within this region help control appetite through the actions of various neurotransmitters and neuropeptides. A deep midline structure, the ARH is approximately 2.5 mm-long rostrocaudally in the adult rat, an area that spans five levels within the Swanson reference atlas (*Brain Maps: Structure of the Rat Brain, 3rd Ed).* However, despite its vast expanse, its chemoarchitecture is poorly characterized and often treated as being uniformly homogenous. To characterize ARH chemoarchitecture across its 3-dimensional extent, here we analyzed the spatial organization of six major neuropeptides and signaling molecules within the ARH: hypocretin/orexin, melanin-concentrating hormone, phenylethanolamine-*N*-methyltransferase, tyrosine hydroxylase, neuronal nitric oxide synthase, and calbindin. Using multi-fluorescence immunohistochemistry, in conjunction with Nissl-based parcellation and mapping, we labeled these molecules in the ARH and obtained fluorescence images of them using high-resolution, wide-field microscopy. The spatial distributions of their expression within neuronal perikarya and axonal fibers were mapped to ARH-containing levels of the Swanson atlas with the aid of a camera lucida. To enhance peptide expression, a 48-hour food deprivation model was also used. Initial results indicate that for several peptides there exists a spatial restriction of expression to either dorsoventral or mediolateral sub-regions of the ARH. In the case of TH and calbindin, somata were densely distributed within the dorsomedial but not ventromedial ARH. In contrast, for PNMT, the opposite was observed, with fiber expression robustly appearing within the

ventrolateral ARH. We also observed parallel spatial distributions of certain molecules across the ARH. MCH and H/O were found to innervate ARH space in complementary patterns, possibly reflecting their redundant actions in fine-tuning physiological operations. Dramatic shifts in the densities and spatial distributions of peptidergic elements were also noted in the normal vs food - deprived animals. By mapping the distribution of distinct neuropeptides and signaling molecules across the ARH to a canonical rat brain atlas, our datasets can be contextualized with other types of data mapped in the same reference space. This data integration, in turn, may improve our understanding of how the ARH integrates signals as part of a node within larger control networks in the brain.

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**Poster**

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**Title:** Mapping the connections between the medial prefrontal cortex and the diencephalon: A combined anterograde and retrograde tract-tracing study in the adult male rat

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**Abstract:** Adaptive changes to ongoing behaviors are supported by the cingulate region (CNG; alternatively, medial prefrontal cortex), a region often described topographically, with dorsal parts associated with adaptive actions and ventral parts with emotional and autonomic outcomes. However, CNG chemoarchitecture and connectivity also vary rostrocaudally; a feature that has received less attention. In order to examine CNG rostrocaudal topography in a standardized spatial framework, *P. vulgaris* leucoagglutinin and cholera toxin B subunit were co-injected into circumscribed CNG areas. Tracer immunoreactivities were plotted to the Swanson atlas (LW Swanson, *Brain Maps, 3rd ed.)* with the aid of an adjacent Nissl series. Here we report results following injections centered in mid-rostrocaudal (ILAm) and caudal (ILAc) parts of the infralimbic area and the dorsal part of the anterior cingulate area (ACAd).
Thalamic CNG connections were found mostly along midline structures. Dense bidirectional connectivity was observed throughout the mediodorsal thalamic nucleus (MD). Tracers from both ILA injections were concentrated in the medial part of the MD (MDm) along the border

with the paraventricular thalamic nucleus (PVT), while ACAd connections were concentrated in the lateral part (MDl) at its boundary with the central lateral nucleus. CNG connections with the nucleus reuniens (RE) were observed predominantly from the ILA, including bidirectional connections with ventrolateral RE sub-regions bordering the perireuniens nucleus (PR). Laterally, a similar clustering of ACAd connections was observed in the ventromedial tip of the ventral medial nucleus (VM). Hypothalamic connectivity with the CNG was restricted mainly to the ILA, although sparse ACAd axons and retrogradely labeled neurons were also observed. Notably, most of the observed CNG projections in the lateral hypothalamic area (LHA) originated from the ILAc and, to a lesser extent, the ILAm. Dense terminal fields from ILAc projections were seen in the dorsal (LHAd), suprafornical (LHAs) and posterior (LHAp) regions of the LHA. Within the tuberal nucleus, we characterized for the first time dense ILAc axonal terminals throughout the rostrocaudal extent of the terete nucleus (TUte).
The observations made here support and extend previous work done on CNG connections. Additionally, our atlas-based spatial framework co-extends with a larger body of anatomical studies in the rat. Together with tools for streamlining anatomical data (e.g., Axiome; Hahn & Swanson, 2016; SfN #467.01), these data will aid in the assembly of spatially precise connectomes.

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**Poster**

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**Title:** Efferent projections of thyrotropin-releasing hormone-synthesizing neurons from the tuberal region of the lateral hypothalamus impinge on histaminergic neurons of the tuberomammilary nuclei

**Authors: *E. SANCHEZ JARAMILLO**[1], G. WITTMANN[2], E. SÁNCHEZ-ISLAS[1], M. LEÓN-OLEA[1], R. M. LECHAN[2]
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**Abstract:** Thyrotropin-releasing hormone (TRH) is a tripeptide widely distributed in the mammalian brain. Besides its critical role in the central regulation of the hypothalamic-pituitary-

thyroid axis, TRH has been proposed as a metabolic sensor that regulates appetite and energy homeostasis. Hypophysiotropic and non-hypophysiotropic TRH neurons originating from the hypothalamic paraventricular (PVN) nucleus, respond to highly demanding conditions as such food deprivation and cold exposure. However, little is known about other hypothalamic cells that synthetize TRH and may also be involved in eating behavior.
Central administration of TRH decreases food intake, but increases histamine in the tuberomammilary nucleus (TMN), where all the histaminergic neurons of the brain reside. As TMN histamine neurons are densely innervated by TRH fibers from an unknown origin (Sarvari et al., 2012), we mapped the TRH afferences using two complementary tracers.
The retrograde tracer, Cholera toxin B subunit (CTB, 0.5%) was injected by iontophoresis under stereotaxic guidance from a glass micropipette placed into the TMN E1-E2 and E4 subdivisions of 8 week old male Sprague Dawley rats (n=7). After 1 week of transport interval, animals were perfused and brain sections were prepared for double-labeling immunofluorescence using an antibody directed against pro-TRH(178-199) and CTB. The origin of CTB afferents to the TMN included the septum, medial, central and lateral preoptic area, bed nucleus of the stria terminals, perifornical area, anterior parvocellular PVN, lateral, anterior and ventromedial hypothalamus, peduncular part of the lateral hypothalamus, suprachiasmatic nucleus, medial amygdala and tuberal lateral hypothalamus (TuHL). However, Only double-labeled neurons were found in the TuLH suggesting that TRH neurons innervating histaminergic neurons in the TMN originate in the TuLH. To confirm the specificity of the retrograde tract-tracing result, we administered iontophoretically the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHAL, 2.5%) in the TuLH of 8 week old male Sprague Dawley rats (n=7). PHAL injections hit rostral, mid or caudal TuHL. After a 10 day transport interval, we identified double labeled PHAL- and pro-TRH-immunofluorescence cells on the TMN. We are currently determining the number of PHAH/pro-TRH-ir containing axon terminals and en-passant boutons in the E1-E5 subnuclei of the TMN. The data suggest, therefore, that TuLH TRH neurons may function as an important metabolic sensor in the brain to regulate the effects of neuronal histamine on energy homeostasis and thermogenesis.

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**Poster**

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**Title:** High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory

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**Abstract:** More Americans are consuming diets higher in saturated fats and refined sugars than ever before, and based on increasing obesity rates, this is a growing trend among older adults as well. While high saturated fat diet (HFD) consumption has been shown to sensitize the inflammatory response to a subsequent immune challenge in young adult rats, the inflammatory effect of HFD in the already-vulnerable aging brain has not yet been assessed. Here, we explored whether short-term (3 days) consumption of HFD would serve as a neuroinflammatory trigger in aging animals, leading to cognitive deficits. HFD impaired long-term contextual (hippocampal-dependent) and auditory-cued fear (amygdalar-dependent) memory in aged, but not young adult rats. Short-term memory performance for both tasks was intact, suggesting that HFD impairs memory consolidation processes. Microglial markers of activation (Iba1 and cd11b) were increased in the aged rats, but were not further amplified by HFD. However, these HFD-induced long-term memory impairments were accompanied by IL-1beta protein increases in both hippocampus and amygdala in aged rats. Central administration of IL-1RA in aged rats following conditioning mitigated both contextual and auditory-cued fear memory impairments caused by HFD, strongly suggesting that IL-1beta plays a critical role in these effects. Voluntary wheel running, known to have anti-inflammatory effects in the hippocampus, rescued hippocampal-dependent but not amygdalar-dependent memory impairments caused by HFD. Together, these data suggest that short-term consumption of HFD can lead to memory deficits and significant brain inflammation in the aged animal, and strongly suggest that appropriate diet is crucial for cognitive health.

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**Poster**

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**Topic:** F.10. Food Intake and Energy Balance

**Support:** DK104897

**Title:** Convergence of 1st- and 2nd-order projection pathways from the ventral hippocampus to the medial prefrontal cortex and the lateral hypothalamic area

**Authors: *C. LIU**[1,2], A. N. SUAREZ[1], J. D. HAHN[1], S. E. KANOSKI[1,2]
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**Abstract:** The ventral hippocampus (vHP) plays a central role in memory and affective processes such as anxiety and fear conditioning. Recent studies from our group show that vHP neurons also regulate learned aspects of feeding behavior via direct pathways to the lateral hypothalamic area (LHA) and medial prefrontal cortex (mPFC). The present study sought to deepen understanding of these feeding-relevant vHP signaling pathways by utilizing conditional dual viral-mediated pathway tracing strategies to identify collateral 1st-order and 2nd-order targets of LHA- and mPFC-projecting vHP neurons.
First-order outputs of vHP (field CA1; vCA1) neurons were investigated using viral (AAV1-hSyn-eGFP-WPRE-bGH) and nonviral (Phaseolus vulgaris-leucoagglutinin) anterograde pathway tracers. Analyses confirmed the LHA and mPFC as two principal vHP targets. To characterize these neural pathways further, collateral projections were investigated using a conditional dual viral approach: Cre-recombinase (CRE) was expressed in LHA- and mPFC-projecting vHP neurons following injection of a retrograde vector (AAV2retro-hSyn1-eGFP-2A-iCre-WPRE) into the LHA or mPFC, and a CRE-dependent anterograde vector (AAV1-CAG-Flex-eGFP-WPRE-bGH) was then injected into vCA1. Axon collaterals of vHP > LHA neurons were found in the nucleus accumbens (ACB), lateral septum (LS), and subfornical organ, whereas collaterals of the vHP > mPFC pathway were found in the ACB, LS, LHA, and lateral amygdala. Next, we examined 2nd-order outputs of these vHP pathways by injecting a transsynaptic anterograde vector (AAV2/1-hSyn-Cre-WPRE-hGH) into vCA1 that drives CRE expression in both 1$^{st}$-order (vCA1 injection site) and 2$^{nd}$-order neurons, and then a CRE-dependent anterograde vector (AAV1-CAG-Flex-eGFP-WPRE-bGH) was injected into the LHA or mPFC. Preliminary data indicate that many 2nd-order outputs of vCA1 > LHA and vCA1 > mPFC pathways converge on the same regions, including the medial and lateral septal nuclei. Interestingly, LHA neurons that receive direct input from vCA1 also project to mPFC, and mPFC neurons that receive input from the vCA1 project to LHA.
Collectively, these results reveal substantial convergence of the vHP > LHA and vHP > mPFC neural pathways with regards to both 1$^{st}$-order collateral and 2$^{nd}$-order projections. Considering our recent work identifying an important role for vHP signaling in learned and hedonic aspects of feeding behavior, future studies are needed to determine the functional implications of these newly identified signaling routes with regards to the control of food intake.

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**Poster**

**604. Mapping Central Hypothalamic Pathways**

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**Topic:** F.10. Food Intake and Energy Balance

**Title:** Natural and artificial hunger gate social behavioral choice

**Authors: *J. BURNETT**, S. C. FUNDERBURK, C. LI, J. NAVARRETE, M. J. KRASHES
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**Abstract:** Complex motivated behaviors must be prioritized and executed according to appraisal of internal drive states. When strong enough, these drives can suppress expression of other motivated behaviors. In mice, caloric starvation states can suppress normal self-preservation, social interaction, and water-seeking behaviors to favor foraging and food consumption behaviors. Starvation-sensitive Agouti-related peptide (AgRP) neurons expressed in the arcuate nucleus of the hypothalamus (ARC) may be integral to this process: stimulation of this population induces an artificial hunger-like state that also can suppress non-feeding-related behaviors. It is unclear, however, how different hunger states dynamically coordinate multiple types of motivated behaviors; $ARC^{AgRP}$ neurons' role in this process is also unclear. Here we sought to investigate the influence of natural hunger states, as well as an artificial hunger-like state through stimulation of $ARC^{AgRP}$ neurons, on an animal's drive to engage with a male or female conspecific.

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**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

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**Topic:** G.01. Appetitive and Aversive Learning

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**Title:** TAAR1 partial agonist RO5263397 prevented the extinction of lithium chloride-induced conditioned taste aversion

**Authors: *J. LIU**, R. SEAMAN, Jr., B. JOHNSON, J.-X. LI
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**Abstract:** Trace amine-associated receptor 1 (TAAR1), a G protein-coupled receptor, is a modulator of monoaminergic neurotransmission. Selective TAAR1 agonists showed antipsychotic-like properties, antidepressant-like effects, and pro-cognitive properties. In addition, activation of TAAR1 inhibited the expression of drug and palatable food rewarding memories. However, few study investigated the role of TAAR1 in aversive learning and memory. Here, we examined the effects of TAAR1 partial agonist RO5263397 on lithium chloride (LiCl)-induced conditioned taste aversion (CTA). We showed that LiCl (0.075 or 0.15 M, 7.5 ml/kg, i.p.) significantly induced taste aversion to saccharin in rats. Pretreatment of RO5263397 (5.6 mg/kg, i.p.) significantly prevented the extinction of LiCl-induced CTA. Sub-chronic administration of RO5263397 delayed the extinction but had no effect on the extinction rates during later extinction. Further experiments should be conducted to confirm the effects of activation of TAAR1 on the expression and extinction of LiCl-induced CTA.

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**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

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**Topic:** G.01. Appetitive and Aversive Learning

**Support:** HHMI grant to Swarthmore College

**Title:** Reducing retention of learned fear: Prediction error versus lability

**Authors: *A. M. SCHNEIDER**[1], J. SUN[1], C. G. YAO[1], J. KANG[1], R. ABISHEK[1], D. J. KALAMARIDES[2], P. E. SIMSON[3], L. G. KIRBY[4]
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**Abstract:** Extinction-based procedures for eliminating fear-related memory have met with limited success in that fear memory often returns with the passage of time. Accordingly, more recent approaches have sought to prevent recovery of fear by targeting it directly, manipulating either a) the *lability* of newly retrieved fear memory prior to extinction training or b) *prediction error* during extinction training. In the present experiment, a modified extinction procedure was

used after training that incorporates features of both the lability and prediction error approaches, to determine the effectiveness of each approach in reducing retention of fear. In the modified procedure, animals were exposed to the conditioning apparatus for a brief period (30 sec) in the absence of shock, that is, they were removed from the apparatus before an opportunity to recall fear or equivalently to experience prediction error developed to a significant degree. Specifically, 24 hrs after contextual fear conditioning, rats were divided among 5 groups and received one of the following procedures: a single 30 sec exposure, a single 60 sec exposure, two 30 sec exposures separated by 10 min, two 30 sec exposures separated by 6 hrs; a control group was not exposed to the apparatus. Each of the 5 groups received two retention tests to determine the effectiveness of the extinction procedure in reducing retention of fear, the first test administered 24 hrs after the extinction procedure (short-term retention), the second test administered 11 days after the extinction procedure (long-term retention). If *prediction error* determines the effectiveness of the extinction procedure, then the second exposure in the 10-min and 6-hr conditions, accompanied by weak fear or equivalently weak prediction error, should not only limit retention of fear, it should do so *regardless of the inter-exposure interval*. In contrast, if *lability* determines the effectiveness of the extinction procedure, then the second exposure in the 10 min and 6 hr condition, introduced within or outside the window of lability, should not only limit retention of fear, it should do so *depending on the inter-exposure interval*. The results indicated that all exposure conditions reduced retention of fear both in the short term and long term, but only the 30 sec exposure administered within the window of lability (10 min condition) reduced retention in the long term to a level approaching the pre-training baseline. The results support the efficacy of the brief exposure procedure in attenuating long-term retention but suggest that its effectiveness may depend on lability rather than prediction error.

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**Poster**

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**Topic:** G.01. Appetitive and Aversive Learning

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**Title:** Hippocampal-prelimbic cortex pathway activation curbs fear after recent but not remote extinction

**Authors: *W. A. SZADZINSKA**, J. BUKOWCZAN, K. ROKOSZ, K. KONDRAKIEWICZ, M. MIKOSZ, E. A. KNAPSKA
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**Abstract:** With the passage of time after fear extinction an extinguished fear returns. Spontaneous recovery of fear is well described at the behavioral level; however the neuronal basis of the phenomenon is not well understood. The prelimbic region (PL) of the prefrontal cortex, which integrates signals from the basolateral part of the amygdala (BL) and the ventral hippocampus (vHIP), has been implicated in retrieval of both fear and fear extinction memories. Its involvement has been studied shortly after fear extinction; it is not known however how the BL and vHIP signals in the PL impact retrieval of fear extinction memory at later times after extinction. To investigate the role of the BL and vHIP inputs to the PL during extinction memory retrieval we used immediate early gene c-Fos mapping, functional anatomy tracing and optogenetic stimulation. We found two distinct subpopulations of neurons in the PL activated by successful extinction and relapse of fear; the fear neurons received predominantly the BL inputs, whereas the extinction neurons received mainly the vHIP projections. Consistently, optogenetic activations of the BL projections increased and the vHIP inputs decreased levels of freezing to the CS during retrieval of the recent but not remote memory. At the later times after extinction fear expression could be reduced by vHIP-PL pathway stimulation only after extensive extinction training, which is supposed to strengthen the vHIP-PL connectivity. The number of neurons activated by successful extinction and spontaneous fear recovery was similar. Thus, the results suggest that over time fear memory is no longer gated by the vHIP inputs to PL and the BL signals can play a dominant role, resulting in spontaneous recovery of fear.

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**605. Fear and Aversive Learning and Memory: Extinction**

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**Title:** In the pursuit of the fear engram: Identification of neuronal circuits underlying the treatment of anxiety disorder

**Authors: *O. KHALAF**, L. DIXSAUT, V. GORDEN, L. GLAUSER, J. GRÄFF
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**Abstract:** Fear and other anxiety disorders are extraordinarily robust and difficult to treat. Among the most effective treatments for anxiety disorders are exposure-based therapies, during which a patient is repeatedly confronted with the originally fear-eliciting stimulus in a safe environment so that the once fearful stimulus can be newly interpreted as neutral or safe. A fundamental element for successful exposure-based therapies is the reactivation/recall of the traumatic memory, which initiates a time-limited process called memory reconsolidation, during which a memory becomes susceptible to disruption.
Presently, the neuronal subpopulations and molecular mechanisms underlying successful fear memory attenuation remain completely unknown, which represents a big gap in memory research. Therefore, the first aim of this work is to identify the neuronal subpopulations that are causally implicated in effective attenuation of remote fear memories. This will help to determine whether the original traumatic memory trace has been permanently modified or a new memory trace of safety has been superimposed over the original one. The second aim is to develop a tool that allows for the isolation of the neuronal subpopulations causally implicated in remote memory attenuation, in order to be able to delineate the epigenetic and transcriptional mechanisms at play within these subpopulations. This will help to identify a molecular signature of effective remote fear memory attenuation.
The results of my research suggest for the first time that there is a small population of neurons in the dentate gyrus – that was active during the recall of fear – that needs to be reactivated during extinction to attain successful remote fear attenuation. While the inactivation of such population during extinction impairs fear attenuation, its activation ameliorates behavioral extinction. Furthermore, we have successfully established a method to isolate this neuronal subpopulation from the brain, namely by fluorescence-activated cell sorting. This tool will allow follow up studies to pursue the quest for the molecular signature of successful remote memory attenuation. Overall, these findings could help us to better understand the intricate principles of effective remote fear memory attenuation, and thus to develop new strategies that improve the treatment of anxiety disorder.

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**Title:** Elucidating the mechanisms of fear extinction in developing animals: a special case of NMDAR-independent extinction in adolescent rats

**Authors: *M. A. BISBY**, K. D. BAKER, R. RICHARDSON
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**Abstract:** NMDARs are considered critical for the consolidation of fear extinction. However, recent work has challenged this assumption. Namely, extinction retention is NMDAR-independent in infant rats and when extinction training occurs for the second time (i.e., re-extinction) in adult rats. In this study, a possible third instance of NMDAR-independent extinction was tested. Although adolescents typically exhibit impaired extinction retention, some recent work has shown that rats that are conditioned as juveniles and then given extinction training as adolescents (JuvCond-AdolesExt) have good retention of extinction (indicating successful extinction consolidation). However, this good extinction retention is observed in the absence of an upregulation of the synaptic plasticity marker phosphorylated mitogen activated protein kinase (pMAPK) in the medial prefrontal cortex, a region considered critical for extinction consolidation. In the current study, rats received either the non-competitive NMDAR antagonist MK801 (0.1mg/kg, s.c.) or saline 10 minutes prior to extinction training. Although juvenile rats exhibited impaired extinction retention after MK801 compared to saline, this effect was not observed in JuvCond-AdolesExt rats (*n*s = 11-12). Further experiments ruled out a number of alternative accounts for the lack of an effect of NMDAR antagonism on extinction retention in JuvCond-AdolesExt rats (e.g., that the animals were adolescents or had a delay between conditioning and extinction). These results provide evidence for yet another circumstance in which NMDARs are not required for successful extinction retention - that is, when rats learn fear as juveniles and undergo extinction as adolescents. Furthermore, our findings highlight the complexity of fear inhibition across development, particularly in adolescence.

**Disclosures: M.A. Bisby:** None. **K.D. Baker:** None. **R. Richardson:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.06/OO33

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** NIH Grant R01AG052934

**Title:** Distinct neuronal activation in medial prefrontal cortex between wild-type inbred mouse strains during fear extinction learning

**Authors: *V. A. CAZARES**[1], R. PARENT[2], L. OUILETTE[3], S. J. MOORE[4], G. G. MURPHY[5]
[1]Mol. and Behavioral Neurosci. Inst., [2]Mol. and Behavioral Neurosciences Inst., [4]Mol. & Behavioral Neurosci Inst., [5]MBNI/Physiology, [3]Univ. of Michigan, Ann Arbor, MI

**Abstract:** The medial prefrontal cortex (mPFC) has emerged as a brain region capable of gating the expression and extinction of fear memories. Subdivisions of the mPFC are proposed to exert orthogonal effects on the expression of fear. The prelimbic (PL) division is believed to enhance fear; whereas, the infralimbic (IL) is believed to diminish fear and thereby facilitate extinction. However, the dynamics of how mPFC neural activity in these brain regions can exert overall net enhancement or suppression of fear expression remains unclear. Moreover, synthesis of results from lesion, microstimulation and single-unit recordings of IL and PL have yielded somewhat paradoxical conclusions that do not support a straightforward division of labor between IL and PL. In part, this may be a consequence of the methodological difficulty in discerning unique vs. overlapping contributions of two closely positioned brain regions. To address this, we expand our previous work (Temme et al., 2014), along with work from the Holmes laboratory (i.e. Camp et al., 2009; Fitzgerald etl al., 2014) which has characterized *wild-type* inbred mouse strains that differ in their capability for fear extinction learning. Specifically, we have found that the 129 inbred mouse strain is significantly impaired in extinction learning and also shows greater fear generalization to novel environments relative to the C57BL/6. Our goal is to leverage this distinction between these strains to visualize differences in neural activation in IL and PL during extinction. To do this, we compare mice of each strain that have been fear conditioned and then received a varying number of presentations of the CS during extinction (0, 30, or 60 presentations). We use both *in situ* and *in vivo* measures for neural activation in IL and PL during and after this extinction learning. First, we compare fractions of neurons in mPFC that show immunoreactivity for expression of immediate early genes, which are upregulated in response to increases in neural activity (*cFos, Zif268*). Furthermore, we use miniaturized endoscopes together with a genetically encoded calcium sensor (GCaMP6) to visualize fluorescence changes associated with neuronal activation in single cells of IL or PL during presentations of a CS during extinction training. Using these approaches, we may elucidate distinct cellular and network mechanisms that confer different extinction learning capabilities.

**Disclosures: V.A. Cazares:** None. **R. Parent:** None. **L. Ouilette:** None. **S.J. Moore:** None. **G.G. Murphy:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.07/OO34

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** NHMRC Career Development Fellowship APP1083309

Baker Foundation Fellowship

Melbourne University ECR grant

**Title:** Sex differences in extinction of conditioned fear in adolescent rats: Estrus cycle effects

**Authors: *J. H. KIM**[1], D. L. D. NGUYEN[1], S. WHITTLE[2], D. E. GANELLA[1]
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**Abstract:** Over 70% of those suffering from anxiety disorders are diagnosed during childhood or adolescence. Despite these findings, adequate treatments are lacking, with over 50% of young people with anxiety disorders not responding to first-line treatments (i.e. cognitive behavioural therapy involving exposure training). While female children, adolescents, and adults are more than twice as likely to suffer from anxiety disorders as compared to males, the biological mechanisms underlying sex differences in anxiety disorders, especially during puberty, are poorly understood. Therefore, we examined fear conditioning and extinction in female and male postnatal day 35 rats. Rats first received 6 tone-shock pairings and then 60 tone alone presentations the next day for 2 days. Following each behavioural session, all female rats received a vaginal swab to monitor the estrus cycle (metestrus, diestrus, proestrus, and estrus). We observed comparable conditioning between male and female adolescent rats, and the estrus cycle on conditioning day did not affect tone-elicited freezing during conditioning and subsequent extinction days. However, the estrus cycle on the first day of extinction had a significant effect. Specifically, proestrus females overall froze significantly more (main effect of group, significant Tukey post-hoc) and showed a significantly delayed extinction (group x tone trials interaction) compared to male rats. The delayed extinction was further supported by Tukey post-hoc tests that showed higher lever of freezing in proestrus female rats at the final 7-12$^{th}$ block of extinction. Interestingly, this effect of proestrus during extinction day 1 carried over to the next day, even though many of the proestrus rats were no longer in that stage during extinction day 2. No other estrus cycle had effects on both extinction days, indicating that metestrus, diestrus, and estrus female adolescent rats fear condition and extinguish similarly to adolescent male rats. These findings are contrary to previous reports on the dampening effects of high estrogen levels in adult female rats on conditioned fear, and provide a rat model to study

why adolescent females may be more susceptible to developing anxiety disorders compared to males.

**Disclosures:** **J.H. Kim:** None. **D.L.D. Nguyen:** None. **S. Whittle:** None. **D.E. Ganella:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.08/OO35

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** Discovery Early Career Research Award from the Australian Research Council DE140100243

**Title:** Reproductive experience alters the involvement of N-methyl-D-aspartate receptors in fear extinction, but not fear conditioning, in female Sprague Dawley rats

**Authors: *S. TANG**, B. M. GRAHAM
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**Abstract:** Recently, evidence has emerged showing that the behavioural and hormonal features of fear extinction are altered as a result of reproductive experience (Milligan-Saville & Graham, 2016). The current set of experiments sought to determine whether reproductive experience also alters the molecular features of fear extinction. In adult male rats, it has been widely demonstrated that the activation of N-methyl-D-aspartate receptors (NMDAr) is essential for fear extinction. We therefore compared the involvement of NMDAr in fear extinction between nulliparous (virgin) and primiparous (reproductively experienced) female rats. Nulliparous and primiparous females received systemic administrations of either MK-801 (a non-competitive NMDAr antagonist) or saline prior to extinction training. MK-801 was found to impair extinction recall in nulliparous females, but not primiparous females. When the same dose of MK-801 was administered prior to conditioning, both groups of rats showed impaired recall of conditioning the following day. Although further research is necessary, the results of these experiments indicate that the extinction, but not the acquisition of fear, may become NMDAr-independent following reproductive experience.

**Disclosures:** **S. Tang:** None. **B.M. Graham:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.09/OO36

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** National Health and Medical Research Council grant APP1086855

National Health and Medical Research Council grant APP1054642

Australian Research Council DP150104835

Australian Government Research Training Program Scholarship

**Title:** Improving extinction retention and reducing relapse in adolescent rats with a TrkB agonist

**Authors: *A. A. STYLIANAKIS**[1], R. RICHARDSON[2], K. D. BAKER[3]
[1]Sch. of Psychology, Univ. of New South Wales - Kensington Campus, Sydney, Australia; [2]Univ. of New South Wales, Sydney, Australia; [3]UNSW Australia, Sydney, Australia

**Abstract:** A number of preclinical studies have demonstrated that fear extinction is impaired during adolescence. Evidence from studies with adult rodents suggests that the neurotrophic factor BDNF, or brain-derived neurotrophic factor, is important for extinction retention. However, there is some evidence that BDNF signaling does not function effectively during adolescence, which could be a contributing factor to the poor extinction retention exhibited in rats in this developmental period. One of the receptors that BDNF binds to and activates is the TrkB receptor. Past work has reported that a TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF), injected systemically before a sub-optimal extinction session facilitated retention of extinction and reduced fear relapse (renewal) in adult mice. Here we examined whether 7,8-DHF also improves extinction retention and reduces renewal in adolescent rats. Adolescent rats (33 days of age) were given 3 CS (white noise)-US (.45 mA shock) pairings in Context A. The next day, rats were injected (i.p.) with 7,8-DHF (5 mg/kg) or vehicle one hour before extinction training in a second context (B). Over the next two days, rats were tested for extinction retention (in Context B) and ABA renewal. Adolescent rats given the TrkB agonist before extinction training exhibited improved extinction retention and less renewal than those injected with vehicle. These results demonstrate that activating TrkB receptors can ameliorate the extinction retention deficit typically exhibited by adolescent rats, as well as reduce fear relapse (renewal) in these animals. Considering that the impaired extinction retention exhibited by adolescents can be ameliorated by activating TrkB receptors, the receptors through which BDNF functions, the results of this experiment provide support for the hypothesis that dysfunctional BDNF signaling could be contributing to the extinction retention deficit of adolescents.

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**Disclosures: A.A. Stylianakis:** None. **R. Richardson:** None. **K.D. Baker:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.10/PP1

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** EMBO ALTF 1605-2014

**Title:** A cortico-thalamic-hippocampal circuit for remote fear memory attenuation

**Authors: *B. A. SILVA**, M. KINTSCHER, R. SCHNEGGENBURGER, J. GRÄFF
École Polytechnique Fédérale De Lausanne (EPFL), Lausanne, Switzerland

**Abstract:** The experience of strong traumata can lead to the formation of over-enduring fear memories that risk to degenerate into a pathological state known as post traumatic stress disorder (PTSD). When recalled, previously acquired memories can enter a labile state where new information can be incorporated. This memory update process forms the basis of the most successful treatments for PTSD, where subjects are repeatedly exposed to the trauma-inducing stimulus in a safe environment, resulting in an attenuation of the fearful component of trauma-related memories. Traditionally, the recall of recently acquired fearful memories is thought to be dependent on the hippocampus, whereas remote memory storage is said to rely more on higher cortical areas such as the medial prefrontal cortex. Nevertheless, here we hypothesize that hippocampal reactivation is necessary for remote memory updating. In particular, we posit that a bisynaptic cortico-thalamic-hippocampal circuit, involving the anterior cingulate cortex, the nucleus reuniens of the thalamus, and hippocampal area CA1, is critically involved in this process. To test this hypothesis, we are combining virus-based tracing and inducible chemogenetic tools for the specific manipulation of the neural activity of the neuronal population constituting such bisynaptic system during remote fear memory attenuation.

**Disclosures: B.A. Silva:** None. **M. Kintscher:** None. **R. Schneggenburger:** None. **J. Gräff:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.11/PP2

**Topic:** G.01. Appetitive and Aversive Learning

**Title:** Chronic alcohol after fear extinction augments cued freezing

**Authors: *H. C. BERGSTROM**, J. DISHART, A. HILLER, G. MINTZ, Z. WANG
Dept. of Psychological Sci., Vassar Col., Poughkeepsie, NY

**Abstract:** Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) often co-occur. Some evidence suggests that alcohol may augment PTSD symptomatology. An inability to extinguish traumatic fear has been linked with the development of PTSD. In all prior studies modeling the impact of alcohol on fear extinction, alcohol was administered prior to extinction, leaving open the question of how alcohol directly interacts with the retrieval of a previously extinguished fear memory. Adult male C57BL/6 mice underwent auditory fear conditioning, context fear extinction, and cued fear extinction (massed presentations) on subsequent days. The next day, mice were administered daily intraperitoneal injections of ethanol (EtOH; 2.5 g/kg in 0.9% saline) or vehicle control over 5 days. Four days following alcohol exposure, CS-evoked freezing behavior in the extinction context was tested. Adult mice in the EtOH group (n=14) exhibited more CS-evoked freezing behavior relative to controls (n=13). There were no differences in contextual fear (pre-CS), context renewal and the effect of alcohol diminished with time (16 days). These data suggest that chronic alcohol impairs the retrieval of a previously established fear extinction memory and/or enhances the expression of the original fear memory. Analysis of neurons expressing the activity-regulated cytoskeletal protein Arc/arg 3.1 following fear extinction retrieval is ongoing in the basolateral amygdala, infralimbic, and prelimbic cortices

**Disclosures: H.C. Bergstrom:** None. **J. Dishart:** None. **A. Hiller:** None. **G. Mintz:** None. **Z. Wang:** None.

**Poster**

**605. Fear and Aversive Learning and Memory: Extinction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 605.12/PP3

**Topic:** G.01. Appetitive and Aversive Learning

**Support:** NARSAD Distinguished Investigator

Brain Health Institute, Rutgers

**Title:** MAP Training the brain with meditation and aerobic exercise

**Authors: *T. J. SHORS**, E. M. MILLON, H. M. CHANG
Behavioral and Systems Neuroscience, Dept. of Psychology, Rutgers Univ., Piscataway, NJ

**Abstract:** MAP Training[SM] is a novel brain health program designed to enhance neurogenesis—the production and survival of new neurons in the adult brain (maptrainmybrain.com; Shors et al., 2014, Curlik and Shors, 2013; Alderman et al., 2016; DiFeo and Shors, 2017). MAP Training[SM] begins with 20 minutes of silent sitting meditation followed by 10 minutes of slow walking meditation and ends with 30 minutes of aerobic exercise. Individuals who complete 8 weeks of training (twice a week) experience significant increases in mental and brain health, as assessed by decreases in depression, anxiety along with fewer ruminations about the past and increases in synchronized brain activity (Shors et al., 2014; Alderman et al., 2016; Shors et al., 2017). In this set of studies, we provided MAP Training to young adult women with and without trauma related to sexual violence history. Before training, women with sexual violence history reported more symptoms of depression, anxiety and post-traumatic stress disorder (PTSD) than women without a history. Moreover, those symptoms were highly related to ruminative thoughts and autobiographical memories about a stressful life event in their past. We propose that the MAP Training intervention is effective because the meditation component reduces trauma symptoms through extinction learning for trauma-related thoughts and memories while the aerobic exercise component targets depressive symptoms. We present the combination of mental and physical activity during MAP Training as a compelling new approach for enhancing brain health in people from all walks of life, but especially those who suffer from trauma and depression associated with stressful life events.

**Disclosures: T.J. Shors:** None. **E.M. Millon:** None. **H.M. Chang:** None.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.01/PP4

**Topic:** G.02. Motivation

**Support:** NIH Grant K01 DA03444

**Title:** Activational differences in neurons from the paraventricular nucleus of the thalamus in binge eating prone and binge eating resistant rats

**Authors: *K. A. RICHARDSON**[1], A. D. KIMBLE[2]
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**Abstract:** The paraventricular nucleus of the thalamus (PVT) functions as a communication center between the ventral and dorsal striatum and the lateral hypothalamus. It receives innervation from neuropeptides that affect feeding. However, it is not clear what influence the PVT has on binge eating behavior. The purpose of this study is to determine whether there are activational differences in the PVT of binge eating prone (BEP) and binge eating resistant (BER) rats. It is hypothesized that BEP rats have significantly more c-Fos-activated neurons versus BER rats. We used a rodent, binge eating protocol to identify female Sprague Dawley rats (250-300g, n=7/group) that display BEP or BER phenotype. After the completion of nine feeding tests, the animals were processed for c-Fos immunoreactivity. Rats classified as BEP consume significantly more palatable food (high fat, sugar) than BER rats. Quantification of c-Fos cells is still underway, but preliminary results indicate a trend in increased c-Fos in the PVT of BEP versus BER rats. The activation of PVT neurons after palatable food consumption may indicate an involvement of these neurons in mediating binge eating behavior.

**Disclosures: K.A. Richardson:** None. **A.D. Kimble:** None.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.02/PP5

**Topic:** G.02. Motivation

**Support:** ZIA-AA00421

**Title:** D2 receptor independent modulation of iMSN synaptic transmission by dopamine in the nucleus accumbens

**Authors: *D. A. BURKE**[1,2], V. A. ALVAREZ[1]
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**Abstract:** Medium spiny neurons (MSNs) in the nucleus accumbens express high levels of dopamine D1 or D2 receptors and receive dense innervation of dopamine fibers from the ventral tegmental area. Dopamine, acting on these receptors, modulates synaptic transmission and plasticity through a combination of pre- and postsynaptic effects. However, dissecting the specific function dopamine receptor activation has proven difficult due to the presence of

dopamine receptors on multiple interconnected cell types within the accumbens. In particular, D1 receptors are Gs/olf coupled receptors expressed on the half of the MSN population that forms the direct projection pathway (dMSNs), and D2 receptors are Gi/o coupled receptors expressed on the half of the projection MSNs that form the indirect pathway (iMSNs), as well as on interneurons, and presynaptic afferent terminals to this region. Understanding the specific synaptic consequences of dopamine receptor activation on MSNs in the accumbens has been difficult using conventional tools.
The goal of the current experiments is to determine how dopamine affects synaptic transmission between different combinations of MSN subtypes (i.e. iMSN->dMSN, iMSN->iMSN, etc.). Using Adora2a-cre/Drd1-tdTomato or Drd1-cre/Drd1-tdTomato mice, we expressed channelrhodopsin-2 in either subpopulation of MSN to allow selective optogenetic stimulation of either iMSN or dMSN terminals, while measuring GABA IPSCs onto red-fluorescent labeled dMSNs or negative (putative) iMSNs using whole cell voltage clamp recordings. When stimulating iMSNs, application of D2-like agonist quinpirole inhibits IPSCs onto dMSNs and iMSNs to a similar degree. Interestingly, the endogenous agonist, dopamine, inhibits iMSN->MSN IPSCs to a much greater degree than maximal doses of quinpirole. This differential effect is due to a component of the overall inhibition that is not blocked by D1 and D2 antagonists or genetic deletion of D2 receptors from iMSNs. Current experiments are aimed at determining the means by which dopamine is inhibiting iMSN synaptic transmission that does not involve D1 or D2 receptors. Understanding exactly how dopamine modulates MSN->MSN lateral inhibition is a critical step for gaining full knowledge of how dopamine ultimately shapes information flow through the accumbens.

**Disclosures: D.A. Burke:** None. **V.A. Alvarez:** None.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.03/PP6

**Topic:** G.02. Motivation

**Title:** $\Delta^9$-tetrahydrocannabinol (THC) produces bi-phasic rewarding and aversive effects in the anterior vs. posterior nucleus accumbens shell through dissociable μ vs κ opiate receptor mechanisms and differential modulation of medium spiny neuron activity

**Authors: *C. NORRIS**, H. J. SZKUDLAREK, S. R. LAVIOLETTE
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**Abstract:** The phytochemical in cannabis primarily responsible for its psychoactive properties, $\Delta^9$-tetrahydrocannabinol (THC), produces seemingly contradictory effects of reward and aversion at different doses. In addition, previous studies demonstrate that cannabinoid receptor

transmission can strongly modulate opiate-related motivational processing. Emerging evidence suggests that there are anatomical and functional differences between the poles of the shell region of the nucleus accumbens (NASh). Stimulation of a so-called “hedonic hotspot” on the anterior pole produces reward while stimulation of the rest of the structure either has no effect or produces aversion. Using a combination of conditioned place preference, social interaction, and *in vivo* electrophysiology we sought to elucidate how the unique properties of the NASh could be responsible for the biphasic effects of THC. Targeted microinfusions of THC into the anterior shell (+2.5mm from bregma) produces reward but microinfusions into the posterior shell (+1.5mm from bregma) produces aversion. Both these effects were challenged by either κ-opioid or μ-opioid receptor blockade. Reward in the anterior NASh was selectively blocked by co-administration of a μ-opioid receptor antagonist whereas aversion in the posterior NASh was selectively blocked by a κ-opioid receptor antagonist. Next, we wanted to examine if the effects of THC in the NASh generalized beyond producing reward and aversion. Using a social interaction test we demonstrated that infusions into the posterior (but not anterior) NASh induces deficits in natural sociability and social recognition memory. Conversely, THC infused into the anterior (but not posterior) NASh potentiates the reward salience of a normally subthreshold dose of morphine. Neither infusions into the anterior or posterior NASh have any effect on sucrose consumption, indicating these effects are specific to drug-related reward processing. Finally, using *in vivo* neuronal recording in rats, we demonstrate inter-cranial ventricle (ICV) infusions of THC produce a predominate decrease in medium spiny neuron (MSN) activity in the anterior NASh but produce a predominate increase in MSN activity in the posterior NASh. Collectively, these data further categorize the functional differences present in the NASh and suggest that interaction with endorphin receptors in the area are important for the effects of THC on affective processing.

**Disclosures:** **C. Norris:** None. **H.J. Szkudlarek:** None. **S.R. Laviolette:** None.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.04/PP7

**Topic:** G.02. Motivation

**Support:** Canadian Institutes of Health Research

Natural Sciences and Engineering Research Council of Canada

Ontario Mental Health Foundation

**Title:** Delta-9-tetrahydrocannabinol and cannabidiol exert differential effects on aversive and rewarding emotional memory formation and salience attribution through actions in the ventral hippocampus

**Authors: *R. M. HUDSON**[1], W. J. RUSHLOW[2], S. R. LAVIOLETTE[2]
[1]Neurosci., [2]Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

**Abstract:** Disturbances in emotional processing and salience attribution are core features of schizophrenia and other neuropsychiatric disorders. The ventral hippocampus (VHipp) contains large distributions of cannabinoid CB1 receptors (CB1R) that regulate emotional memory formation by modulating signaling in mesocorticolimbic structures such as the ventral tegmental area (VTA) and medial prefrontal cortex (mPFC). Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are phytocannabinoids that differentially impact dopamine (DA) activity and emotional processing. We have shown previously that intra-VHipp CB1R activation strongly modulates both fear and reward-related associative memory formation through modulation of mesolimbic neuronal activity states. In addition, we demonstrated that CBD can prevent amphetamine-induced amplification of DA activity and psychotomimetic behaviours through control of the rapamycin (mTOR) signalling cascade. Nevertheless, the mechanisms through which intra-VHipp THC and CBD may modulate emotional memory remain unknown. We used a combination of behavioural pharmacology, molecular analyses and in-vivo electrophysiology in rats to examine whether intra-VHipp THC and CBD differentially control the salience of rewarding and aversive emotional associative memory using a morphine place conditioning procedure (CPP), social reward assays and associative fear conditioning. In addition, we characterized the effects of intra-VHipp THC and CBD on simultaneous VTA DAergic and mPFC pyramidal neuronal activity states using single unit, extracellular electrophysiological recordings under urethane anesthesia. Intra-VHipp THC induced deficits in social memory formation but enhanced memory for morphine CPP and aversive associative cues. CBD alone had no effect, but when combined with THC increased social memory and disrupted THC-induced effects on reward and aversion-related emotional memory formation. We further report that CBD alone and co-administered THC control downstream phosphorylation of the mTOR signalling cascade within the VHipp. Additionally, intra-VHipp THC altered firing patterns in VTA DA and mPFC neurons by reducing phasic bursting levels and inducing opposing actions on population activity. Collectively, our findings implicate the VHipp as a critical site of action for phytocannabinoid-induced modulation of both reward and aversion-related emotional processing and memory formation. In addition, our findings have important implications for understanding potential positive and negative effects of specific cannabis-derived phytochemicals in neuropsychiatric symptomology.

**Disclosures:** **R.M. Hudson:** None. **W.J. Rushlow:** None. **S.R. Laviolette:** None.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.05/PP8

**Topic:** G.02. Motivation

**Support:** Department of Psychology (BGSU)

John Paul Scott Center for Neuroscience

Center for Undergraduate Research and Scholarship (BGSU)

**Title:** Order effects alter work effort in rat model of free choice

**Authors: *Z. T. KNAUSS**[1], M. M. QUEENER[1], J. A. LUBERA[1], M. FILIPOVIC[1], N. M. BOLDEN-HALL[1], J. P. SMITH[1], R. S. GOLDSMITH[1], K. A. SMITH[1], J. E. BISCHOFF[1], A. PRICE[1], M. C. MILLER[1], H. C. CROMWELL[2]
[2]Dept. of Psychology, [1]Bowling Green State Univ., Bowling Green, OH

**Abstract:** Previous research on effort is sparse, mainly showing weak effects to obtain consistent work by animal subjects. Studies have obtained consistent preference for the higher effort option but only after extensive training with forced choice. Moreover, no studies that we are aware of have shown animals will persist choosing the higher effort option despite obtaining relatively fewer rewards by making this choice. The sequence of effort experience could have a major impact on the intensity of work emitted by the subjects and even lead to this form of 'suboptimal' persistence. One of the goals is to create a more effective method to embed effort in order to test the impact of drugs of abuse on motivation and choice behavior. The present study used a paradigm that couple's elements of reward order during free foraging choice with the goal of forming a more naturalistic model of how order effects relate to work motivation/output in the rat model of choice. Previous work using this model has been successful at delineating components of choice behavior and comparing different contexts of choice (Ricker et al., 2016). Animals choose between a high effort option (5 lever press) with shifting reward outcome value either magnitude descending (5,4,2,1) or magnitude ascending (1,2,4, 5). The opponent low effort option (one lever press) contained a constant reward (one pellet) for all weeks. Work motivation/output was determined through analysis of high effort reward discrimination, preference and incentive contrast between weeks. An order effect was observed with animals working harder and providing more effort when rewards were in descending order as compared to rewards in ascending order. Further analysis will focus on a set of dependent variables including place preference, approach and consumption measures. In addition, the results allow for an analysis of error rates when obtaining reward options in different choice contexts. These findings suggest that rats that experience higher levels of work first, will choose to work and

prefer working even when the food reward is decreased to a point where it is costly in comparison to the constant one lever press one pellet reward. The implications of developing a consistent method to induce preferences for effort and work include: 1) the ability to test the impact of drugs of abuse on effort and work output; 2) to enable an examination of the environmental factors involved in how organisms choose to work harder and persist in choice evaluation toward effort vs. reduced effort options and 3) examining the neural substrates for effort as a key parameter in motivation and the brain basis of decision-making and choice.

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.06/PP9

**Topic:** G.02. Motivation

**Support:** Center for Undergraduate Research and Scholarship (BGSU)

**Title:** Effects of anandamide administration on components of reward processing during free choice

**Authors: *B. R. FRY**, L. C. ZONA, J. A. LALONDE, H. C. CROMWELL
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**Abstract:** Previous research has implicated the positive modulation of anandamide, an endocannabinoid neurotransmitter, on feeding behavior. Anandamide is particularly noteworthy as it shares a similar mechanism of action with tetrahydrocannabinol, the primary psychoactive component in Cannabis. Cannabis legalization in North America has presented with a need to study endocannabinoid agonists and their effects on behavior. Much has yet to be determined in terms of the role of the endocannabinoid system in decision-making scenarios. The research presented here tested the hypothesis that anandamide would augment motivation and reward processing via appetitive and consummatory measures during an operant, foraging task. A three-box design was used in order to provide the animals with a free choice, exploratory foraging environment. Discrimination, preference, and incentive contrast were analyzed as discrete measures of decision-making in the three-box paradigm. Anandamide administration (1mg/kg) was found to significantly increase motivation for the optimal foraging outcome and alter basic processing of reward information involved in discrimination and relative valuation. The positive effects of anandamide on eating behavior and motivation have implications toward possible treatment modalities for patient populations presenting with disorders of motivation. These

findings suggest the need for continued investigation of the endocannabinoid system as a central component of motivated behavior.

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.07/PP10

**Topic:** G.02. Motivation

**Support:** Blackthorn Therapeutics, Inc.

**Title:** Electrophysiological characterization of novel selective and reversible kappa opioid receptor antagonists in VTA neurons

**Authors: *E. B. MARGOLIS**[1], T. L. WALLACE[2], L. J. VAN ORDEN[2], W. J. MARTIN[2]
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**Abstract:** Kappa opioid receptors (KORs) modulate physiological conditions of stress, reward, and mood, and are localized within neuronal circuits connecting midbrain monoaminergic neurons with forebrain limbic structures. Because activation of KORs within the ventral tegmental area (VTA) inhibits VTA dopamine neuron activity (Margolis et al., 2003 & 2006), blockade of KOR may be a useful approach to treating a variety of neurobehavioral disorders in which dopamine neuronal function is suppressed. Here we used an acute midbrain slice preparation from rat and whole cell electrophysiology to evaluate the potency, selectivity, and reversibility of four different KOR antagonists: BTRX-335140, BTRX-395750, PF-04455242, and LY2456302/CERC-501. Each compound reduced the outward current induced by the KOR selective agonist, U69593, in a concentration-dependent manner. BTRX-335140 acted as a full antagonist with an $IC_{50}$ of 1.1 nM in DA neurons, consistent with the potency measurements observed in a recombinant cell line stably expressing rat KORs ($IC_{50}$ = 3.2 nM). Similarly, BTRX-395750 ($IC_{50}$ of 5.8 nM) exhibited potent, full antagonist properties. Interestingly, LY2456302 showed an absolute $IC_{50}$ = 0.3 nM in this preparation, yet also had an unusually shallow sloped concentration response curve. In contrast, we found PF-04455242 exhibited partial antagonist activity in this VTA preparation, with a maximum of 60% blockade of the U69593 effect. PF-04455242 also generated an outward current and a decrease in membrane resistance in a subset of neurons, consistent with a channel opening. BTRX-335140 had no effect on responses to saturating doses of the mu opioid receptor agonist DAMGO or the delta opioid receptor agonist DPDPE in VTA neurons at a concentration that fully blocked the U69593 responses. Compared to the KOR antagonist nor-BNI, which as expected did not show any washout during slice experiments, responses to U69593 at least partially recovered following

washout (20 min) of all tested antagonists except PF-04455242. In particular, BTRX-335140 showed complete washout within 10 min of the termination of bath application. Together these data provide electrophysiological evidence that BTRX-335140 is a potent, selective, and reversible KOR antagonist in neurons within a key circuit implicated in many neurobehavioral disorders.

**Disclosures: E.B. Margolis:** 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.; Blackthorn Therapeutics. **T.L. Wallace:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics, Inc. **L.J. Van Orden:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics, Inc. **W.J. Martin:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics, Inc..

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.08/PP11

**Topic:** G.02. Motivation

**Support:** BlackThorn Therapeutics

NIH Grant UH2 NS 09030

**Title:** Pharmacological characterization of BTRX-335140, a potent, selective and reversible kappa opioid receptor antagonist

**Authors: *T. L. WALLACE**[1], L. J. VAN ORDEN[1], M. GUERRERO[2], S. RILEY[2], S. BROWN[2], F. PORRECA[3], H. ROSEN[2], E. ROBERTS[2], W. J. MARTIN[1]
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**Abstract:** Kappa opioid receptors (KORs) are expressed throughout the brain including within mesolimbic and mesocortical circuits and are involved in reward processing, mood and cognition. Under conditions of stress, activation of KORs by the endogenous peptide dynorphin negatively impacts cognitive and affective processing leading to the hypothesis that antagonizing

KORs could be a therapeutic approach for treating mood disorders. We have developed a novel series of potent and selective molecules that exhibit KOR antagonist properties and here within show the pharmacological characterization of BTRX-335140. In radioligand binding studies using [$^3$H]Diprenorphine, BTRX-335140 demonstrated potent inhibition at human KOR ($K_i$ = 1.5nM) and showed 300-fold selectivity over human opioid mu receptor (MOR; $K_i$ = 0.45μM). Subsequently, BTRX-335140 was assessed for functional activity in a recombinant cell line stably expressing human KORs in which it demonstrated potent antagonism ($IC_{50}$ = 0.8nM) in response to challenge with the synthetic KOR agonist, U-50,488, and was modestly less potent following challenge with the endogenous ligand, Dynorphin A ($IC_{50}$ =6.1nM). BTRX-335140 demonstrated an approximate 125-fold separation between the KOR and the MOR ($IC_{50}$=0.1μM), and >8000-fold separation between KOR and delta (DOR; $IC_{50}$=6.5μM). In addition, BTRX-335140 potently blocked dynorphin A-induced activation of the β-arrestin pathway ($IC_{50}$ =4.8nM) suggestive of balanced antagonist properties, and antagonized dynorphin A-induced receptor internalization ($IC_{50}$=8.8nM). BTRX-335140 was tested for in vivo KOR antagonist properties using an agonist-induced prolactin challenge test in mice and rats. In these studies, KOR-agonists significantly increased the plasma prolactin concentration, and this increase was blocked by the oral administration of BTRX-335140 (minimally effective dose □10 mg/kg). Additionally, BTRX-335140 (1 mg/kg, intraperitoneal) blocked U-50,488-induced analgesia in naïve ICR mice in the tail flick test at 0.5h, but not 24h following administration, demonstrating the reversible nature of this compound. BTRX-335140 has excellent brain penetration properties as shown in an in vivo microdialysis evaluation in rats with striatal exposures approximately 4-times those observed from jugular vein samples collected in the same animals. Collectively, these studies demonstrate BTRX-335140 is a novel, potent, and reversible KOR antagonist with in vivo properties suitable for investigation in neurobehavioral disorders.

**Disclosures:** **T.L. Wallace:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **L.J. Van Orden:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **M. Guerrero:** A. Employment/Salary (full or part-time):; The Scripps Research Institute. **S. Riley:** A. Employment/Salary (full or part-time):; The Scripps Research Institute. **S. Brown:** A. Employment/Salary (full or part-time):; The Scripps Research Institute. **F. Porreca:** 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.; co-PI UH2 NS 093030. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **H. Rosen:** A. Employment/Salary (full or part-time):; The Scripps Research Institute. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. F. Consulting Fees (e.g., advisory boards); BlackThorn Therapeutics, Celgene, Kyorin Pharma. **E. Roberts:** 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.; PI on NIH grant UH2 NS 093030. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **W.J. Martin:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics.

**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.09/PP12

**Topic:** G.02. Motivation

**Title:** Impulsivity an hyperdopaminergic disorders in Parkinson's disease: From behavioral to cellular approaches in a rodent model

**Authors: *R. MAGNARD**[1], C. CARCENAC[1], Y. VACHEZ[1], S. BOULET[1], D. J. BELIN[2], S. CARNICELLA[1]
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**Abstract:** Impulse control disorders (ICDs) are a complex group of behavioral addictions found in 10 to 14% of Parkinson’s disease (PD) patients under dopamine replacement therapies. Importantly, impulsivity appears as a core symptom of ICDs. In the present study, we wonder whether denervation of the dopaminergic (DA) nigrostriatal system would promotes the development of ICDs when combined with DA agonist treatments. To answer this question, we used a rodent model of non-motor symptoms of PD recently developed in our team (Drui et al., Mol Psychiatry, 2014; Magnard et al., Transl Psychiatry, 2016). Briefly, rats were injected bilaterally with the neurotoxin 6-OHDA into the SNc in order to induce a selective, and partial denervation of the dorsal striatum. We treated them with the DA $D_2/D_3$ receptors agonist pramipexole, a medication known to favor the development of ICDs in PD patients. Two different tasks were used to measure cognitive and motor impulsivity respectively: the delay discounting task (DDT) and the 5-choice serial reaction time task (5-CSRTT). In the former, rats have to press a lever and choose between a smaller, but immediate, reward or a larger, but delayed, reward. In the later, they have to inhibit a prepotent response until the stimulus light appear. In the DDT, chronic administration of pramipexole increases impulsive choices in non-lesioned rats. Interestingly, SNc DA lesion render rats indifferent to the delay, which may reflect a lack of behavioral flexibility or a problem of choice between the magnitude of the reward and the delay. In the 5-CSRTT, pramipexole increases progressively premature responses, reflecting a pro-impulsive effect when the inter trial interval (ITI) is constant. However, when the ITI

punctually increased, pramipexole reduces premature responses, therefore exhibiting here an anti-impulsive effect. Interestingly, this modulation of motor impulsivity by pramipexole is only observed in rats with a high level of impulsivity, suggesting that an impulsive endophenotype may be an important factor of vulnerability to the effects of pramipexole. Moreover, we showed that the mammalian target of rapamycin complex 1 (mTORC1) pathway is long lastingly overactivated in the nucleus accumbens, as already observed in drug addictions. Such mechanisms may potentially be responsible for synaptic dysfunctions related to impulsive behaviors. Taken together, these results suggest that impulsivity in PD may occur through an abnormal activation of the mTORC1 pathway induced by pramipexole.

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.10/PP13

**Topic:** G.02. Motivation

**Title:** Cholinergic antagonists do not alter reinforcer devaluation in macaques

**Authors: *H. F. WAGUESPACK**[1], C. CASTANON[2], E. WICKER[3], L. MALKOVA[5], P. A. FORCELLI[4], J. N. TURCHI[6]
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**Abstract:** Reinforcer devaluation is a measure of goal-directed behavior, reflecting an animal's ability to adapt behavioral responses to changes in reward value. The neuronal circuitry associated with reinforcer devaluation has been extensively studied in nonhuman primates. This circuitry includes the thalamus, nucleus accumbens, orbitofrontal cortex, and amygdala (Mitchell et al., 2007; Izquierdo & Murray, 2010; Malkova et al., 1997; Wellman et al., 2005; West et al., 2011). Because these regions receive dense cholinergic input, we asked whether systemic injection of either nicotinic or muscarinic acetylcholine receptor antagonists, mecamylamine and scopolamine, respectively, would impair performance on the reinforcer devaluation task. For this purpose, we tested four rhesus macaques (*Macaca mulatta*) on a reward devaluation task. In this task, one set of 20 objects was consistently rewarded with one food (e.g., a peanut) and another 20 objects were rewarded with an alternative food (e.g., a fruit snack). Reinforcers were devalued through selective satiation: i.e., *ad libitum* access to one of the two foods for 30 minutes. After satiation, the animal typically will shift selection preference towards objects associated with the non-sated food. Animals were tested on a baseline choice session prior to

each devaluation session, in which their preference for objects associated with one of two rewards was examined. We tested animals after intramuscular injection of vehicle (0.9% USP grade saline), mecamylamine hydrochloride (1 mg/kg), or scopolamine hydrobromide (13.8 µg/kg); in each case, injections occurred 20 minutes prior to the selective satiation and 50 minutes prior to reinforce devaluation task. Under all three conditions, a clear devaluation effect was observed; thus, in the presence of either a nicotinic or muscarinic antagonist, animals are able to shift their behavioral responses in an appropriate manner. These data are consistent with previous studies, including a rodent study showing that systemic injection of either mecamylamine or scopolamine had no effect on reinforcer devaluation (Ostlund et al., 2014). Furthermore, these results corroborate a previous primate study, which showed that selective depletion of acetylcholine in the prefrontal cortex had no effect on reinforcer devaluation (Baxter et al., 2009).

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.11/PP14

**Topic:** G.02. Motivation

**Title:** Ketamine increases sexual motivation in female rats

**Authors: *F. A. GUARRACI**[1], H. ABDEL-RAHIM, 78626[1], J. DEVORE[1], C. M. F. GONZALEZ[1], M. N. KUNKEL[2], D. LUCERO[1], J. SMAT[1], M. STINNETT[1], P. D. WOMBLE[1], E. QUADLANDER[1], J. A. BOYETTE-DAVIS[2]
[1]Psychology, Southwestern Univ., Georgetown, TX; [2]Psychology, St. Edward's Univ., Austin, TX

**Abstract:** Antidepressant medication often produces a host of side-effects, including sexual dysfunction and changes in anxiety (Ferguson, 2001). Recent research indicates that ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, may be an alternative drug that can rapidly reduce the symptoms of depression in patients unresponsive to standard treatments (Serafini et al., 2014). However, the side-effect profile of ketamine needs to be more fully investigated. Our study investigated the effects of ketamine on sexual behavior and anxiety in female rats. In Experiment 1, adult female rats received either saline or ketamine (10 mg/kg) 30 min prior to a sexual partner preference test during which each female subject was given the opportunity to interact with a sexually vigorous male or female stimulus. Immediately afterwards, female subjects were tested for locomotion in an open field. Female subjects treated with ketamine spent significantly more time with the male stimulus than saline-treated subjects.

No other measures of mating behavior (i.e., paced mating behavior, lordosis) were affected by ketamine. Ketamine also had no effect on line crossings during the open field test. In Experiment 2, female subjects from Experiment 1 received saline or ketamine (10mg/kg), counterbalanced for previous drug exposure, and were tested for anxiety on an elevated plus maze (EPM). Ketamine had no significant effect on time spent in the open or closed arms of the EPM. Taken together the results from these two experiments indicate that ketamine does not have side-effects like other medications for depression, and may have beneficial effects on sexual motivation.

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 606.12/PP15

**Topic:** G.02. Motivation

**Title:** Deep Brain Stimulation, apathy symptom and dopaminergic system in Parkinson's disease: Preclinical study in the rat

**Authors: *Y. VACHEZ**[1], C. CARCENAC[1], R. MAGNARD[1], M. SAVASTA[2], S. CARNICELLA[1], S. BOULET[1]
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**Abstract:** Among the neuropsychiatric symptoms expressed by Parkinson’s disease patients, apathy, or decreased motivated behaviors, is the most disabling and one of the most frequently reported, especially in patients under Subthalamic Nucleus High Frequency Stimulation (STN-HFS). While the most accepted explanation for this deleterious effect is a maladaptive reduction of dopaminergic treatments, some studies report that STN- HFS, by itself, could induce apathetic behavior by activating non-motor territories of STN or surrounding structures. The aim of this study is to clinch the question of the deleterious motivational effects of STN-HFS and to determine the underlying neurobiological basis.
Innovative wireless microstimulators allow continuous stimulation of STN of freely moving rats during several weeks. Motivation and several other aspects of behavior such as motor skills have been evaluated with state- of-the-art specific tests. We have thus shown, for the first time, that STN-HFS induces a motivational deficit without altering either the sensitivity for the reward or the motor capacities of the animals.
In the clinic, post STN-HFS apathy is reversed by dopaminergic treatments, especially those targeting the D2 and D3 receptors (D2/3R) such as pramipexole. This therapeutic approach has

been translated to our model and we have found that pramipexole perfectly reversed the motivational deficit induced by STN-HFS. These results seem to confirm that apathy can be induced directly by STN-HFS alone. They bring evidence that this could be due to downregulation of D2/3R in specific brain structures, taking us one step closer to understanding the neuropsychiatric effects of STN-HFS.

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**Poster**

**606. Reward: Neuropharmacology**

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**Program#/Poster#:** 606.13/PP16

**Topic:** G.02. Motivation

**Support:** KAKENHI JP26120733

JP15H05917; SRPBS/AMED

**Title:** Distinct roles of serotonergic receptor subtypes in value-based decision processes in monkeys: A behavioral pharmacological study with PET imaging

**Authors: *Y. HORI**, Y. NAGAI, A. OH-NISHI, E. KIKUCHI, T. SUHARA, T. MINAMIMOTO
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**Abstract:** The serotonin (5-HT) system has been implicated in affective and adaptive behavior. Low 5-HT transmission is thought to increase susceptibility to negative mood and impulsive behavior. However, it remains unclear how 5-HT system modulates value-based decision processes. To address this, we manipulated 5-HT transmission in macaque monkeys by systemic treatment with a 5-HT synthetic inhibitor or receptor antagonists while they performed value-based goal-directed tasks. There were two rewarding conditions, either reward size only (1, 2, 4, or 8 drops) or delay duration to reward (0, 3.3, 6.9, or 10.2 s) was manipulated. Each rewarding condition was assigned by an associated visual cue at the beginning of each trial. When monkeys (N = 6) were treated with 5-HT synthetic inhibitor, 4-chloro-DL-phenylalanine methyl ester hydrochloride (150 mg/kg, day), 5-HIAA, a metabolite of 5-HT in CSF was decreased about 50%. The monkeys' task performance in reward size condition got worse (i.e., higher error rates) regardless of reward size. Positron emission tomography (PET) with specific radioligands for 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{2A}$, or 5-$HT_4$ receptors visualized unique distribution of each subtypes in the brain; 5-$HT_{1A}$, medial prefrontal cortex, amygdala and hippocampus; 5-$HT_{1B}$, ventral palladium and globus pallidus; 5-$HT_{2A}$, prefrontal and orbitofrontal cortex; 5-$HT_4$, striatum. We treated

monkeys with systemic injection of each receptor antagonist (WAY100635, GR55562, MDL100907, or SB125487, respectively) and determined the antagonist doses that yielded about 50% occupancy by using PET. Blocking 5-$HT_{1A}$ receptor, but not other, increased error rates regardless of reward size (N = 4), as seen in 5-HT depletion. Blocking 5-$HT_4$ receptor facilitated the tendency of increase in error rates as delay duration increased. These results suggest distinct contributions of serotonergic receptor subtypes to value-based decision processes; attenuation of serotonin transmission via 5-$HT_{1A}$ receptor—probably in medial PFC and amygdala—induces value-independent alteration of decision, whereas reduced activation of 5-$HT_4$ receptor—presumably in the striatum—causes steep temporal discounting.

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**Poster**

**606. Reward: Neuropharmacology**

**Location:** Halls A-C

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**Program#/Poster#:** 606.14/PP17

**Topic:** G.02. Motivation

**Support:** NIDA R01 DA038599 (SBF)

**Title:** The pharmacological antagonism of orexin/hypocretin receptors in the paraventricular nucleus of the thalamus decreases the conditioned reinforcing properties of a reward-associated cue in sign-tracking rats

**Authors: *P. CAMPUS**[1], J. L. HAIGHT[2], A. M. JOHNSON[3], M. S. KLUMPNER[1], I. R. COVELO[1], S. B. FLAGEL[1,4]
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**Abstract:** Conditioned stimuli (CS) associated with both drugs and natural rewards can acquire not only predictive value, but also incentive motivational value. When a CS is attributed with incentive salience it acquires the ability to elicit complex emotional and motivational states that can gain control over one's behavior, potentially leading to maladaptive outcomes. However, individuals vary considerably in the extent to which they attribute incentive value to a CS. When rats are exposed to a Pavlovian conditioned approach (PCA) paradigm, two distinct behavioral phenotypes emerge: some rats preferentially approach the lever (sign-trackers, ST) while others approach the food cup (goal-trackers, GT). While the lever is a predictor for both ST and GT, only for ST does it become an incentive stimulus. Thus, the ST/GT model offers a way to study the neurobiological mechanisms underlying the attribution of predictive vs. incentive value to

reward cues. It has recently been discovered that the paraventricular nucleus of the thalamus (PVT), a small midline thalamic nucleus, is a key part in the neural circuitry underlying the attribution of incentive salience. Previous studies have shown that presentation of an incentive, but not a predictive stimulus is capable of eliciting robust c-fos expression in the PVT. In addition, lesions of the PVT have been shown to increase sign-tracking and decrease goal-tracking behavior. The PVT contains a high density of orexin receptors and several studies have found that orexin transmission plays a role in cue-dependent motivated behaviors. Furthermore, local delivery of orexin into the PVT increases dopamine release in the nucleus accumbens, and sign-tracking behavior is dependent on accumbens dopamine. Based on these data, we hypothesize that orexinergic activity in the PVT is critical for the attribution of incentive salience to reward cues. To test this hypothesis, we trained rats on a PCA task and then evaluated the effects of the intra-PVT infusions of either the orexin 1 receptor (OX-1) antagonist SB334867 or orexin 2 (OX-2) receptor antagonist TCSOX229 on the expression of sign-tracking behavior and on the conditioned reinforcing properties of the CS. We found that neither OX-1 nor OX-2 antagonism affected sign-tracking behavior, but both decreased the incentive motivational value of the CS during the conditioned reinforcement test. These results highlight a role for the orexin system, and specifically, that in the PVT, in mediating individual variation in the propensity to attribute incentive motivational value to cues associated with rewards.

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**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

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**Program#/Poster#:** 607.01/PP18

**Topic:** G.02. Motivation

**Support:** NIH Grant DA034021 to RMC

NIH Grant DA033773 to EAW

**Title:** A history of cocaine alters prelimbic neuronal activity during learning and impairs subsequent reinforcer devaluation

**Authors: *E. A. WEST**, M. NIEDRINGHAUS, H. K. ORTEGA, R. M. HAAKE, R. M. CARELLI
Dept. of Psychology and Neurosci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

**Abstract:** A history of cocaine impairs the ability to adjust behavior away from reward-predictive cues following reward devaluation, a canonical test of flexible behavior. Here, we

used electrophysiology (multineuron) methods to record activity from prelimbic cortex (PrL), a key region necessary for flexible behavior, in cocaine-exposed rats (n=5; 14 days cocaine self-administration; 0.33 mg/inf) and controls (n=6, 14 days of saline/water self-administration) during pavlovian conditioning. Briefly, after 3 weeks of abstinence, rats were presented with two distinct cues as conditioned stimuli (CS+; one predicting a sugar pellet and one predicting a food pellet) and two cues that did not predict a reward (CS-); 10 trials each. After 10 sessions, rats underwent a devaluation procedure to induce a conditioned taste aversion to the sugar pellets. Rats were then tested on the same pavlovian task (under extinction) to evaluate their ability to avoid CS+ associated with the devalued outcome. On the last day of conditioning, control and cocaine-exposed rats spent significantly more time in the food cup during both CS+ compared to the CS- (day X cue interaction: controls, $F_{(3,15)}=11.4$, cocaine $F_{(3,12)}=11.3$, $p<0.05$), showing successful discrimination of rewarded vs unrewarded cues. Post-devaluation, controls successfully avoided the CS+ associated with the devalued reward; however, cocaine rats continued responding to both CS+ equally suggesting impaired behavioral flexibility (determined by a Devaluation Index, DI, DI=0.31 for controls vs. 0.009 for cocaine; t=2.4, *p <0.05). Recordings of PrL neurons on the last day of pavlovian conditioning revealed distinct populations of cells that were excited or inhibited during cues (classified as “phasic”). The percentages of phasic PrL neurons were similar in cocaine-exposed and controls; however phasic PrL neurons in the controls were predominately excited (15/22) whereas phasic Prl neurons in cocaine rats were predominately inhibited (9/15). We have previously shown phasic activity in the nucleus accumbens core (NAc), a predictor of flexible behavior (West and Carelli, 2016) and a primary target of PrL, is abolished in cocaine-exposed rats during pavlovian conditioning (Saddoris and Carelli 2014). We hypothesize the shift to an inhibition of PrL neurons following cocaine drives the loss of phasic activity in the NAc, leading to an inflexible phenotype. In support, pilot studies that inhibited the PrL to NAc pathway during cues using optogenetics during pavlovian conditioning resulted in impaired behavioral flexibility (control, n=3, DI=0.32; halo, n=4, DI=0.10), similar to that observed following cocaine.

**Disclosures: E.A. West:** None. **M. Niedringhaus:** None. **H.K. Ortega:** None. **R.M. Haake:** None. **R.M. Carelli:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.02/PP19

**Topic:** G.02. Motivation

**Support:** NIH Grant DA014339 (RMC)

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NIH Grant DA007244 (RMH)

**Title:** Effects of abstinence from cocaine self-administration on basal cell firing dynamics in prelimbic cortex and nucleus accumbens core

**Authors: *R. M. HAAKE**, M. NIEDRINGHAUS, E. A. WEST, R. M. CARELLI
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**Abstract:** Withdrawal and prolonged abstinence from cocaine is associated with numerous neuroadaptations within corticostriatal circuitry that mediate incubation of cocaine craving (Wolf, Nat Rev Neurosci, 2016). We have previously reported that 30 days of abstinence from cocaine self-administration is associated with robust increases in the number and strength of neurons that are responsive to cocaine-associated cues in the nucleus accumbens (NAc) core and prelimbic cortex (PrL), as well as an increase in drug seeking (Hollander and Carelli, J Neurosci, 2007; West et al., Eur J Neurosci, 2014). It is not clear, however, how basal (i.e., spontaneous, not task-related) activity in PrL and NAc core neurons changes as a function of cocaine abstinence. This is of crucial importance given the well-documented decrease in prefrontal cortical function (i.e., hypofrontality) in human cocaine addicts. In preliminary studies, adult male Sprague Dawley rats were trained to self-administer cocaine (n=3; 0.33 mg/inf, 2 h per session) or received yoked saline (n=2) during 14 daily sessions, before undergoing 30 days of experimenter-imposed abstinence (i.e., home cage, no drug). Using in vivo electrophysiological methods, we simultaneously recorded basal cell firing in the PrL and NAc core during 30 min sessions on the first (day 1) and final (day 29) days of abstinence. Our preliminary analysis revealed that abstinence from cocaine self-administration, but not yoked saline, was associated with a decrease in basal cell firing in PrL (mean firing rate: day 1, 5.5 ± 1.3 Hz; day 29, 3.3 ± 0.5 Hz), but no changes were observed in the NAc core (day 1 mean firing rate, 2.3 ± 0.6 Hz; day 29, 3.0 ± 1.0 Hz). Next, on day 30, cell firing was recorded during a test session consisting of three phases: 1) cocaine-associated cue probes, 2) extinction (i.e., lever press for cues only, no drug), and 3) cocaine self-administration. Preliminary data show that rats exhibited an abstinence-induced increase in cocaine seeking, i.e., ~5-fold increase in lever pressing under extinction on test day compared to last day of self-administration (day 14 mean lever presses = 23 ± 4.4; test day mean extinction presses = 107 ± 24.5), consistent with our previous work (Cameron et al., Neuropharmacology, 2016). Ongoing analyses are examining if abstinence-induced decreases in basal PrL cell firing are correlated with cell firing dynamics to cocaine-associated cues and during resumption of cocaine self-administration following abstinence, as well as increased cocaine seeking under extinction. Finally, future analyses will also examine whether abstinence leads to changes in PrL to NAc core connectivity.

**Disclosures:** **R.M. Haake:** None. **M. Niedringhaus:** None. **E.A. West:** None. **R.M. Carelli:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.03/PP20

**Topic:** G.02. Motivation

**Support:** NIH Grant 5R01DA014339-14 to RMC

**Title:** A distinct subpopulation of neurons in the anterior insula tracks cocaine-induced devaluation of natural rewards

**Authors: *T. M. MOSCHAK**, 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 reinforcement, 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 0.15% saccharin (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 previously showed that nucleus accumbens neural activity tracks this shift, but the involvement of other brain regions 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 = 13) received infusions of saccharin paired with delayed cocaine access as mentioned above. On Day 1, 7, and 14 of the task, neural activity in the AI was recorded and orofacial behavior during saccharin delivery was measured and quantified as appetitive or aversive. Replicating previous work in our lab, our preliminary findings show that rats primarily exhibited appetitive behavior on Day 1 and aversive behavior on Days 7 and 14. Neural activity in the AI tracked this shift, but only in neurons that fired within the first 2 s of saccharin delivery. On Day 1, the majority of these “early-firing” neurons exhibited an excitatory response following saccharin infusion (26 of 37 neurons excitatory, 70%). However, by days 7 and 14, this shifted to a predominantly inhibitory response (day 7: 15/37 excitatory, 41%; day 14: 10/25 excitatory, 40%). Furthermore, infusion of an aversive tastant (quinine) resulted in a similar neuronal profile as Day 1 saccharin in “early-firing” neurons (27/36 excitatory, 75%), suggesting that these neurons may be tracking the devaluation of taste rather than aversive taste

per se. In total, 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. **X. Wang:** None. **R.M. Carelli:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

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**Program#/Poster#:** 607.04/PP21

**Topic:** G.02. Motivation

**Support:** DA034021

DA042721

**Title:** Dynamics of prelimbic cortical neuron activity during delay discounting behavior

**Authors: *D. A. SACKETT**, R. M. CARELLI
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**Abstract:** Delay discounting is a form of decision making in which individuals discount the value of a reward based on the amount of delay to its receipt. As the delay to reward increases, the subjective value of the reward decreases. As such, delay discounting is a measure of impulsivity, since individuals will eventually “lose patience” and choose an immediate small reward over a larger, delayed reward. Delay discounting recruits many brain regions, including the dorsolateral prefrontal cortex in humans, which is the correlate of the rodent prelimbic cortex (PrL). While the PrL has been implicated in impulsivity, its specific contributions to delay discounting are unknown. Here, we utilized multineuron recording methods in male, Long-Evans rats (n=9) to characterize the firing dynamics of PrL neurons during discrete cues and lever press events in a delay discounting task that consisted of three trial types. On forced-choice delay trials, a cue light predicted the opportunity to press for a large reward (three sucrose pellets) delivered after a period of delay. During forced-choice immediate trials, another cue light predicted the opportunity to press for a small (one sucrose pellet) immediate reward. On Free Choice trials, rats were presented with both cue lights and were rewarded based on the contingency of the lever chosen. The task consisted of 20 forced choice and 10 free choice trials, each within three trial blocks: “no-delay” (large reward presented immediately), “short-delay” (10 s delay to large reward), and “long-delay” (20 s delay to large reward). Rats’ initial preference for the large reward decreased as delays for that outcome increased across blocks, reflecting classic discounting behavior. Preliminary electrophysiological findings indicate that the overall percentage of cue-evoked phasic vs. nonphasic cells (n = 178 cells, 77% nonphasic,

23% phasic) did not change across blocks. However, unique, excitatory PrL cell populations responded to discrete forced choice cues across each block, indicating a shift in cell firing dynamics across delays. Further, during the long-delay block, a greater percentage of phasic neurons tracked *both* forced-choice cues (40 cells, 52%) compared to the no-delay block (32 cells, 41%). Ongoing studies will confirm a role of the PrL during the delay for reward and reward receipt. Though preliminary, these data suggest that as the delay to reward increases, discrete neuronal populations in the PrL may track the predictive cue's discounted value. This cellular activity may serve to inform and update downstream structures, such as the nucleus accumbens, about the predicted value of rewards during delay discounting.

**Disclosures: D.A. Sackett:** None. **R.M. Carelli:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.05/PP22

**Topic:** G.02. Motivation

**Support:** DA014339

**Title:** Processing of hedonic valence by the infralimbic cortex

**Authors: *S. W. HURLEY**, R. M. CARELLI
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**Abstract:** Processing the hedonic valence of stimuli is integral for the performance of adaptive behavior. Many psychiatric disorders, including drug addiction, are associated with the emergence of negative affect and maladaptive hedonic processing (*e.g.*, anhedonia). The infralimbic cortex (IL) sends a robust glutamatergic projection to the nucleus accumbens shell (NAcSh) and both the IL and NAcSh have been implicated in the control of hedonic processing. Here, we began probing the role of the IL in hedonics using electrophysiological (multineuron) recording methods in behaving rats. Specifically, we recorded neuron activity and objective hedonic taste reactivity (TR) responses over the course of conditioned taste aversion (CTA) learning and its extinction. Naïve rats (n=4) that received intraoral (IO) infusions of sucrose predominantly exhibited appetitive TR (appetitive: 70.75 ±6.5; aversive: 0), but after a pairing with lithium chloride, rats displayed primarily aversive TR to the sweet (appetitive: 0.25 ±0.25; aversive: 65.25 ±19.61). During extinction (5 days of IO sucrose without a LiCl pairing), appetitive TR reemerged and aversive TR ceased (appetitive: 92.5 ±24.73; aversive: 0.75 ±0.48). Preliminary electrophysiology data suggests that IL neurons track the hedonic value of sucrose. Specifically, IL neurons exhibited 'phasic' responses (inhibitions or excitations in firing rate) in naïve rats (10 of 16 cells; 62.5% phasic), but the percentage of phasic neurons decreased during

CTA (3 of 18 cells; 16.6%), and then phasic activity reemerged during extinction (4 of 13 cells; 30.8%). Further, the excitatory and inhibitory response profile of phasic IL neurons changed over the course of CTA learning and its extinction. In the naïve state there was a mixed excitatory and inhibitory neural profile (58.33% excitatory, 41.66% inhibitory), while during CTA 100% of phasic neurons were excitatory. A mixed inhibitory and excitatory response profile reemerged during extinction (75% excitatory, 25% inhibitory). These preliminary data indicate that a subset of IL neurons track the rewarding value of sucrose in the naïve and extinction state, but not when it is devalued during CTA. Future experiments will employ optogenetic tools to determine whether the projection from the IL to the NAcSh plays a causal role in the regulation of hedonic processing in this task.

**Disclosures: S.W. Hurley:** None. **R.M. Carelli:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.06/PP23

**Topic:** G.02. Motivation

**Support:** Kakenhi 17H01758

**Title:** White matter integrity is associated with individual difference in locus of control

**Authors: *S. IWAKI**[1,2], Z. DUO[1,2], T. KUMADA[3]
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**Abstract:** The subjective sense to which extent people feel they have control on the outcome of events in their daily life is often referred to as Locus of Control (LoC). The LoC is considered to be an important aspect of personality especially in academic or health-related behavior (Rotter 1966). Persons with high internal LoC have belief that events that occur in their life are caused by their own action. Conversely, low internal LoC (i.e., high external LoC) corresponds to the individual tendency to associate the cause of events to external or uncontrollable factors. In this study, we test the hypothesis that the individual differences in LoC is correlated with the differences in brain structure, specifically, the white matter integrity in young adults. Fifteen male subjects (23.4 +- 2.6 years of age) participated in the study. Written informed consent was obtained from all subjects before the experiment complying with the policies of the internal review board of AIST. Subjects' locus of control was assessed by Japanese version of Locus of Control Questionnaire (Kamahara et al., 1982), which consists of 18 items with 4-point scales. The MRI data were acquired on a 3-T MR scanner (Philips Ingenia 3T). Fractional anisotropy (FA) maps were derived from diffusion tensor imaging (DTI) scans (TR=18,496 ms, TE=60 ms,

FA=90 deg, b-factor=1,000, 70 axial slices with 2 mm slice thickness) and used as a measure of white matter fibre integrity. Results of the correlation analysis between the internal LoC score and the FA maps show significant positive correlation in the bilateral posterior cingulate and the precuneus. The cortical areas adjacent to these regions are reported to be responsible for self-consciousness, self-related mental representation (Cavanna et al., 2006) and have dense connection to hippocampus where significant correlation between its volume and LoC have been observed (Pruessner 2005). These regions are also known as central nodes of the human default mode network which regulates internally-directed thought (Buckner et al., 2008). The current results indicate that the white matter integrity in the posterior cingulate and the precuneus might affect default mode network which explains individual difference in LoC.

**Disclosures: S. Iwaki:** None. **Z. Duo:** None. **T. Kumada:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

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**Program#/Poster#:** 607.07/PP24

**Topic:** G.02. Motivation

**Title:** Lateral prefrontal neuronal activity during a 2 interval forced choice task

**Authors: *R. FALCONE**[1], D. WEINTRAUB[3], T. SETOGAWA[2], B. RICHMOND[2]
[1]NIMH, Bethesda, MD; [2]NIMH, BETHESDA, MD; [3]Functional Neurosurgery, North Shore Univ. Hosp., NEW YORK, NY

**Abstract:** Among its functions, lateral prefrontal cortex is involved in assessing rewards. It projects to the striatum that in turn, together with midbrain areas, projects back to the prefrontal cortex via medial dorsal nucleus of the thalamus. Monkeys with lesions in lateral prefrontal cortex (lPFC) lose the ability to integrate the reward value information across multiple domains. We recorded neuronal responses from lateral prefrontal cortex (probably 9/46v based on MRI) of two monkeys while they performed a task in which 9 combinations of reward were offered by mixing 3 sizes (2, 4 or 6 drops of water) and 3 delays (1, 5 or 10s). A visual cue predicting the combination being offered was presented throughout the trial. The monkeys were required to respond in one of two periods represented by the appearance of a yellow or a purple dot. On the appearance of yellow dot the monkeys could refuse the offer by releasing a bar immediately or accept by releasing when a purple dot appeared. If a purple dot appeared, the monkeys could accept by releasing immediately or refuse by waiting for the yellow dot. In the period immediately following the onset of the visual cue, before the dot appeared, i.e., before the monkey could plan when to make the motor response, we found that more than 50% of recorded neurons were modulated by the factors reward size and/or delay (factors in 2-way ANOVA). We asked whether this subpopulation of neurons modulated its activity according to the value that

the animal assigned to each offer (approximately subjective value) more than by strict measure of reward size and delay per se. For each monkey we estimated the subjective value of each offer from the observed behavioral data using the hyperbolic and the exponential discounting models. The estimated subjective value was used to correlate with the mean firing rate for each offer. We found that about one third of the neurons modulated their activity (either increasing or reducing linearly their firing rate) according to the subjective value. We think that the presence of neural representation of the reward value is important for making the desired choice action, such as accept or refuse. For this reason, we are trying to understand how the lPFC neurons convert the information of reward value in the non-motor planning of abstract choices.

**Disclosures:** **R. Falcone:** None. **D. Weintraub:** None. **T. Setogawa:** None. **B. Richmond:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.08/PP25

**Topic:** G.02. Motivation

**Support:** NYS DOH SCIRB

**Title:** Varying levels of reward, punishment, and motivation in the primary sensory and motor cortices of non-human primates

**Authors:** ***J. P. HESSBURG**[1], A. TARIGOPPULA[1], D. B. MCNIEL[1], J. T. FRANCIS[2]
[1]Physiol. and Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Univ. of Houston, Houston, TX

**Abstract:** Signals of reward, punishment, and motivation have been recorded in a number of areas in the brain, including a reward signal in the primary motor cortex (M1). This work is continuing this investigation in the hand and arm regions of M1 and the primary somatosensory cortex (S1), as well as the dorsal premotor cortex (PMd). Two non-human primates (NHP) were trained to complete a gripping task on a virtual robotic arm, where the animal gripped and held a given level of force for a specified period of time. Prior to each trial, visual cues were displayed to inform the NHP if the trial would result in a juice reward if completed successfully, a punishment consisting of a five-second timeout if completed unsuccessfully, or no reward or punishment, where the task would move immediately to the next trial. Subsets of trials with no cues and with catch trials, where a cue was presented but no reward or punishment delivered, were included to investigate reward and punishment prediction and error. Multiple levels of reward and punishment were incorporated to investigate how the value of reward and punishment were represented in these regions, and how the interplay between the two was

represented as motivation. Neural data were recorded from the hand and arm regions of M1, S1, and PMd, and spike sorted to isolate individual units. We hypothesize that in addition to reward and punishment, motivation is represented by subpopulations of neurons in M1 and S1. Investigating the intricacies of these signals in M1 and S1 will allow future brain-machine interfaces (BMI) to capture the breadth of these signals in one or two brain regions that also contain sensorimotor information, rather than requiring multiple implants in multiple regions. These data will be useful in creating algorithms for more robust and nuanced BMI control, taking greater advantage of the range of information available in these regions for better neural control of robotic prostheses.

**Disclosures: J.P. Hessburg:** None. **A. Tarigoppula:** None. **D.B. McNiel:** None. **J.T. Francis:** None.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.09/PP26

**Topic:** G.02. Motivation

**Support:** BlackThorn Therapeutics

**Title:** The effects of the NOP-receptor antagonist BTRX-246040 on the sensitivity to losses during decision-making and on spatial working memory performance in non-human primates

**Authors: *H. SEO**[1], K. S. ABEDRABBO[2], M. N. BOUCHER[2], T. L. WALLACE[3], W. J. MARTIN[3], A. F. T. ARNSTEN[2], D. LEE[2]
[1]Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [2]Neurosci., Yale Sch. of Med., New Haven, CT; [3]BlackThorn Therapeut., South San Francisco, CA

**Abstract:** Nociceptin/orphanin FQ and its receptor (NOP-R) are widely distributed in cortical and subcortical brain regions associated with reward processes, anxiety, food intake and pain. Evidence from clinical and preclinical studies has suggested an antidepressant-like effect of the selective NOP-R antagonist BTRX-246040 (aka LY2940094; Post et al., 2016). However, the effect of antagonizing the NOP-R system on higher cortical functions relevant to depression has yet to be characterized in detail. In the present study, we evaluated the effects of BTRX-246040 in monkeys using a task that required representations of gains and losses, and compared it with those in a visuospatial working memory task that depends on neural representations by the dorsolateral prefrontal cortex. We trained rhesus monkeys (Macaca mulatta, n=2) in the token-based binary choice task where appetitive and aversive consequences of choice (e.g. gains and losses) were quantitatively manipulated in the identical stimulus modality, and tested the effect of BTRX-246040 on the animal's choice behavior. During the task, animals played a biased-

matching pennies game against a computerized opponent (Seo and Lee, 2009). Animals gained a token only when they matched the opponent's choice, and the number of tokens remained unchanged or decreased otherwise. A juice reward was delivered when the animal accumulated 6 tokens. The tokens owned by the animal at the beginning of a trial were displayed as small disks in a circular array at the center of the computer monitor. Empty circles served as placeholders indicating the number of tokens needed for the reward. Behavioral analysis using a logistic regression model showed that animals tended to repeat/switch their choice when it was followed by gain/loss of tokens, respectively, demonstrating the reinforcing and punishing effect of gains and losses in the task. Once animals were trained, they were tested approximately 1-hour after BTRX-246040 (0.001-1.5mg/kg, intramuscular) or saline (vehicle) administration. Compared to control sessions, animals switched their choice subsequent to losses significantly less frequently when they received BTRX-246040 (1.0 mg/kg). Animal's choice behavior following gains was not affected, suggesting that BTRX-246040 might selectively reduce the punishing effect of losses. In contrast, a lower dose of BTRX-246040 (0.01 mg/kg; n=8) significantly improved animals' performance in the spatial working memory test. These results suggest that NOP-R system might mediate adaptive behavioral choice subsequent to losses, via a pathway distinct from the one that mediates spatial working memory.

**Disclosures:** **H. Seo:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. **K.S. AbedRabbo:** None. **M.N. Boucher:** None. **T.L. Wallace:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **W.J. Martin:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BlackThorn Therapeutics. **A.F.T. Arnsten:** None. **D. Lee:** A. Employment/Salary (full or part-time):; BlackThorn Therapeutics. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; BlackThorn Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BlackThorn Therapeutics.

**Poster**

**607. Motivation: Cortical Neurocircuitry**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 607.10/PP27

**Topic:** G.02. Motivation

**Title:** rTMS modulation of prefrontal cortex affects salience coding of food cues

**Authors: *T. KAMMER**[1], M. ULRICH[2], S. LORENZ[2], L. STEIGLEDER[2], M. W. SPITZER[2], G. GRÖN[2]
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**Abstract:** Curbing the potential of superficially seductive environmental cues motivating behavioral tendencies with increased risk of long-term negative consequences has attracted great interest in addiction neuroscience. We investigated the effects of two transcranial magnetic stimulation (TMS) protocols on blood oxygen level-dependent (BOLD) signals while processing high-caloric (HC) and low caloric (LC) food images. Location of TMS application was over the right mid-ventrolateral prefrontal cortex (mid-VLPFC) which had been predetermined by seed-based resting-state fMRI with seed voxels located in the ventral tegmental area (VTA) which were differentially more active for HC than LC food stimuli. In a first sample of 15 healthy male participants, BOLD signals for both food categories were comparably modulated by continuous (cTBS) and intermittent theta-burst (iTBS) TMS in bilateral orbitofrontal cortex, mid-VLPFC, right amygdala, and the VTA. Effects of TMS protocols differed quantitatively in right basolateral amygdala, left dorsal caudate nucleus and left anterior insula. Compared to sham stimulation, iTBS, but not cTBS also affected subjective ratings of wanting HC food items in a second, separate sample of 24 participants. Results are discussed from the perspective of putative regional interactions for which neurostimulation of right mid-VLPFC is the entry point of downstream signal changes for HC and LC food cues, indicating a shift in valuation stimuli of initially different incentive salience.

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.01/PP28

**Topic:** G.03. Emotion

**Title:** Hemisferic asymetry link between attachment styles

**Authors: *E. VARLIK ÖZSOY**[1], E. GÜLBETEKIN[2]
[1]Psychology Dept., [2]Dept. of Psychology, Akdeniz Univ., Antalya, Turkey

**Abstract:** Attachment is a very common issue that is suggested healthy childhood development on the basis of the phenomenon of attachment (Bowlby, 1979). It refers to the relationship between the child and caregiver. These early interactions with mother directly shape the architecture of the growing brain (Siegel, 2012). The two sides of the human brain are different in their processing abilities and preferences. According to approach-withdrawal model,

happiness, surprise, and anger are referred to as approach emotions and sadness, disgust, and fear are referred as withdrawal emotions. Davidson et al. (2000) proposed that there is cerebral asymmetry in the neural representation of approach and withdrawal emotions. Accordingly, approach emotions are related to left hemisphere activity while, withdrawal emotions are related to right hemisphere activity. For instance Davidson et al. (2000) found that infants with more dominant right-hemisphere activity at rest were more likely to cry and fuss when separated from their mothers in comparison to infants with more dominant left hemisphere. The main aim of this study is to find out if there is a link between hemispheric asymmetry and attachment styles of adults. 26 male and 38 female undergraduate students (totally 64) who had no psychological or neurological disorders participated the study.We presented 32 photos -which were selected according to a pilot study- showing positive and negative relationships with parents, partners and self in three viewing conditions: right visual field/left hemisphere (RVF/LH), left visual field/right hemisphere (LVF/RH) and inter-hemispheric/center. The participants were asked to rate the stimuli according to feeling of closeness. It was aimed to investigate whether the ratings and the reaction time of the participants change in respect to the attachment styles of the participants and viewing conditions. Participants also filled Attachment-Based Mental Representation Scale, Experiences in Close Relationships R, The Brief Symptom Inventory, Hand Use Test with an information form. In order to determine the effects of sex and visual half field/hemisphere (VHF/H) on subjects' responses, 2 x 3 (sex × VHF/H [RVF/LH, LVF/RH, center]) repeated measures ANOVA was conducted. We found a significant main effect of VHF/H, $F(1, 186) = 5{,}60$, $p = .001$, $\eta p^2 = .08$ and a significant interaction effect of sex and VHF/H $F(3, 186) = 3{,}28$, $p = .02$, $\eta p^2 = .05$. Following analysis for the interaction effect indicated that in LVF/RH condition men responded most negatively to negative mothers and women responded most negatively to negative fathers. We did not observe the same effect in RVF/LH and center conditions.

**Disclosures: E. Varlik Özsoy:** None. **E. Gülbetekin:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.02/PP29

**Topic:** G.03. Emotion

**Title:** Moral cognition and reasoning in relation with prosocial and aggressive behaviors for elementary students

**Authors: *L. UCHIYAMA**
Houai Res. Inst., Warabi-Shi, Japan

**Abstract:** In the recent education of elementary school, it has been important to grow a rich spirit in ruling oneself, and cooperation with others, but no clear solution has been made to aggressive behaviors amid growing concerns yet. This research shows which actions school kids choose, prosocial behaviors or offensive actions in cooperative playing scene like doing puzzle. Several studies have shown that prosocial moral reasoning is related positively to prosocial behaviors (Eisenberg, et al,1995; Eisenberg, et al, 2001, Calro, et al, 2010) but is negatively related to aggressive behaviors (Laible, et al, 2008; Calro, et al, 2010). Despite the previous evidences on the relationships between prosocial moral reasoning, prosocial behaviors, and aggressive behaviors, few studies have disclosed these links with real performance by kids due to choice questionnaires in the previous studies. In this research, normal elementary school kids, whose number included 8 people with an average of years in the present time, did puzzle over 2 trials with both disneys under the two way videos, pairing four children in a group. About seven days later, they had to answer several questions, which said what they did, whether they thought they helped and offended with their friends, and why they thought so, watching their videos. It was reported that means and standard deviations for each behavior variable were included in Table. Mean differences between prosocial behavior(physical help, linguistic help, and cooperation) and aggressive behavior(aggression and taking away) were shown. The values of prosocial behaviors were higher than those of aggressive behaviors. Also, mean differences for each behavior variable were examined using ANOVAS. There was a significant difference in five behavior variables ($F(4,35)=5.06$). For the third analysis, mean differences across behavior variables were examined using multiple comparison with ryan method. The values of linguistic help were higher than those of taking away and those of physical help. Concerning questionnaires for the cooperative reasons over their behaviors, what students said was that they wanted to complete their puzzle as quickly as possible, and their friends searched for the pieces and don’t know where to place the pieces. On the other hands, they regard the aggressive reason as their friends’ disturbances. If this study collects more data by the academic conference, it analyses correlations among variables at the scoring point with questionnaires and frequency of their behaviors during the puzzle, which will lead the research result.

**Disclosures: L. Uchiyama:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.03/QQ1

**Topic:** G.03. Emotion

**Support:** NSFC 31530032

**Title:** Jealousy is positively associated with fronto-striatal responses to angry faces

**Authors: *X. ZHENG**, B. BECKER, L. LUO, J. LI, K. KENDRICK
Key Lab. for NeuroInformation of Ministry of Educ., Univ. of Electronic Sci. and Technol., Sichuan, China

**Abstract:** Jealousy is a complex social emotion in close relationships and has been related to self-esteem and self-identification. Extreme jealousy in the pathological range (e.g. delusional jealousy) can promote aggression in terms of domestic violence, self-mutilation and even murder. Accumulating evidence has associated pathological jealousy with frontostriatal dopaminergic circuits (Marazziti et al., 2013). However, currently little is known about the underlying neuro-functional basis of non-pathological jealousy.

To identify the neuro-functional systems associated with trait jealousy, 85 healthy subjects (43 males, 18-27 years) underwent fMRI during an emotional face recognition paradigm. A total of 150 faces (happy, angry, fearful, sad and neutral, 30 faces each) were presented to subjects for 1500ms and after each one they were required to identify the emotion expression and rate its emotional intensity. Before the fMRI experiment, trait jealousy was assessed using the Multidimensional Jealousy Scale (MJS). To control for potential influences of trait aggression, all subjects were additionally administered the Buss-Perry aggression Questionnaire (AQ). Results of a correlation analysis showed that intensity ratings given by subjects for angry faces were positively associated with their MJS score ($p=0.043$, $r=0.220$). The fMRI results from a whole brain analysis demonstrated that higher trait jealousy was associated with increased activity in right hippocampus, bilateral inferior frontal gyrus, and the left dorsal striatum (putamen and caudate) ($p<0.05$, FWE-corrected) in response to angry emotional faces relative to neutral faces, controlling for participant gender and trait aggression as covariates. Further Psychophysiological Interaction analysis revealed an association between higher trait jealousy and increased functional connectivity between the right inferior frontal gyrus and left caudate ($p=0.034$, FWE-corrected) for angry versus neutral faces, again controlling for gender and trait aggression.

The present study provides the first evidence for an association between trait jealousy and the dorsal striatal-inferior frontal circuit specifically during processing of angry expression faces. The dorsal striatum and inferior frontal cortex share dense dopaminergic projections, suggesting a potential interaction between these dopaminergic pathways and trait jealousy during social-threat situations.

Reference

Marazziti, D et al. (2013). CNS Spectrums, 18(01), 6-14.

**Disclosures: X. Zheng:** None. **B. Becker:** None. **L. Luo:** None. **J. Li:** None. **K. Kendrick:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.04/QQ2

**Topic:** G.03. Emotion

**Support:** Mind and Life Francisco J. Varela Award

**Title:** Electroencephalographic evidence that mindfulness training dampens emotion sharing but increases approach orientation toward racial outgroup members' distress

**Authors: *D. R. BERRY**, D. BUSTAMANTE, K. W. BROWN
Dept. of Psychology, Virginia Commonwealth Univ., Richmond, VA

**Abstract:** Perceiving differences between oneself and others, which are often defined by racial and other social group divisions, can attenuate emotion sharing, a putative automatic response to others' distress (Gutsell & Inzlicht, 2012). Recent theory and research indicates that mindfulness training may decrease this perceived psychological distance between oneself and others, and can increase empathy and kindness toward outgroup members (Berry & Brown, 2017). This study asked whether brief mindfulness training would increase emotion sharing in interracial interactions. White female students ($N$=79) were shown video clips depicting racial ingroup and outgroup members expressing sadness. During these tasks 64-channel EEG was recorded, and self-report measures of empathic concern and empathic sadness felt for the sad individuals were taken after each video. Participants were then randomized to either a 4-day mindfulness training (MT), or sham mindfulness training (ST). The video viewing task, and EEG and self-report measures were completed again post-training. Homologous frontal EEG channel pairs (F1/2, F3/4, F5/6, F7/8) were created by subtracting log right alpha power from log left alpha power, and we expected that greater emotion sharing would be reflected in right frontal alpha suppression (cf., Gutsell & Inzlicht, 2012). Participants showed less post-training emotion sharing for racial outgroup members, relative to racial ingroup members ($p < 0.05$). MT, however, decreased emotion sharing toward both ingroup and outgroup members, indicated by greater alpha asymmetry scores on medial frontal channel pairs ($p < 0.05$). Yet MT increased self-reported empathic concern during interracial video observation ($p < 0.05$). To disambiguate approach and withdraw orientation in the alpha asymmetry measure, channel homologues were unpaired and analyzed separately. Relative to ST, MT decreased left frontal alpha asymmetry scores ($p < 0.01$), but did not change right frontal alpha scores. Importantly, decreases in post-training left frontal alpha scores predicted greater empathic concern ($p < 0.01$). These results indicate that mindfulness may increase empathy in interracial interactions not by increasing emotion sharing, but by increasing approach orientation to others' sadness.

**Disclosures: D.R. Berry:** None. **D. Bustamante:** None. **K.W. Brown:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.05/QQ3

**Topic:** G.03. Emotion

**Support:** NSERC

**Title:** Neuroanatomical correlates of personal space preferences: A voxel-based morphometry study

**Authors: *J. VIEIRA**, T. P. TAVARES, D. G. V. MITCHELL
Univ. of Western Ontario, London, ON, Canada

**Abstract:** Keeping and appropriate distance from the surrounding world is a defensive mechanism that helps to prevent injuries (Graziano & Cooke, 2006) and ensures the preservation of personal space boundaries (Hayduk, 1983). Research has shown that atypical personal space preferences are characteristic of several psychiatric and personality disorders (e.g. autism, schizophrenia, psychopathy); yet, the neuroanatomical bases of personal space regulation remain poorly understood. In this study, we used voxel-based morphometry (VBM) to investigate the structural brain correlates of personal space preferences. Twenty-three healthy volunteers underwent Magnetic Resonance Imaging (MRI) scanning and high-resolution T1-weighted structural scans were acquired. Outside the scanner, participants performed a Stop-distance task, wherein they adjusted the distance between themselves and an experimenter walking towards them, across a series of trials. Preprocessing and analyses of anatomical data for VBM were performed using Statistical Parametric Mapping software (SPM12) and included visual inspection and realignment, segmentation, bias correction and spatial normalization (Ashburner and Friston, 2005). Images were registered using DARTEL to increase the accuracy of inter-subject alignment (Ashburner, 2015), smoothed using a 10 mm Gaussian FWHM, and normalized to Montreal Neurological Institute (MNI) space. To investigate the association between personal space preferences and regional grey matter volume, we performed voxel-wise regression analysis using average preferred distance in the Stop-Distance task as predictor and controlling for total intracranial volume. Results showed grey matter volume in the right premotor cortex (BA 6; xyz=57, 0, 29; 1513 voxels) was positively correlated with preferred distance (FWE-corrected $p < .05$). These results are consistent with neurophysiological data from non-human primates (Cléry et al., 2015) and functional MRI data from humans (e.g., Ferri et al. 2015) highlighting the role of the premotor cortex in responding to looming objects that enter one's personal space. Our results suggest the premotor cortex is also implicated in responding to personal space intrusions by social stimuli, and provide a structural correlate of personal space preferences in humans.

**Disclosures:** **J. Vieira:** None. **T.P. Tavares:** None. **D.G.V. Mitchell:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.06/QQ4

**Topic:** G.03. Emotion

**Support:** Swedish Research Council FYF-2013-687

**Title:** BOLD correlates of endogenous oxytocin release during affective touch

**Authors: *I. MORRISON**[1,2], H. LINDHOLM[3], G. NOVEMBRE[2], L. HANDLIN[2,3]
[1]Linköping Univ., Linkoeping, Sweden; [2]Ctr. for Social and Affective Neurosci., Linkoeping Univ., Linkoeping, Sweden; [3]Sch. of Hlth. and Educ., Univ. of Skoevde, Skoevde, Sweden

**Abstract:** Touch in social relationships can have a strong affective and hedonic dimension, which may play a role in the formation and maintenance of social bonds. A candidate mediator of such a role is the neuropeptide oxytocin. Oxytocin has been implicated in numerous aspects of social interaction and bonding, and has been particularly associated with tactile stimulation. However, little is known about the contribution of endogenously-released oxytocin during social touch interactions in humans, and what cortical regions may be implicated in the context-dependent modulation of oxytocin release. To address this, we collected serial samples of plasma oxytocin while female participants were caressed on the arm and the palm, by either their romantic partner or by a stranger they had never met before. Peak oxytocin levels were higher for partner than for stranger touch, demonstrating that touch by familiar vs unfamiliar others has differential effects on oxytocin release. A larger group (n=36) underwent this paradigm in the fMRI scanner. To investigate any effects on stress- and autonomic-related hormones, plasma cortisol and catecholamine levels were serially sampled alongside oxytocin. Blood samples were collected during the scanning session via an indwelling, magnet-safe catheter. A total of nine samples per participant was gathered: baseline (1), during partner touch (3), intermediate (1), during stranger touch (3), and final. The order of partner/stranger touch was counterbalanced across participants. Partner touch was rated significantly more pleasant and relaxing than stranger touch. Consistent with previous findings, touch to the arm compared to palm elicited activation in operculoinsular and superior temporal cortex regardless of whether the participant was touched by partner or stranger. Specific effects of oxytocin and the other sampled hormones were investigated in these regions of interest, as well as in the whole brain. These findings elucidate the relationships between brain networks for affective touch, endogenous oxytocin levels, and stress-related variables.

**Disclosures:** **I. Morrison:** None. **H. Lindholm:** None. **G. Novembre:** None. **L. Handlin:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.07/QQ5

**Topic:** G.03. Emotion

**Title:** Flow: The application of psychophysiology to understand user experience in video games

**Authors:** ***M. M. NIEDZIELA**[1], M. ROSAZZA[2]
[1]HCD Res., Flemington, NJ; [2]Neurosci. Technol., HCD Res., Philadelphia, PA

**Abstract:** Usability research involves understanding human limits of perception, attention, and memory in ease of use and learnability of human-made objects such as websites or video games. Cognitive modeling involves creating a computational model to estimate usability based on psychological principles and experimental studies. Cognitive models can be used to improve user interfaces or predict problems, errors, and pitfalls during the design process. Flow is the mental state of operation in which a person performing an activity is fully immersed in a feeling of energized focus, full involvement, and enjoyment in the process of the activity, a theory pioneered by psychologist Csikszentmihalyi. The aim of our study was to use electrophysiology (fEMG, HRV, GSR) and eye tracking to identify flow events in physiological responses linked to in-game events. Twelve male participants (18-35 yrs) were measured with electrophysiology (fEMG, HRV, GSR), eye tracking while playing a novel video game, Darksiders 2, and given a post-game survey. Flow was calculated by amount greater than average arousal (GSR) generated and attention/motivation experienced (HRV) and then compared with performance in video game play at that time interval. On average, we found gamers entered a flow state 20.3% of the time coinciding with intense moments during the game with 24.6% of this time being a positive experience. Our study revealed that it is possible to predict the occurrence of flow during video game play. The ability to model flow using physiological measures may help game designers create better games and help researchers better understand game play.

**Disclosures:** **M.M. Niedziela:** A. Employment/Salary (full or part-time):; HCD Research. **M. Rosazza:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.08/QQ6

**Topic:** G.03. Emotion

**Support:** brain research program through NRF of Korea (NRF-2016M3C7A1905475)

MCST and KOCCA in CT research & development program in 2017

**Title:** Affective auditory stimuli using simultaneous MEG and EEG — Source localization study

**Authors: *M. KWON**[1], H. CHO[2], S. AHN[3], K. KIM[4,5], S. JUN[1]
[1]Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [2]Wadsworth Ctr., New York State Dept. of Hlth., Albany, NY; [3]Dept. of Psychiatry, UNC at Chapel Hill, Chapel Hill, NC; [4]Ctr. for Biosignals, Korea Res. Inst. of Standards and Sci., Daejeon, Korea, Republic of; [5]Dept. of Med. Physics, Univ. of Sci. and Technol., Daejeon, Korea, Republic of

**Abstract:** Listener often misinterprets speaker's intention during conversation, because the same words or sentences may deliver different meanings depending on speaker's voice tone, facial expression, and body language and so on. Different understanding of the same words comes from gender, culture, age, background. In this study, we focused different brain signal responses between male and female using magnetoencephalography (MEG) and electroencephalography (EEG) during affective auditory stimuli are given. Twenty-three native Korean speakers (11 females; age 23.8 ± 2.9) participated in this study; their brain signals were recorded while accordance or discordance affective stimuli between word and voice tone. The Korean words include five positive and five negative words; each words were spoken in positive, negative or reading tone. Subjects were given the stimuli, and they pressed one of three buttons depending on their feelings. The brain responses were measured simultaneously using MEG 152 sensors (KRISS) and EEG 21 electrodes (Biosemi), electrooculogram, and electrocardiogram with 1024 Hz sampling rate. The signals were filtered between 1 - 55 Hz, and some artifacts were removed. Boundary element method with three-spherical-shell head model was used for array-gain beamformer, one of well-known localizers. In accordance cases between words and tones yielded rather similar patterns, although female responses appeared in wider activation regions than male responses using MEG. Left posterior lobe was more activated on emotional stimuli than neutral stimuli. Right hemisphere and frontal area were activated on negative words, but right hemisphere was deactivated on positive words. In discordance cases, common patterns of male and female were more activation in wider areas on negative words with positive voice. Right hemisphere was more activated in male, while both hemispheres yielded bigger amplitude in female on negative words with positive tone. When positive words with negative tone were given, male results were similar to those of positive word and tone, however, female results were

similar to those of negative word and tone. On the other hand, EEG results showed that temporal lobes were activated regardless of conditions. From these results, it is inferred that the affective auditory stimuli may yield different brain responses depending on meaning of the words or voice tone, especially, the stimuli are more influenced to female than male, and negative stimuli are more impacted on the brain signal. In the mismatch of the emotion between words and voice tone, voice tone may be more effective to female, however, meaning of words may be more effective to male.

**Disclosures:** **M. Kwon:** None. **H. Cho:** None. **S. Ahn:** None. **K. Kim:** None. **S. Jun:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.09/QQ7

**Topic:** G.03. Emotion

**Title:** Unique electrocortical responses associated with attentional bias to fearful facial expressions and auditory distress signals

**Authors: *J. STERR**[1], S. CONGER[2], B. DIMARIA[2], J. ANDRZEJEWSKI[2], J. M. CARLSON[2]
[1]Dept. of Psychology, [2]Psychology, Northern Michigan Univ., Marquette, MI

**Abstract:** Unimodal emotionally salient visual and auditory stimuli capture attention and have been found to do so cross-modally. However, little is known about the combined influences of auditory and visual threat cues on directing spatial attention. In particular, the neural mechanisms associated with the allocation of attention to multimodal fearful facial expressions accompanied by human auditory distress signals are unknown. Based on previous research indicating that fearful faces enhance attention by increasing the amplitude of the N170 event-related potential (ERP) in the contralateral visual cortex, it was hypothesized that visual cortical responses to fearful faces would be enhanced when co-presented with auditory signals of human distress. To test this hypothesis, we used a modified multimodal dot-probe task where fearful faces were paired with two sound categories: non-distressing human vocalizations and distressing human vocalizations. At a behavioral level, fearful faces captured attention across both sound conditions. In addition, distressing sounds resulted in faster reaction times than non-distressing sounds. These behavioral effects were mirrored in the ERP data. N170 amplitudes were enhanced in the hemisphere contralateral to the visual field of the fearful face. N170 amplitudes were also bilaterally enhanced by auditory distress signals. The results provide initial evidence suggesting that emotional attention is facilitated through unique mechanisms for auditory and visual signals in multimodal audiovisual distress signals.

**Disclosures:** **J. Sterr:** None. **S. Conger:** None. **B. DiMaria:** None. **J. Andrzejewski:** None. **J.M. Carlson:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.10/QQ8

**Topic:** G.03. Emotion

**Title:** Resting state functional connectivity associated with alexithymic personality type

**Authors: *S. TUKAIEV**[1], M. CHERNYKH[1], I. ZYMA[1], Y. HAVRYLETS[3], V. RIZUN[3], M. MAKARCHUK[2]
[1]Dept. of Physiol. of Brain and Psychophysiology, [2]Dept. of Human and Animals Physiol., Natl. Taras Shevchenko Univ. of Kyiv, Inst. of Biol., Kyiv, Ukraine; [3]Dept. of Social Communication, Natl. Taras Shevchenko Univ. of Kyiv, Inst. of Journalism, Kyiv, Ukraine

**Abstract:** The alexithymia construct is characterized by impairment of emotional processing and reduced interaction between different brain areas during various experimental conditions. Yet little known about permanent alteration of functional connectivity associated with alexithymia in resting state. The aim of current study was to detect the psychological determinants of alexithymia and investigate the resting state cortical networks of alexithymic personality type. 232 volunteers, first-third year students from the Taras Shevchenko National University of Kyiv aged 18 to 24 years participated in this study. EEG was registered during the rest state (3 min). We estimated the interhemispheric and intrahemispheric average coherence across all EEG segments in all frequencies from 0.2-45 Hz. To determine the level of alexithymia we used 26-item Toronto Alexithymia Scale (TAS-26). Alexithymic personality type was found in 43 volunteers (TAS-26 total score ≥74, alexithymia group, AG). A control group consisted 113 subjects with low alexithymia (TAS-26 total score ≤62, non-alexithymia group, NAG). 85 participants formed borderline group (BG, TAS-26 total score 62> and <74). The main factors related to alexithymia were marked extraversion, high level of trait anxiety and outward euphoric activity. The emotional exhaustion and reduction of personal achievements (Maslach Burnout Inventory), the Resistance Phase (Boyko's Syndrome of Emotional Burnout Inventory) were the most pronounced within the alexithymia group. The alexithymic personality type demonstrated less developed spatial anticipation. In background EEG activity during the development of the alexithymia variations in EEG spatial synchronization were observed in low- and high-frequency EEG components. Alexithymic personality type includes breaking of interhemispheric anterior frontal-frontal (alpha1,2-subbands) and formation central-temporal links (alpha1-subband) (awareness and cognitive processing of incoming information). We demonstrated left lateralization of intrahemispheric links in alpha3 (occipital-parietal area) and beta (central area)

subbands (inner image formation, external attention). Inter and intrahemispheric coherence in low-frequency EEG components (theta2-subband) indicates the influence of alexithymia on attention focusing, working memory, and emotional processes. As such, topographical reorganization of functional connectivity under alexithymia had specific features reflecting information and emotion-activating processes.

**Disclosures: S. Tukaiev:** None. **M. Chernykh:** None. **I. Zyma:** None. **Y. Havrylets:** None. **V. Rizun:** None. **M. Makarchuk:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.11/QQ9

**Topic:** G.03. Emotion

**Support:** NIMH Intramural Research Program

**Title:** Emotion processing in Moebius syndrome

**Authors: *S. JAPEE**[1], *S. JAPEE[1], S. LOKEY[3], J. JORDAN[4], C. I. BAKER[2], L. G. UNGERLEIDER[5]
[2]Lab. Brain and Cognition, [1]NIH, Bethesda, MD; [3]Lab. of Brain and Cognition, Natl. Inst. of Mental Hlth., Bethesda, MD; [4]NIMH/NIH, Bethesda, MD; [5]Natl. Inst. of Mental Hlth., Bethesda, MD

**Abstract:** Many researchers believe that humans learn to recognize facial expressions by mimicking the expressions of others, thereby experiencing the emotion themselves. But what happens when one cannot generate or mimic facial expressions due to congenital facial palsy, such as that seen in Moebius Syndrome (MoS)? MoS, a rare congenital neurological disorder, is characterized by abnormality of the VI$^{th}$ and VII$^{th}$ cranial nerves, resulting in paralysis of the face and diminished skeletal muscle feedback. Thus, it is possible that individuals with MoS have trouble identifying or processing emotion. To investigate this question, we used a set of computer-based behavioral tasks to characterize the ability of MoS patients to detect and label emotional facial expressions.
Individuals with MoS and a group of healthy controls were shown morphs of neutral to fearful and neutral to happy faces, and were instructed to distinguish between fearful and neutral, and happy and neutral faces, with a button press. A one-up, three-down staircase procedure was used to determine each participant's threshold for 79% accuracy. The same morph stimuli and staircase procedure were used in a feature-detection control task, where participants were instructed to indicate with a button press whether the face depicted an open or closed mouth. For the emotion-labeling task, participants were shown faces depicting each of 7 emotions (happy,

sad, anger, disgust, fear, surprise, neutral) and instructed to press a button corresponding to each emotion, with “other” and “don't know” as additional response options.
Analysis of threshold levels for the emotion-detection task revealed that individuals with MoS, compared to controls, showed a deficit in detecting fearful faces, but not happy faces. Individuals with MoS performed similar to controls for labeling of angry, happy, neutral, sad and surprised faces, but worse than controls for labeling disgusted and fearful faces, often confusing the former with sadness and the latter with surprise. MoS individuals also performed similar to controls on the feature-detection control task. These results suggest that an emotion-detection deficit may be present in MoS, but further testing with additional patients is needed. Further, to test if the neurocircuitry underlying emotion processing is different in MoS compared to controls, we plan to use fMRI while subjects perform these tasks in the scanner. Results from these experiments will shed light on whether the paralysis of, and lack of feedback from, the facial muscles in MoS impairs emotion perception and its underlying neurocircuitry.

**Disclosures:** **S. Japee:** None. **S. Lokey:** None. **J. Jordan:** None. **C.I. Baker:** None. **L.G. Ungerleider:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.12/QQ10

**Topic:** G.03. Emotion

**Support:** Undergraduate Research Opportunity Program (UROP) Grant

**Title:** Autonomic and central correlates of empathic response and sub-clinical psychopathy as reflected in heart rate variability and cerebral activity

**Authors: M. J. SCHUMACHER**, R. HJELLE, *R. L. LLOYD
Dept. of Psychology, Univ. of Minnesota, Duluth, MN

**Abstract:** Finding out the cause of conduct disorder has been a growing concern for researchers, and there is now evidence to support that it may be related to lack of empathic response. Humans are social creatures, and empathy is normally classified as a social emotion, therefore those who lack the ability to be empathic have trouble associating with others. The purpose of this study is to examine elevated sympathetic tone (relative to parasympathetic output) as reflected by HRV (heart rate variability) and to examine power and hemispheric distribution of frontal and temporal lobe activity as reflected by EEG in participants viewing an empathy-inducing video clip. The hypotheses of this study are: (1) that a diminished parasympathetic contribution to cardiac tone would be negatively associated with higher scores on the Levenson Self-Report Psychopathy scale (LSRP); (2) lower frontal lobe neurological activity (higher alpha activity in

EEG) would be associated with higher scores on the LSRP; (3) less lateralization of cerebral activity to the right temporal and frontal lobes will be associated with higher scores on the LSRP. Support of these hypotheses may indicate that these procedures could be used to help predict psychopathological traits based upon empathic responses as reflected by HRV and frontal/temporal lobe activity.

**Disclosures:** **M.J. Schumacher:** 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.; Undergraduate Research Opportunity Project Grant, University of Minnesota Duluth. **R. Hjelle:** None. **R.L. Lloyd:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.13/QQ11

**Topic:** G.03. Emotion

**Support:** Defense Advanced Research Projects Agency (DARPA) under Cooperative Agreement Number W911NF-14-2-0043

**Title:** Electrical stimulation of human orbitofrontal cortex engages limbic circuits and acutely improves negative mood

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**Abstract:** Mood disorders characterized by depression and anxiety are highly prevalent causes of morbidity and mortality worldwide. In 20-30% of patients, mood symptoms do not respond to medications or cognitive therapy, underscoring the need for new treatments. Deep brain stimulation (DBS), the direct electrical stimulation of specific brain regions, is a promising therapeutic strategy for mood disorders refractory to medical therapy[1], but the efficacy of DBS hinges on identification of optimal stimulation targets and parameters. Converging lines of evidence implicate frontostriatal and limbic circuits in emotion regulation[2,3], but DBS at sites within these networks shows variable efficacy for treatment-resistant depression. Prefrontal cortical regions, including orbitofrontal cortex (OFC), are thought to regulate emotion by exerting 'top-down' influence on reward pathways[4], but it is unclear whether electrical stimulation in OFC can elicit therapeutic relief of mood symptoms. Furthermore, how stimulation at a given site modulates circuit physiology remains poorly understood.

Here, we assess the effects of direct electrical stimulation in OFC on mood in 12 human participants with epilepsy implanted with intracranial electrodes for seizure localization. We demonstrate that unilateral stimulation of OFC acutely improves mood[5] in participants who have moderate to severe anxiety or depression symptoms at baseline. We found a stimulation-amplitude dose-dependent improvement in mood. Stimulated regions of the OFC were anatomically and functionally connected to distributed brain networks implicated in emotion processing, demonstrated using probabilistic tractography based on diffusion imaging and measuring electrically evoked potentials, respectively. Finally, we demonstrate the feasibility of using neural-based control signals to develop circuit-based closed-loop stimulation paradigms. These results suggest OFC as a promising target for neuromodulatory treatment of mood disorders.

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5 Nahum, M., Van Vleet, T. M., Sohal, V. S., Mirzabekov, J. J., Rao, V. R., Wallace, D. L., Lee, M. B., Dawes, H., Stark-Inbar, A., Jordan, J. T., Biagianti, B., Merzenich, M., Chang, E. F. JMIR Mhealth Uhealth 5, 1-15, doi:10.2196/mhealth.6544 (2017).

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.14/QQ12

**Topic:** G.03. Emotion

**Support:** Center of Innovation Program from Japan Science and Technology Agency

**Title:** Evaluation of unpleasant emotions during cursor control from fMRI brain activity signals

**Authors: *Y. OGATA**, Y. KATSUI, N. YOSHIMURA, Y. KOIKE
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**Abstract:** Recently, decoding emotions from brain activity signals has been drawn attentions to develop better communication tools based on computer devices. However, most existing studies focus on emotions elicited by passively viewing of emotional images or recollection of autobiographical memory, hence possibility of evaluating or decoding emotions elicited in daily-

life situation such as using devices was remain unclear. In this study, we aimed to identify brain regions related to emotions, especially frustration in daily-life situation by functional magnetic resonance imaging (fMRI).Fourteen healthy volunteers participants were asked to perform a target-reaching task within an MRI scanner and their brain activity was measured during the task. They moved a computer cursor to a target randomly appearing on one of eight positions. The task was consisted of three conditions: normal condition (NC), normal-small condition (NSC), and rotation and acceleration condition (RAC). NC was a control task that participants could control the cursor as they usually use. In NSC and RAC the cursor was not controlled as the subject was expected. After the task, participants answered a questionnaire relating emotions they had during the task. We separated participants into 2 groups (NSC and RAC) according to their answer which condition they felt more frustration and analyzed the fMRI data set for each group separately. The analysis revealed activation in supramarginal gyrus, insular cortex and middle frontal gyrus within NSC group and in dorsolateral prefrontal cortex within RAC group ($p<.001$, uncorrected). Additionally, we found that activation in the middle frontal gyrus was significantly correlated with evaluation score of irritation ($p<.05$), and that in supramarginal gyrus and insular cortex had marginally significant correlation ($p<.10$). In addition, amygdala and anterior cingulate cortex, that are known as emotion-related area, showed correlation with irritation score in NSC group ($p<.05$). These brain areas, especially middle prefrontal cortex and dorsolateral prefrontal cortex, seemed to relate to regulation of frustration. Our results suggested that unpleasant emotion in daily-life situation such as usage of devices could be evaluated by measuring brain activity from these emotional-related areas.

**Disclosures:** **Y. Ogata:** None. **Y. Katsui:** None. **N. Yoshimura:** None. **Y. Koike:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.15/QQ13

**Topic:** G.03. Emotion

**Title:** Familiarity effects on musical appraisal

**Authors:** ***N. H. SPILKA**[1], S. J. PHILIBOTTE[2], S. SPIVACK[1], I. PASSMAN[1], P. WALLISCH[3]
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**Abstract:** We are interested in the mere-exposure effect in music. Previous research, (Zajonc, 1968; Hunter & Schellenberg, 2011), suggests thatthere is a mere-exposure as well as an over-exposure effect for a variety of stimulus materials, including music. However, most of thisprior research is underpowered, thus making it difficult to ascertain for any given study, whether any or both of these effects (whichcombined would create an “inverted-U” trajectory) are present. In

thisexperiment, 600 participants provided familiarity and musical appraisal ratings for entire songs as well as 12 repeats of musicclips from any given song. Using this high powered sample - and musicthat was not disliked, on average - we can show that there is noevidence for a mere exposure effect in music. Instead, repeatedexposure to musical stimuli results in an immediate and near-lineardrop in preference on the order of 1.3% per exposure. This researchsupports the prevalence of over-exposure effects for musical stimuli and puts the generality of the mere-exposure effect in question.

**Disclosures:** **N.H. Spilka:** None. **S.J. Philibotte:** None. **S. spivack:** None. **I. passman:** None. **P. Wallisch:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.16/QQ14

**Topic:** G.03. Emotion

**Title:** Sex differences in alterations in the mood states of university athletes with a history of concussion

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**Abstract:** Concussion or mild traumatic brain injuries are known to cause alterations in mood states (Mainwaring, 2004). Females comprise an increasing percentage of the athlete population and relative to males, they are thought to be at greater risk for concussion, as well as for reporting more intense symptoms following concussion (Covassin, 2007). Despite the results of these research, few studies comprehensively evaluated mood states and even fewer evaluated mood states between males and females beyond the acute phase of injury (Barnes, 1998; Covassin, 2007). This study aim to examine whether sex influences the alterations in the mood states following concussion in a longitudinal manner. To reach this goal, 31 collegiate athletes (15 females, age = 20.79 ± 1.37; 16 males, age = 20.93 ±1.12) completed the Beck Depression Inventory-II (BDI-II) and the Profile of Mood States (POMS) at 7 and 30 days following concussive injury. On the POMS subscales, all athletes, irrespective of sex, reported greater anger ($p$=0.05), vigor ($p$=0.03), fatigue ($p$=0.01), confusion ($p$=0.01) and the total mood disturbances ($p$=0.03) at day 7 compared to day 30. Analyses failed to reveal any sex differences for any of the POMS subscales or for total mood disturbance at either time point. All athletes, irrespective of sex, also exhibited greater intensity of depressive symptoms on the BDI-II ($p$<0.01) at day 7 compared to day 30. However, at day 7, female athletes reported significantly greater scores on the BDI-II than male athletes ($p$=0.05). The current results suggest that sex

differences in mood states alterations following concussion are selective to depressive symptoms. Further this difference appears relegated to the acute phase of injury. Thus time since injury, not sex, appears to be the most important factor moderating the intensity of mood states alterations following concussion.

**Disclosures: W. Sauve:** None. **D. Ellemberg:** None. **R.D. Moore:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.17/QQ15

**Topic:** G.03. Emotion

**Support:** JSPS KAKENHI Grant Number JP26870465

**Title:** The neural basis of emotion induced by real playing in sports games

**Authors: *M. OTOMO**[1], J. SHINOZAKI[1], H. NAGAHAMA[2], Y. SAKURAI[2], T. NAGAMINE[1]
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**Abstract:** The neural basis of emotions caused by participating real sports games(Gms) was not fully understood. We aimed to clarify the neural basis of emotion such as disappointment and pleasantness by watching real sports matches where subjects participated themselves.
Participants are 9 karate players and 1 dancer. Four types of video clips of 14 seconds including the following moments were prepared from two-person competing Gms: Three video clips when the subject got (1) or lost (2) a point each taken from Gms in which the subject participated. Other three clips when a teammate of the subject got (3) or lost (4) a point taken from the teammate's Gms. Three video clips of got and lost each from the subject were interleaved and repeated three times to construct a set of 18 moments for one fMRI run. Another set of 18 moments of video clips from the teammate was also similarly prepared. Eight out of 10 subjects joined two fMRI runs watching sets of the subject and the teammate one by one, resulting perceiving 4 different kinds of moments. The remaining two subjects participated only one scan watching the set of subject oneself including 2 kinds of moments. After each fMRI run, subjects rated their memory confidence, disappointment and pleasantness for six moments each by 5-point Likert scales. Scanned functional images were checked by individual and group analyses, using Statistic Parametric Mapping (SPM) 12.
The activities in the bilateral anterior insular cortices (AI) were significantly stronger in lost than got moments during own Gms. However, these activities were not detected during teammate's Gms. We found significant activity in the left nucleus accumbens (NAcc) in got moments compared to lost moments during own Gms. However, these activities were not found during

teammate's Gms. Rating results showed that there was no significant difference in memory confidence between got and lost ($p = 0.203$). Pleasantness was significantly higher in got than lost moments ($p = 0.005$), and disappointment was vice versa ($p = 0.005$).
Although memory confidence was comparable between got and lost moments, the subjects experienced different degree of disappointment and pleasantness depending on the emotional moments. These results indicated that these emotions were not produced by memory confidence, but successfully evoked by moments of emotion during fMRI. Previous reports have provided the evidence of AI activation by disappointment in decision making task and NAcc activation in pleasant visual stimuli. Therefore, activities in the bilateral AI and left NAcc in the present study may be related to emotions of disappointment and pleasantness, respectively, in real sporting circumstances.

**Disclosures: M. Otomo:** None. **J. Shinozaki:** None. **H. Nagahama:** None. **Y. Sakurai:** None. **T. Nagamine:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.18/QQ16

**Topic:** G.02. Motivation

**Title:** Young adult neural responses to viewing gun violence videos

**Authors: *A. ADEBIMPE**[1], D. S. BASSETT[2,3], D. ROMER[1]
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**Abstract:** Many popular movies feature high levels of gun violence in which characters use firearms to repel and destroy adversaries. The excessive gun violence in PG-13 movies is often acceptable to the general public if the violence appears justified. Justified gun violence involves shooters who are responding to the actions of characters who have harmed them; whereas, unjustified gun violence involves shooters who are inflicting harm on other characters for sinister purposes. Neural responses to viewing violent movies are largely unknown, and it is possible that viewing justified gun violence creates greater identification and empathy with the shooter. To investigate neural activity while viewing violent movies, we obtained fMRI data of 26 young adults (mean age: 20.08 years, std: 1.08 years, 13 females) while watching 4 justified and unjustified video clips for 12 minutes each. General linear model analysis was performed with FSL. The results show that middle temporal gyrus, a known sensory input to the auditory cortex which plays an important role in memory and encoding, was activated for both justified and unjustified video clips. The precuneus - known for its role in visual-spatial imagery and episodic

memory retrieval-was activated while watching justified but not unjustified clips. High activation at the precuneus suggests that participants identified with the justified violence with self-consciousness. Watching unjustified clips produced brain activation in the superior frontal gyrus and inferior parietal lobules, implicating attention and emotion-processing networks. Dorsal striatum was activated while watching unjustified clips, a finding that is particularly interesting in light their crucial role in decision making, especially in regard to action selection. The differences between justified and unjustified clips were found in primary visual cortex and inferior temporal gyrus, areas essential for conscious processing of visual stimuli and perception. The cuneus, dorsal prefrontal cortex and anterior cingulate gyrus were activated while watching unjustified clips in comparison to justified clips. This suggests that unjustified violence elicited more cognitive and emotional conflict than justified violence. Our results indicate that neural responses to justified and unjustified violence differ in young people and exist in high level cortical areas where information is abstracted beyond sensory constraints. Our results support the notion that justified violence elicits a greater identification with the shooter that can override constraints to violent action against particular opponents

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.19/QQ17

**Topic:** G.03. Emotion

**Title:** Anger increases dominance seeking in young male

**Authors: *R. M. DE ALMEIDA**, J. C. CENTURION CABRAL
UFRGS, Porto Alegre, Brazil

**Abstract:** Dominance is an agonistic behavior that have a direct impact on the organization of social groups and on interpersonal relationships. Although the association between emotions and dominance has a strong empirical basis, most studies on the subject are restricted to effects on perception, overlooking other possible implications of this relationship. Thus, we tested the hypothesis that anger increases dominance seeking in those who feel it, and fear decreases this agonistic behavior pattern. One hundred and eighty-four male students voluntarily completed the experiment; they were randomly selected and individually contacted at Federal University of Rio Grande (FURG) and Federal University of Rio Grande do Sul (UFRGS), Brazil. Volunteers were automatically and randomly assigned to one of the four groups: anger (n = 38), sadness (n = 41), fear (n = 36) or control (n = 47). After watching the film clip, the participants filled out the emotional assessment, and then, BIS/BAS scales and dominance measurement. The anger group showed the highest scores for 3 out of 4 dominance measures used, differing significantly from

the fear group (ps < 0.043), which had the lowest levels of dominance for all scores. These findings reinforce the notion that feelings of anger can cause an increase, and fear a decrease, in dominance motivation and agonistic behaviors, leading possibly to action tendencies for the establishment and maintenance of dominance hierarchy.

**Disclosures: R.M. De Almeida:** None. **J.C. Centurion Cabral:** None.

**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

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**Program#/Poster#:** 608.20/QQ18

**Topic:** G.03. Emotion

**Support:** NIH Grant 5F32HD079143

Jacobs Foundation

Gates Foundation

**Title:** Amygdala-medial prefrontal functional connectivity relates to stress exposure and mental health in early childhood

**Authors: *A. T. PARK**[1], P. SAXLER[2], A. B. CYR[2], J. A. LEONARD[2], J. D. E. GABRIELI[2], A. P. MACKEY[1]
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**Abstract:** Functional connectivity between the amygdala and medial prefrontal cortex (mPFC) has been previously shown to be important for emotion regulation. Early life stress has been shown to disrupt this neural circuitry in older children, adolescents, and adults, thereby increasing later vulnerability to psychopathology. Critically, it is unknown how early in development stress-induced differences in amygdala-mPFC connectivity emerge, and whether such differences are linked to latent changes in mental health. In a resting-state functional connectivity analysis with 4- to 7-year-olds, we found significant negative correlations between two measures of stress–parent perceived stress and stressful life events experienced by the child–and amygdala functional connectivity with medial and orbital frontal regions. Further, decreased amygdala connectivity with orbitofrontal cortex (OFC) was associated with more behavioral problems, even within a subclinical range. Amygdala-OFC connectivity mediated the relationship between stressful life events and behavioral problems. These results suggest that the impact of stress on emotional circuitry is detectable in young children, and therefore could be used as a marker for the efficacy of interventions for preschool-aged children.

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.21/QQ19

**Topic:** G.03. Emotion

**Title:** Understanding the relationship between neural activity and stress response in a subsequent immersive target detection task

**Authors: *H. ROY**[1], N. WASYLYSHYN[1,3], J. O. GARCIA[2,3], K. GAMBLE[1], C. DAVIS[4,5], D. PATTON[1], M. EDDY[4], J. BROOKS[1], J. M. VETTEL[1,6,3]
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**Abstract:** The capacity to control and regulate our emotions is essential for our ability to adapt to the environment and circumstances surrounding us. We recruited 25 male participants with marksmanship training and asked them to participate in two sessions: 1) an emotion regulation task while measuring EEG and 2) a target detection task with two stress conditions during which we measured ECG. Our analysis then examines the relationship between neural responses during the emotion regulation task and the participant's stress response, indexed by heartrate variability, during the target detection task in a 300-degree immersive simulator. During the emotion regulation task, participants were instructed to either passively attend or reinterpret 60 high arousal negative images from the Military Affective Picture System (Goodman et al., 2015). The continuous EEG data was then preprocessed using EEGLAB (Delorme & Makeig, 2004), and functional connectivity was computed between channel pairs by fitting multivariate autoregressive models and calculating the short-time direct directed transfer function (sdDTF). During the target detection task, participants were instructed to distinguish presentations of friendly and foes presented on a 300-degree immersive simulator. Participants wore a ThreatFire belt and completed two stress conditions, one where task errors were penalized by a vibration and another where errors resulted in shock. Throughout the target task, electrocardiogram was collected via the LifeMonitor vest and used to calculate heart rate variability. To examine the relationship between brain activity and stress response, we employed a Lasso regression analysis method to reduce the number of features and identify any EEG connections during the emotion regulation task that predicted heart rate variability (HRV) during the target detection task. Results demonstrated that the theta band connectivity detected during the emotion regulation

task, specifically during the “attend” and “reinterpret” phases occurring within the parietal lobe, successfully predicted low frequency HRV (LFHRV) during the shock condition. Connectivity was negatively correlated with LFHRV, such that stronger connections predicted lower LFHRV on average. These results have important implications towards understanding individual variability and trait based information regarding predicting a physiological response during a task from brain connectivity.

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.22/QQ20

**Topic:** G.03. Emotion

**Support:** KAKENHI 15H02502

**Title:** Acute mental fatigue modulates cognitive processing of negative emotion

**Authors: *K. WATANABE**[1,2], A. T. SASAKI[1,4], K. TAJIMA[1,4], K. MIZUNO[1,4,3], Y. WATANABE[1,4]
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**Abstract:** Chronic fatigue syndrome (CFS) is frequently accompanied by depression or anxiety disorders. Although there might be some relationship between such emotional disorders and fatigue, the details are still unclear. A number of reports revealed that depressive or anxiety patients exhibit biased attention to negative emotional stimuli such as sad or threat. In our previous behavioral study with healthy adults, we found that participants, fatigued by long lasting working memory task, exhibited opposite biased attention: they avoided paying attention to sad facial stimuli at acute mental fatigue state. Based on the finding, we hypothesized that cognitive processing of negative emotional stimuli is selectively suppressed at acute mental fatigue state. To verify the hypothesis, we conducted functional magnetic resonance imaging (fMRI) during an emotional attention task.
During fMRI scans, 31 healthy adults performed face dot probe (FDP) task as an emotional attention task before and after 2-back task, lasted for 45 minutes, as a fatigue-inducing mental task. In FDP task, participants were asked to view a pair of faces, out of which one was a neutral and the other was an emotional (angry, happy or sad) or neutral, and to press one of the two buttons corresponding to the side the dot appeared immediately after the face presentation, as

quickly as possible.
As a result of fMRI analysis for FDP task, we found that activations of the bilateral inferior occipital gyrus (IOG) and the right fusiform gyrus (FuG) were significantly different between before and after fatigue-inducing mental task. On parameter estimates (PEs) extracted from these three regions, we conducted a repeated measures analyses of variance. After the fatigue-inducing mental task, PEs of the bilateral IOG increased regardless of emotional conditions, and PEs of the right FuG decreased only under sad and angry conditions. Further, whole brain voxel-wise comparison with each emotional condition revealed that activations of the right thalamus, left superior parietal lobule (SPL) and right FuG significantly decreased under sad condition, and that of the left SPL significantly decreased under angry condition after fatigue-inducing mental task. In contrast, under happy and neutral conditions, there is no significant difference. It has been reported that the IOG contributes to early visual processing and the FuG includes fusiform face area. Taken together, the present results may indicate that, at acute mental fatigue state, much more resources are recruited for early visual processing, whereas the processing of negative emotional face perception is selectively suppressed.

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.23/QQ21

**Topic:** G.03. Emotion

**Support:** ZIA NR000020-06

**Title:** Measuring fatigue in a mouse model of radiation therapy

**Authors: B. S. WOLFF**[1], S. RAHEEM[1], *K. FUKUHARA[3], L. SALIGAN[2]
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**Abstract:** Fatigue is a common and distressing symptom following radiation therapy for cancer, and it can be frequently undertreated in clinical practice. It is commonly defined as a subjective feeling of exhaustion that is not alleviated by rest or sleep, and it is likely a symptom with very complex underlying biology. Consequently, it has proven difficult to study objectively. Our understanding of causes and potential therapies for fatigue may benefit from the systematic study of an animal model. To develop such a model, we have investigated in mice several quantitative behavioral methods to measure fatigue that develops after irradiation. Mice received irradiation targeted to the lower abdomen at a dosage of 8 Gy once per day for three days. We recorded

voluntary wheel running in their home cages, and used video cameras to monitor activity in their home cages and in the open field test. We found that irradiation caused a large and long-lasting reduction of activity in their home cages, measured in distance by wheel-running or video tracking. Irradiation also produced a shift in circadian activity patterns, with activity levels more heavily effected in the latter half of the dark (waking) hours. Video monitoring appeared to be the most sensitive measure of fatigue, with changes in behavior consistently seen in individual mice without averaging the results. In contrast, the open field test showed only modest effects of irradiation on distance travelled; center-time and other measurements were more heavily affected by irradiation, but they also may be more difficult to interpret. Overall, these results show that irradiation produces a fatigue-like reduction in mouse home cage activity that in important ways can mimic the fatigue symptoms experienced by cancer patients after radiation therapy. Future studies using this model may investigate causes of and treatments for cancer-related fatigue.

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**Poster**

**608. Emotional Processes**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 608.24/QQ22

**Topic:** G.03. Emotion

**Support:** ZIA NR000020-06

**Title:** Metabotropic glutamate receptor 5 mediates post-radiotherapy fatigue development in cancer patients

**Authors: L. FENG**[1], *S. D. DETERA-WADLEIGH[2], L. SALIGAN[3]
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**Abstract:** Background Cancer-related fatigue is a common source of cognitive burden in cancer patients and little is known about its underlying mechanism. The aim of this study is to identify gene signatures predictive of post-radiotherapy fatigue in prostate cancer patients. Method Fisher Linear Discriminant analysis (LDA) was performed using whole genome microarray data from 36 men with prostate cancer, yielding a reduced base of the most discriminatory genes. Ingenuity Pathway Analysis was performed to identify the predictive network of genes. In vitro testing was performed using a T lymphocyte cell line, Jurkat E6.1. Cells were pretreated with mGluR5 agonist (DHPG), antagonist (MPEP), or control (PBS) for 20min before irradiation at 8Gy in a Precision X-Ray Irradiator. Cell death was accessed using the MTT assay. Confocal microscopy and IncuCyte quantitative analysis were used to assess mGluR5 receptor distribution post-radiation. Inflammatory cytokines released into the medium were measured using ELISA. Results The LDA model achieved 83.3% accuracy in predicting fatigue phenotype post-

radiotherapy. “Glutamate receptor signaling” was the most statistically significant (p = 0.0002) pathway among all the predictive genes as revealed by pathway analysis. The predictive gene, metabotropic glutamate receptor 5 (mGluR5) was found in Jurkat cells. Pretreatment with mGluR5 agonist resulted in mGluR5 receptor clustering after radiation, as well as increased inflammatory cytokine RANTES production, whereas inhibition of mGluR5 activity decreased RANTES concentration after radiation. Conclusions These results suggest that mGluR5 signaling in T cells may play an important role in the development of chronic inflammation resulting in fatigue, and it may also contribute to the individual differences in immune responses to radiation. Moreover, modulating mGluR5 provide a novel therapeutic option for treating this debilitating disorder.

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**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.01/RR1

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Mellon Foundation

**Title:** Ketamine effects on temporal discrimination in a rodent model of depression

**Authors: *M. A. FRIAR**, P. JANER, A. ROMERO, M. NASH, M. GOMEZ
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**Abstract:** Depression and subjective slowing of temporal perception has been linked by many studies since the 1960s (Thones and Oberfeld, 2015). Depressed patients will often report, “every hour seems like a year to me” or that “things seem to take forever”. While the debate continues as to whether individuals who suffer from depression perceive time objectively different, trends in recent studies indicate a distinct over-production of "short" and an under-production of "long" time production tasks by depressive patients. Ketamine has been found to produce a rapid and robust antidepressant effects in treatment-resistant patients. It has also has been known to affect time perception when used recreationally. Clinical studies have shown that a sub-anesthetic dose of ketamine can have effects within an hour, which can last up to a week without a subsequent dose (aan het Rot, Zarate, Charney, & Mathew, 2012; Ibrahim et al., 2012). In the current experiment, we studied if sub-anesthetic ketamine (10mg/kg i.p.) had any effects on time perception in depressed rodents. Specifically, female rats were trained on a fixed interval procedure (MacInnis and Guilhardi , 2006). Following the temporal training, we induced depression by a daily injection reserpine (.2mg/kg i.p.) for two weeks. Depressed state was confirmed by a forced swim test 24 h after the last reserpine injection. Subsequently, to establish

ketamine's antidepressant effects, rodents were injected with ketamine and retested in the fixed interval and forced swim procedure. This study confirmed that both drugs exerted its prescribed effects in rodents. Notably, reserpine induces depression and the single injection of ketamine significantly reduced depressive symptoms as shown by an increased escaping behavior in the forced swim test. Interestingly, although reserpine induced depression in forced swim test it did not cause distortion in time perception.

**Disclosures: M.A. Friar:** None. **P. Janer:** None. **A. Romero:** None. **M. Nash:** None. **M. Gomez:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.02/RR2

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** A novel ketamine analogue methoxetamine produced rapid and sustained antidepressant effects probably via glutamatergic and serotonergic mechanisms

**Authors: *C. D. BOTANAS**[1], J. I. DE LA PENA[2], R. CUSTODIO[2], I. I. DELA PENA[3], M. KIM[4], H. KIM[4], Y. LEE[5], J. CHEONG[4]
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**Abstract:** Major depression afflicts approximately 16% of the world population and has become one of the leading causes of disability and economic burden. A number of monoamine-based antidepressants are available today; however, the delayed onset time and low remission rate of these treatments are still a major challenge. Thus, there have been efforts to find rapid-acting and effective antidepressants. The discovery of ketamine producing rapid and potent antidepressant effects paved the way for a new generation of glutamate-based antidepressants. Methoxetamine (MXE) is an N-methyl-D-aspartate (NMDA) receptor antagonist and has been shown to have pharmacodynamic similarities with its analogue ketamine. Furthermore, it was found that MXE also acts as a serotonin reuptake inhibitor. However, no studies have evaluated whether MXE can produce antidepressant effects. In the present study, we assessed whether MXE produces rapid-onset and sustained antidepressant effects in mice. We also investigated the probable mechanisms underlying such effects. ICR mice were acutely treated with intraperitoneal MXE (2.5, 5, or 10 mg/kg) and animal behavior was evaluated in the forced-swimming test (FST), tail-suspension test (TST), novelty-suppressed feeding (NSF), and elevated plus-maze (EPM). A separate group of mice were pretreated with NBQX, an AMPA receptor antagonist, or

ketanserin, a 5HT2 receptor antagonist, during the FST. Parallel experiments were also performed in mice treated with ketamine. MXE reduced the immobility time of mice during the FST and TST, and this effect lasted for 24 hours. MXE also decreased the latency to eat during the NSF and increased the time spent in the open arms of the EPM. These MXE-induced behavioral effects were comparable to that produced by ketamine. NBQX pretreatment significantly blocked the effects of MXE and ketamine in the FST. Interestingly, ketanserin only blocked the effects of MXE and not that of ketamine. Altogether, the results of the present study indicate that the ketamine analogue, MXE, has rapid-onset and sustained antidepressant effects and that its antidepressant effects might be mediated through glutamatergic and serotonergic mechanisms.

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**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.03/RR3

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH grant MH107615

NIH grant P50-MH103222

**Title:** Endogenous kynurenic acid mediates ketamine- and (2R,6R)-hydroxynorketamine-induced increases in extracellular glutamate and antidepressant actions

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**Abstract:** (*R,S*)-ketamine (ketamine), a non-competitive NMDA receptor (NMDAR) antagonist, manifests rapid and sustained antidepressant effects in treatment-resistant patients. Production of ketamine's metabolite (*2S,6S*; *2R,6R*)-hydroxynorketamine (HNK) is essential in this regard, and (*2R,6R*)-HNK itself is sufficient to reproduce the antidepressant effects of ketamine in rodent models. These antidepressant actions of (*2R,6R*)-HNK are independent of NMDAR inhibition. Therefore, the mechanism underlying the rapid antidepressant actions of ketamine and (*2R,6R*)-HNK remain unclear. By *in vivo* microdialysis in rats, we show that an acute, systemic administration of either ketamine or (*2R,6R*)-HNK (both at 20 mg/kg) significantly reduces the extracellular levels of the astrocyte-derived tryptophan metabolite kynurenic acid (KYNA; maximal effect of -30% after 2 hours), concomitant with an increase in extracellular glutamate

levels (maximal effect of +179% after 3 hours), in the medial prefrontal cortex (mPFC). Pre-treatment with KYNA's bioprecursor L-kynurenine (5 mg/kg, ip), or the kynurenine 3-monooxygenasease inhibitor Ro 61-8048 (20 mg/kg, ip) prevented both of these acute effects of ketamine and (*2R,6R*)-HNK. Similarly, pre-treatment with L-kynurenine prevented ketamine- and (*2R,6R*)-HNK-induced decreases in forced swim test immobility time, and the effect of (*2R,6R*)-HNK to reverse learned helplessness, in mice. Reverse dialysis of the sodium channel blocker tetrodotoxin (TTX; 5 μM), used to prevent neuron excitability, did not modify ketamine- or (*2R,6R*)-HNK-induced effects on either KYNA or glutamate in the rat mPFC, suggesting a central role of astrocytes in the phenomena described here. Together, these data indicate that the antidepressant actions of ketamine, via the production of (*2R,6R*)-HNK, are mediated by KYNA-induced modulation of glutamate neurotransmission.

**Disclosures: H. Wu:** None. **P. Zanos:** None. **T.D. Gould:** None. **R. Schwarcz:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.04/RR4

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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Barnard College Provost Scholarship

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National Institutes of Health T32 MH015174-36

**Title:** Antidepressant and prophylactic ketamine administration differentially impact adult hippocampal neurogenesis

**Authors: *C. T. LAGAMMA**[1], W. TANG[2], A. MORGAN[3], J. C. MCGOWAN[3], R. A. BRACHMAN[4], C. A. DENNY[1,4]
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**Abstract:** Ketamine, an N-methyl-D-aspartic acid receptor (NMDAR) antagonist, has been shown to have rapid, long-lasting antidepressant effects in treatment-resistant patients with major depressive disorder (MDD). We have recently shown that ketamine acts as a prophylactic and protects against the development of stress-induced depressive-like behavior in mice, indicating that preventative treatment for mental illness is possible. While there is significant investigation into ketamine's' antidepressant mechanism of action, little research has been done to elucidate ketamine's underlying prophylactic mechanism. More specifically, whether the prophylactic actions of ketamine are similar or divergent from its antidepressant actions are entirely unknown. Therefore, the goal of the current study is to determine age-dependent molecular signatures of cell-populations governing ketamine's antidepressant and prophylactic effects. Mice were administered a number of behavioral paradigms as described in Brachman et al., 2016. Post-fixed brains underwent an immunohistochemistry protocol for age-dependent neuronal markers including doublecortin (DCX), a marker of proliferating neurons; calretinin (CR), a marker for immature granule cells; and calbindin (CB), a marker for mature granule cells. The number of $DCX^+$ neurons in the DG, including those with tertiary dendrites, were not affected by prophylactic or antidepressant ketamine treatment, nor were they altered by SD. While prophylactic ketamine had no effect on the number of CR+ cells in the hilus, antidepressant ketamine treatment increased the number of $CR^+$ cells in SD mice to that of control levels. Lastly, antidepressant, but not prophylactic ketamine administration altered CR and CB expression in the HPC. These data suggest that prophylactic and antidepressant ketamine treatment differentially mediate the expression of age-dependent markers of adult hippocampal neurogenesis. The findings further elucidate the neurogenesis based changes associated with MDD pathology and can be used to develop a more targeted therapeutic approach when using ketamine to treat MDD.

**Disclosures: C.T. LaGamma:** None. **W. Tang:** None. **A. Morgan:** None. **J.C. McGowan:** None. **R.A. Brachman:** Other; R.A.B. and C.A.D. are named on a non-provisional patent application for the prophylactic use of ketamine against stress-related psychiatric disorders. **C.A. Denny:** Other; R.A.B. and C.A.D. are named on a non-provisional patent application for the prophylactic use of ketamine against stress-related psychiatric disorders..

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.05/RR5

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** National Institutes of Health DP5 OD017908-01

**Title:** Ventral CA3 deltaFosB mediates prophylactic ketamine efficacy against stress-induced depressive-like behavior

**Authors: *A. MASTRODONATO**[1,2], C. T. LAGAMMA[2], J. C. MCGOWAN[1], A. J. ROBISON[3], C. A. DENNY[1,2]
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**Abstract:** Stress exposure is a major risk factor for mood disorders, such as major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). However, some individuals can successfully adapt to stress and do not develop mood disorders. This ability is known as stress resilience. We previously reported that a single injection ketamine, an NMDA receptor antagonist, prior to stress protects against the development of depressive-like behavior and buffers against a deleterious fear response in mice. However, the cellular and molecular pathways underlying ketamine-induced stress resilience are still largely unknown. Here, we seek to identify the contribution of the deltaFosB transcription factor in mediating ketamine-induced stress resilience. 129S6/SvEv mice were injected with saline or ketamine (30 mg/kg) 1 week before a social defeat (SD) paradigm. Following SD, the animals were sacrificed and the brains were processed to visualize deltaFosB expression. We found that prophylactic ketamine administration increases deltaFosB expression in the prefrontal cortex (PFC), specifically the infralimbic cortex (IL), prelimbic cortex (PrL), and anterior cingulate (Cgl). There was no effect of SD on deltaFosB expression in the PFC. Conversely, we found no effect of Group (Ctrl or SD) or Drug (saline or ketamine) in the dorsal hippocampus. However, interestingly, there was a significant effect of ketamine only in the SD group on deltaFosB expression in the ventral hippocampus. In a second set of experiments, mice were stereotaxically injected into ventral CA3 (vCA3) with viral vectors in order to upregulate or downregulate deltaFosB expression before prophylactic ketamine administration. Inhibition of deltaFosB only in vCA3 prevented ketamine's prophylactic effect on fear responses. Overall, these data indicate that prophylactic ketamine may induce protective effects by altering deltaFosB expression in the ventral hippocampus. Understanding how deltaFosB expression alterations influence depressive-like behavior, and how prophylactic ketamine may prevent these alterations, can elucidate both the pathophysiology of depression and provide insights into potential new treatment targets.

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**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.06/DP13/RR6 (Dynamic Poster)

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Effects of ketamine and its metabolite hydroxynorketamine on synaptic transmission, neurogenesis and behaviour in a depression model of juvenile and adult Wistar rats

**Authors: H. MICHAËLSSON**[1], M. FORSBERG[1], *E. L. HANSE[2], H. SETH[1]
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**Abstract:** Depression affects around 10% of the population. Current treatments including SSRIs and SNRIs have a delayed onset of several weeks to reach effectiveness, if at all effective. Within this timespan there is an increased suicidal risk. Ketamine, a potent anaesthetic, is considered a promising antidepressant with a very rapid onset. Although relatively safe, there are side effects such as induction of a dissociative state as well as addiction. Hydroxynorketamine (HNK), a metabolite of ketamine, could potentially be of clinical value with similar antidepressant actions ridding the side effects. However, knowledge about the mechanism of action is limited. Several recent studies demonstrate effects related to NMDA receptor inhibition, but also unrelated mechanisms, most of which lead to an increased glutamatergic signalling and BDNF-dependent synaptogenesis in the hippocampus and prefrontal cortex.
To investigate the effects of Ketamine and HNK in the hippocampus of juvenile (p30) and adult (p60) Wistar rats we injected pregnant dams with either dexamethasone (150ug/kg) or saline during the last trimester to create a depressive-like phenotype. We then used electrophysiology, behaviour, proteomics, neurogenesis markers to study the effects of ketamine and HNK in vivo, in slices as well as in dissociated subcortical cerebral stem cells. Recordings were also conducted in acute hippocampal sliced exposed to ketamine at different time points.
We observed no effects of ketamine or HNK on hippocampal synaptic transmission and plasticity in juvenile animals, irrespective of treatment (dexamethasone vs saline). This holds true also for AMPA and NMDA receptor expression and differentiation of dissociated subcortical cerebral stem cells. Results were corroborated by no behavioural recovery in the dexamethasone treated animals. There was, however, a decreased proliferation in the dentate gyrus in the depression model. This was reversed after ketamine treatment and proliferation even surpassed the control proliferation.
In general, there seems to be a limited response to ketamine in young humans as well as in juvenile rodents, both with regard to the negative side effects as well as to the antidepressant action. We are currently conducting experiments in adult animals to examine if the effectiveness of ketamine and HNK follow a developmental profile. In conclusion, ketamine and its metabolite HNK have no effect on hippocampal synaptic transmission and plasticity while influencing dentate gyrus proliferation in depressive-like and phenotypically normal juvenile Wistar rats.

**Disclosures: H. Michaëlsson:** None. **M. Forsberg:** None. **E.L. Hanse:** None. **H. Seth:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.07/RR7

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Project Match Foundation

**Title:** Transcriptomic profiling of the antidepressant ketamine in the mouse hippocampus

**Authors: O. H. COX**[1], P. ZANOS[3], L. FLOREA[2], T. D. GOULD[3], *R. LEE[1]
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**Abstract:** Long lag times for efficacy and high non-response rates of currently approved antidepressants necessitate the need for better drugs to treat mood disorders. In this regard, ketamine is a fast-acting, long-lasting NMDAR antagonist and has gained much traction over the past several years for alleviating depressive symptoms such as anhedonia and suicidal thoughts in treatment-refractory depression and bipolar patients. Despite its rapid and robust efficacy, use of ketamine is limited in large part due to its potential for abuse and capacity to produce dissociative effects even when administered at subanesthetic doses. Further, the precise mechanism of action of ketamine is unknown, as its human therapeutic effect via NMDAR inhibition cannot be fully recapitulated using alternative NMDAR antagonists.
We sought to identify ketamine's effects on gene transcription in affect-regulating brain regions, which are likely related to its sustained antidepressant actions. CD-1 male mice were treated with ketamine (i.p., 10mg/kg, N=12) or saline (N=12) and tissue collected 24 hours later. Messenger RNA was extracted from the hippocampus and was used to generate cDNA libraries for RNA-Seq. Several biologically relevant and q-value significant transcripts predicted to be differentially expressed between ketamine vs. saline-treated samples were validated by quantitative real-time PCR (e.g. *Mtor*: 19.7% increase, p=0.022; *Shank2*: 42.3% increase, p=0.021; *Shank3*: 42.0% increase, p=0.010; and *Cdr1*: 48% decrease, p=0.036). In addition, these transcripts were assessed in the HT-22 mouse hippocampal cell line treated with 20 μM ketamine for 24 hours (e.g. *Mtor*: 118% increase, p=0.014; *Shank2*: 335% increase, p=0.070; *Shank3*: 197% increase, p=0.048; and *Cdr1*: 46% decrease, p=0.004). KEGG pathway analysis of q-value significant transcripts identified pathways involved in Axon Guidance, Synaptic Vesicle Cycle, Glutamatergic Synapse, and neurological disorders (Alzheimer's, Parkinson's, and Huntington's Disease).
Our RNA-Seq results have implicated genes and pathways that may be relevant to the antidepressant action of ketamine. We are currently investigating the underlying epigenetic mechanism of ketamine action on these transcripts, and seeking to identify whether ketamine

shares transcriptional and epigenetic targets with (2R,6R)-hydroxynorketamine, a metabolite of ketamine demonstrated to exert antidepressant effects in rodents.

**Disclosures: O.H. Cox:** None. **P. Zanos:** None. **L. Florea:** None. **T.D. Gould:** None. **R. Lee:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.08/RR8

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** R01 MH093320-01

R01 MH106978

T32 NS082145

**Title:** Investigating the role of serotonin transporter and organic cation transporter 3 in the antidepressant-like effects of ketamine

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**Abstract:** Twenty percent of adults are diagnosed with major depressive disorder, which is typically treated with selective serotonin reuptake inhibitors. However, this type of medication takes approximately six weeks to produce therapeutic effects. Recently, low doses of ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, have been shown to produce rapid and long-lasting antidepressant effects. Studies that have begun to examine the mechanisms underlying these effects have focused on intracellular changes mediated by NMDA and/or AMPA receptors. However, the traditional view of the etiology of depression involves the need for an increase in extracellular serotonin to regulate mood. Surprisingly, there appears to be little research into the effects of ketamine on serotonin. One study found an increase in extracellular serotonin while another found an inhibition of serotonin uptake following ketamine treatment. Together, these studies suggest a role for serotonin in the antidepressant-like effects of ketamine, and putatively one involving uptake-1 and uptake-2 transporters for serotonin. A few studies have examined whether ketamine has affinity for the serotonin transporter (SERT; uptake-1) or organic cation transporter 3 (OCT3, uptake-2). In order to investigate this *in vivo*, we performed *in vivo* chronoamperometry. A nafion coated carbon fiber electrode and four barrel micropipette was inserted into the CA3 region of the hippocampus and serotonin, ketamine, or vehicle was applied exogenously. Our results showed a decrease in clearance rate of serotonin

after application of ketamine as well as an increase in signal amplitude. To further elucidate this effect, we examined antidepressant-like effects of ketamine in SERT knockout mice and OCT3 knockout mice bred on a C57BL/6J background. SERT knockout adult mice, OCT3 knockout adult mice, and wildtype adult mice were treated with vehicle or 3.2, 10.0, or 32 mg/kg ketamine and tested in the forced swim test. Ketamine did not produce antidepressant-like effects in the SERT knockout mice or the OCT3 knockout mice indicating that blockade of SERT and OCT3 may be necessary for the antidepressant-like effects of ketamine. Ongoing studies are continuing to examine the relationship between SERT and OCT3 in the antidepressant-like effects of ketamine.

**Disclosures: M.A. Bowman:** None. **M. Vitela:** None. **W.A. Owens:** None. **W. Koek:** None. **L.C. Daws:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.09/RR9

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** 2014 CARIPLO Foundation BIomedical Research conducted by Young Researchers

MIUR (PRIN 2012 prot-2012A9T2S9)

**Title:** Ketamine restores changes in glutamate release, BDNF trafficking and dendrite morphology in the hippocampus of rats vulnerable to chronic mild stress

**Authors: *P. TORNESE**[1], L. MUSAZZI[1], N. SALA[1], M. SEGUINI[1], D. BONINI[2], M. MILANESE[3], T. BONIFACINO[3], G. TRECCANI[4], G. RACAGNI[1], J. R. NYENGAARD[5], G. WEGENER[4], G. BONANNO[3], A. BARBON[2], M. POPOLI[1]
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**Abstract:** Dysfunction of the glutamate system has been associated to stress-related neuropsychiatric disorders. Clinical studies on depressed patients found volumetric and functional changes in glutamatergic brain areas, including hippocampus (HPC) and prefrontal/frontal cortex, while preclinical studies on stress-based animal models of depression reported impaired glutamate neurotransmission and dendritic arborisation in the same regions.

Intriguingly, consistent evidence reported that the NMDA-receptor antagonist ketamine (KET) induces a rapid and sustained antidepressant effect, both in patients and rodent models of depression. However, the mechanisms underlying KET therapeutic effect are still not completely understood.
Using a chronic mild stress (CMS) rat model of depression, we aimed at studying the effect of CMS and KET on glutamate presynaptic release, dendritic trafficking of BDNF transcripts and dendritic morphology.
Rats were subjected to CMS for 5 weeks. Sucrose Preference Test was used to distinguish stress-resilient (CMS-R) from vulnerable (CMS-V) rats. 10 mg/kg KET, acutely administered to CMS-V 24 h before sacrifice, was able to restore sucrose preference. Although significant changes in body weight gain, adrenal glands/body weight and serum corticosterone levels were found in all CMS rats, the increase of corticosterone levels and adrenal glands/body weight was higher in CMS-V. A decrease in basal and depolarization-evoked glutamate release from purified HPC synaptosomes in superfusion was measured in the CMS-V group. KET restored basal, but not evoked, glutamate release in CMS-V. A significant reduction in total-BDNF, BDNF-2 and BDNF-6 splice variant mRNAs was found in HPC of all CMS rats. Moreover, in situ hybridization studies found reduced dendritic trafficking of these transcripts in CA1 and CA3 of CMS-V. KET treatment, although not reversing changes in BDNF mRNA levels, completely rescued dendritic trafficking in CA3 of CMS-V. Morphological analysis of CA3 pyramidal neurons showed a reduction in total length and branching of apical (but not basal) dendrites; KET restored these changes to control levels.
Our results show that chronic exposure to mild stress induces alterations in the hippocampus of vulnerable rats. Interestingly, a single administration of KET was able to reverse most of these deficits. Further investigation of the mechanisms underlying individual resilience or vulnerability to stress and fast KET antidepressant action could help to clarify the neurobiological underpinnings of depression and to identify new pharmacological targets for faster, more efficient antidepressant drugs.

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**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.10/RR10

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH Grant MH 045481

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State of CT

**Title:** Glutamatergic-GABAergic synaptic plasticity within the prefrontal cortex associated with chronic stress and ketamine treatment

**Authors: *C. H. DUMAN**[1], S. GHOSAL[1], R.-J. LIU[1], M. WU[1], M. J. GIRGENTI[1], E. S. WOHLEB[2], R. TERWILLIGER[1,2], M. ALREJA[1], R. S. DUMAN[1]
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**Abstract:** The prefrontal cortex (PFC) plays a central role in stress adaptation, and structural and functional impairments of PFC have been implicated in the pathophysiology of major depression. Exposure to uncontrollable stress causes persistent changes in the synaptic integrity of the principal glutamatergic neurons in the PFC, characterized by neuronal atrophy and loss of synaptic connections (Duman et al., 2016, Nature Medicine). Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine, completely reverse chronic unpredictable stress (CUS) induced depressive behavior and synaptic deficits in layer V pyramidal cells in the PFC. An emerging clinical and preclinical literature indicates that an imbalance in excitatory and inhibitory neurotransmission characterized by deficient GABAergic inhibitory signaling in the PFC could lead to dysfunction of the PFC circuitry after stress exposure. The present study investigates synaptic mechanisms related to inhibitory function in the effects of chronic stress, and if ketamine rapidly reverses these deficits. Reporter mouse lines with specific labeling of pyramidal cells or GABA interneurons, were subjected to a 21-day CUS model to test the actions of ketamine on depression-like behaviors (forced swim and novelty suppressed feeding tests), GABA neurochemistry using western blots and mRNA analyses, and synaptic function of PFC neurons. Our results demonstrate that CUS exposure leads to depression-like behaviors accompanied by reductions in GABA markers at both the mRNA and protein levels. Whole-cell patch recordings of layer V pyramidal cells indicate that CUS decreased frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in PFC pyramidal neurons. Additionally, whole cell voltage clamp recordings in layer II/III somatostatin (SST) positive interneurons show that CUS significantly decreased IPSCs, suggesting that chronic stress causes a reduction in the overall inhibitory tone within the PFC. Moreover, our preliminary anatomical analyses indicate that ketamine administration drives synaptic remodeling in a circuit specific manner in the PFC. Currently, studies are underway to confirm these effects of CUS and reversal by ketamine. Collectively, our findings suggest that impairments of GABA interneurons and inhibitory neurotransmission are key features in a depression-like state induced by stress, and therapeutic approaches that facilitate restoration of impaired inhibitory circuits could provide a novel therapeutic strategy for restoring function and treating depression.

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**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.11/RR11

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Acetylated alpha-tubulin as a plasma biomarker of ketamine efficacy: A time course study in the Wistar Kyoto rat to assess antidepressant and cognitive effects

**Authors: *J. A. PRENDERVILLE**[1], C. W. MCDONNELL[1], A. FISHER[2,1], G. DI CAUPA[1], J. ROUINE[1], C. MOMBEREAU[3], M. BIANCHI[1]
[1]Transpharmation Ireland Ltd, Dublin, Ireland; [2]Trinity Col. Dublin, Dublin, Ireland; [3]Synaptic transmission in vivo, H. Lundbeck A/S, Valby, Denmark

**Abstract:** One third of major depressive disorder (MDD) patients exhibit resistance to antidepressant drugs, an identified MDD subpopulation known as treatment-resistant depression (TRD). The NMDA antagonist ketamine is the only clinically efficacious drug in TRD but delusional and cognitive side effects limit its clinical utilisation. Alterations in microtubule dynamics have been associated with the pathogenesis and treatment of MDD. Here, the Wistar Kyoto (WKY) rat model of TRD was used to investigate the temporal antidepressant and cognitive effects of a clinically relevant dose of ketamine and the feasibility of acetylated alpha-tubulin (Acet-Tub; marker of decreased microtubule dynamics) as a plasma biomarker of ketamine efficacy. Adult male WKY rats (n=20 per group) received a single administration of ketamine (5mg/kg, s.c.) or saline (1ml/kg, s.c.). Immobility in the forced swim test (FST), a measure of depressive-like behaviour, was tested 30min, 24h and 48h post-administration in separate groups of animals. Plasma was isolated immediately after the FST for analysis of Acet-Tub by Infrared Western Blot. The cognitive effects of ketamine (5mg/kg, s.c.) in WKY rats (n=10) was investigated in the working memory delayed non-matching to sample (DNMS) task 30min and 24h post-administration and compared to Sprague Dawley (SD) rats (n=9) representative of a ‘healthy’ control strain. At 30min post-administration ketamine significantly reduced immobility in the FST while no significant change in plasma Acet-Tub expression was observed. WKY rats showed impaired DNMS performance compared to SD rats and further impairment was observed 30min after ketamine administration. Noteworthy, 40% of ketamine treated WKY rats did not complete the task at this time point. Ketamine significantly decreased immobility in the FST 24h post-administration accompanied by decreased plasma Acet-Tub. A pro-cognitive effect of ketamine was observed in DNMS 24h post-administration. No significant effects of ketamine were observed at 48h. Ketamine produced rapid (30min) and long-lasting (24h) effects in the FST in the WKY rat model of TRD. The rapid effect of ketamine was associated with adverse cognitive side effects while the long-lasting effect was accompanied by pro-cognitive efficacy. Plasma Acet-Tub, previously shown to be overexpressed in WKY rats

(Prenderville et al., 2015 Journal of Psychopharmacology Supplement 29 (8): A19), was decreased only when long-lasting antidepressant and pro-cognitive effects of ketamine were observed. Thus plasma Acet-Tub may represent a biomarker of ketamine antidepressant efficacy and a potential indicator of treatment responsiveness.

**Disclosures:** **J.A. Prenderville:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd. **C.W. McDonnell:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd.. **A. Fisher:** None. **G. Di Caupa:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd. **J. Rouine:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd. **C. Mombereau:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **M. Bianchi:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd..

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.12/RR12

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Comparison of selective NR2B antagonism with non-selective NMDA receptor antagonism by quantitative electroencephalography (EEG) recordings in freely moving rats

**Authors: *B. FERGER**, P. VOEHRINGER, H. RAITH, C. DORNER-CIOSSEK
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**Abstract:** Quantitative Electroencephalography (EEG) and vigilance states analysis have been proposed as useful diagnostic biomarkers of mental disorders and an important unit of analysis in the context of the Research Domain Criteria (RDoC) project. Here, we compare a selective NR2B receptor antagonist Traxoprodil with a non-selective NMDA receptor antagonist Ketamine on EEG brain activity and vigilance states. Wireless telemetric transmitters (F40-EET, Data Sciences International (DSI), USA) were implanted into the intraperitoneal cavity of male Wistar rats. EEG electrodes were placed supradurally above the cortex and EMG electrodes were sutured into the neck muscle. Effects of Ketamine (10,30 mg/kg, i.p.) and Traxoprodil (6,18 mg/kg, i.p.) were determined on EEG brain activity, EMG, body temperature and motor activity using the EEG analysis software NeuroScore (DSI, USA). Ketamine but not Traxoprodil increased motor activity whereas body temperature was significantly reduced by Ketamine only. Additionally, Ketamine enhanced the time spent in active and quiet wake and almost completely suppressed REM and NREM states, whereas Traxoprodil did not show any alteration in vigilance states. Power spectra analysis of Ketamine revealed a significant increase in gamma power, whereas Traxoprodil induced an overall decrease in most of the power spectra bands with the major effects on alpha and beta bands. In conclusion, Ketamine and Traxoprodil showed a high

level of central target engagement and different alterations in EEG parameters and changes in vigilance states. The data can be used to differentiate selective NR2B antagonism and non-selective Ketamine-like drugs which is useful for compound profiling in drug discovery projects.

**Disclosures:** **B. Ferger:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG (full-time). **P. Voehringer:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG (full-time). **H. Raith:** None. **C. Dorner-Ciossek:** A. Employment/Salary (full or part-time):; Boehringer-Ingelheim Pharma GmbH & Co. KG (full-time).

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.13/RR13

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** SRPBS and Brain/MINDS from AMED (K.H. and H.H.)

JSPS KAKENHI JP16K08268 (Y.A.)

JSPS KAKENHI JP26293020, JP15H01288 and JP17H03989 (H.H.)

JSPS KAKENHI JP17H050540 (A.K.)

**Title:** Comparative effects of ketamine isomers, R-ketamine and S-ketamine, in mouse models of depression

**Authors: *Y. AGO**[1], M. HIGUCHI[1], W. TANABE[1], K. SEIRIKI[1], H. IGARASHI[1], A. KASAI[1], K. HASHIMOTO[2], H. HASHIMOTO[1]
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**Abstract:** Recent evidence has suggested that the *N*-methyl-D-aspartate receptor antagonist ketamine shows significant therapeutic effects in major depression including treatment-resistant depression. Until recently, clinically available ketamine was a racemic mixture containing equal amounts of two enantiomers, *R*- and *S*-ketamine. Although some preclinical studies report that *R*-ketamine appears to be a potent antidepressant relative to *S*-ketamine, comparative studies on the individual effects of *R*- and *S*-ketamine are limited. Here we aimed to compare the antidepressant potency of *R*- and *S*-ketamine by using two different mouse models of depression: chronic corticosterone and isolation rearing paradigms. Either chronic administration of corticosterone or post-weaning social isolation in male C57BL6/J mice increased the immobility time in the forced swim test. For acute effects of ketamine, *R*-ketamine at doses of 10 and 20 mg/kg reduced

immobility time of isolation-reared, corticosterone-treated, and vehicle-treated control mice at 30 min after the administration, while *S*-ketamine at dose of 20 mg/kg, but not 10 mg/kg, reduced immobility time of these mice. In addition to the acute effects, *R*-ketamine showed sustained antidepressant effects in both isolation-reared and corticosterone-treated mice at 48 h and/or 7 days after the administration. *S*-ketamine at 20 mg/kg also showed sustained antidepressant effects in isolation-reared mice at 7 days after the administration, but it did not affect the immobility time of corticosterone-treated mice at even 48 h after the administration. These results suggest that *R*-ketamine show acute antidepressant effects at lower doses than *S*-ketamine in mouse models of depression used here. Furthermore, we found that both *R*- and *S*-ketamine exerted sustained antidepressant effects in isolation-reared model, but there was a difference between *R*- and *S*-ketamine in corticosterone-treated model. Further analysis on these models might contribute to clarify the common and distinct neural mechanisms for antidepressant effects of ketamine enantiomers.

**Disclosures: Y. Ago:** None. **M. Higuchi:** None. **W. Tanabe:** None. **K. Seiriki:** None. **H. Igarashi:** None. **A. Kasai:** None. **K. Hashimoto:** None. **H. Hashimoto:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.14/RR14

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** R01MH070727

R01MH081060

**Title:** The ketamine metabolite hydroxynorketamine impacts downstream signaling via NMDA receptor inhibition

**Authors: *K. SUZUKI**[1], E. NOSYREVA[1], K. W. HUNT[2], E. T. KAVALALI[1], L. M. MONTEGGIA[1]
[1]Neurosci., [2]Biopharmaceutical Product Develop., UT Southwestern Med. Ctr., Dallas, TX

**Abstract:** Clinical data have demonstrated rapid and sustained antidepressant effects of ketamine, a noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonist. We previously showed that (R,S)-ketamine and other NMDAR antagonists produced fast-acting behavior antidepressant-like effects in mouse models. In addition, we found that ketamine blocked NMDARs at rest, which deactivates eukaryotic elongation factor 2 kinase (eEF2K), dephosphorylating eukaryotic elongation factor 2 (eEF2), resulting in a subsequent desuppression of brain-derived neurotrophic factor (BDNF) protein translation. This signaling

pathway then potentiates synaptic AMPAR responses in the hippocampus via insertion of GluA1 and GluA2 subunits. A recent study suggested that the ketamine metabolite, (2R,6R)-hydroxynorketamine (HNK) is essential for the antidepressant effects of ketamine in mice in an NMDA-independent manner. We assessed (2R,6R)-HNK on NMDAR-mediated miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neurons. We find that (2R,6R)-HNK significantly inhibits NMDAR currents at rest in a manner consistent with open channel block of the NMDARs similar to ketamine. Congruent with the electrophysiology data, the treatment of (2R,6R)-HNK on hippocampal neurons resulted in a significant decrease in phosphorylated eEF2 levels demonstrating that the block of synaptic NMDAR extended to intracellular signaling. Collectively, these data reveal that (2R,6R)-HNK blocks synaptic NMDARs similar to the parent compound, ketamine. We propose that the ketamine metabolite (2R,6R)-HNK acts through direct inhibition of NMDARs and this continued inhibition of NMDARs may explain why ketamine has long lasting antidepressant effects that are not observed by other NMDAR blockers.

**Disclosures: K. Suzuki:** None. **E. Nosyreva:** None. **K.W. Hunt:** None. **E.T. Kavalali:** None. **L.M. Monteggia:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.15/RR15

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH IRP

**Title:** Connectivity-phenotype associations with depressive symptom improvement after ketamine administration

**Authors: *J. W. EVANS**, E. D. BALLARD, C. FARMER, A. NUGENT, C. ZARATE, Jr.
NIH, Bethesda, MD

**Abstract:** Major Depressive Disorder (MDD) is associated with a heavy burden of disability and can also lead to suicidal thoughts, attempts and deaths. The heterogeneity of symptoms associated with MDD has been an obstacle to identifying specific neural correlates of the presence of depression as well as response to treatment. Ketamine, a glutamate modulator, with a rapid antidepressant response, and is therefore an ideal model to assess rapid changes across depressive symptoms. Subscale scores from a previous exploratory factor analysis of several clinical and self-reported depression rating scales (MADRS, HAMD, BDI and SHAPS) were used as measures of unidimensional constructs of depression. Four of the subscales, depressed mood, tension, suicidal thoughts and negative cognitions, were evaluated in resting state scans (8 minutes with eyes closed at 3T) across a clinical trial of ketamine. The aim of the analysis was to

investigate the connectivity-phenotype associations with depressive symptom improvement after ketamine in a sample of patients with treatment-resistant depression. For this study, a cohort of 29 MDD subjects (ages 20-65, 17 female) received resting state fMRI scans over the course of a double blind randomized placebo controlled cross-over ketamine infusion study: once at baseline, and before and after both placebo and drug infusions. We used multivariate distance matrix regression (MDMR) implemented in the Connectir package in R (http://czarrar.github.io/connectir) to compare voxelwise functional connectivity profiles in relation to our variables of interest. The MDMR models tested were overall MADRS score and as well as the individual subscale scores. From these results, we find that there are distinct maps corresponding to each of the latent factors and the MADRS scale. Regions of overlap included the insula, anterior and posterior cingulate and thalamus. Tension and Negative Cognition were associated with the hippocampus and suicidal thoughts being associated with more frontal regions. Depressive mood had significant association with the somatosensory regions. The intrinsic connectivity findings affirming existing regions associated with MDD which suggests neurobiological validity of this analysis.In conclusion, these results may help provide a more data driven approach to understanding the heterogeneity of depression and potential neural underpinnings of symptom profiles both before and after rapid-acting treatment with ketamine.

**Disclosures: J.W. Evans:** None. **E.D. Ballard:** None. **C. Farmer:** None. **A. Nugent:** None. **C. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Zarate is listed as a co-inventor on a patent for the use of (2R,6R)-hydroxynorketamine, (S)-dehydronorketamine, and other stereoisomeric dehydro and hydroxylated metabolites of (R,S)-ketamine met.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.16/RR16

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** JPI AMBROSIAC

**Title:** Oleoylethanolamide modulates depressive like and stress related responses by recruiting histaminergic neurotransmission

**Authors: *A. COSTA**, S. D. SCHMIDT, B. RANI, P. BLANDINA, M. PASSANI, G. PROVENSI
Univ. of Florence, Firenze, Italy

**Abstract:** Oleoylethanolamide (OEA) is a fat sensing molecule that conveys signals from the intestine to the brain by engaging afferent vagal fibers. Recently we provided evidence that the

endogenous histamine is required for OEA to fully exerts some of its anorexic effect[1]. In the present work, we studied the influence of OEA on stress responses and its potential as an antidepressive-like agent, and if central histaminergic system plays a role in such effects. To this end, mice unable to synthesize histamine due to either disruption of the histidine decarboxylase gene (HDC) or injection of alpha-fluoromethylhistidine (a-FMH, an inhibitor of this enzyme, 5µg/5µL, i.c.v.) and WT littermates were tested in two different paradigms: Tail Suspension Test (TST), a predictive model of antidepressant-like effect, and the Social Defeat Stress (SDS) a risk factor for common psychopathologies such as depression. Mice were treated with either OEA (5 or 10 mg/kg, i.p.) or vehicle. In the TST, OEA significantly reduced the immobility time in WT and control mice, but this effect was not observed in HDC-KO or a-FMH-treated mice. Furthermore, to understand the molecular mechanisms responsible for these effects, cortical and hippocampal CREB phosphorylation was measured by Western Blot analysis. OEA-induced increase in cortical and hippocampal CREB phosphorylation was impaired in histamine-deficient mice. To investigate if OEA affects stress reactivity and cognition we examined the behavioral effects of the SDS in WT. Mice treated with OEA (10mg/kg,i.p.) or vehicle were exposed to an aggressive congener until the first attack and then it was separated by a transparent, perforated Plexiglas for 1 hour. The protocol was repeated for 21 consecutive days. Mice were then tested for Social Interaction Test with the aggressor and in the Novel Object Recognition Test (NOR). Preliminary data indicate that OEA reverts the social defeat-induced depressive-like behavioral profile. OEA decreases stress-induced social avoidance, when compared to stressed animals treated with vehicle and ameliorates cognitive performances in the NOR. The same protocol is underway with histamine-deprived mice to evaluate whether histamine plays a role in the cognitive effects of OEA . Collectively, our data provide novel insight into the role of central histaminergic system in OEA induced favorable effect in depressive-like and stress- related responses.

[1]Provensi et al., 2014

**Disclosures:** **A. Costa:** None. **S.D. Schmidt:** None. **B. Rani:** None. **P. Blandina:** None. **M. Passani:** None. **G. Provensi:** None.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.17/RR17

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** RO1 AT009169

VA BX001149

**Title:** Rapid antidepressant action of ketamine: Increase in cAMP independent of NMDA receptor antagonism in glial cells

**Authors: *N. WRAY**[1], J. SCHAPPI[2], M. M. RASENICK[3]
[1]physiology and biophysics, Univ. of Illinois at Chicago Dept. of Physiol. and Biophysics, Chicago, IL; [2]Physiol. & Biophysics, Univ. of Illinois At Chicago, Chicago, IL; [3]Dept Physiol, Biophysics, Univ. of Illinois at Chicago Col. of Med., Chicago, IL

**Abstract:** Ketamine produces robust antidepressant effects in depressed subjects within hours of administration, often when traditional antidepressant compounds have failed to alleviate symptoms even after 2 months of treatment. We hypothesized that ketamine would translocate $G\alpha_s$ from lipid rafts to non-raft microdomains, similarly to other antidepressants but with a distinct, abbreviated treatment duration. C6 glioma and primary glia stably transfected with $G\alpha_s$-GFP were treated with 10uM ketamine for 15 minutes, which translocated $G\alpha_s$ from lipid raft domains to non-raft domains. Similar to the loss of clinical efficacy seen about a week after ketamine infusion, localization of Gas to lipid rafts returns 24 hours after the withdrawal of ketamine. Other NMDA antagonists did not translocate $G\alpha_s$ from lipid raft to non-raft domains and knockdown of NR1 (loss of NMDA receptors) was without effect. However, the ketamine metabolite, 6 OH norketamine, which does not antagonize NMDA receptors, was effective in translocating Gas. The ketamine induced $G\alpha_s$ plasma membrane redistribution allows increased functional coupling of $G\alpha_s$ and adenylyl cyclase, increasing cyclic adenosine monophosphate (cAMP) production. Furthermore, increased intracellular cAMP increased phosphorylation of cAMP response element-binding protein (CREB), resulting in elevated BDNF expression. These actions were reversed in the presence of the cAMP antagonist, Rp-cAMPs. These results reveal a novel antidepressant mechanism mediated by acute ketamine treatment in glial cells that may contribute to ketamine's powerful and rapid antidepressant effect.

**Disclosures: N. Wray:** None. **J. Schappi:** None. **M.M. Rasenick:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PAX neuroscience. F. Consulting Fees (e.g., advisory boards); Otsuka.

**Poster**

**609. Ketamine as an Antidepressant**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 609.18/RR18

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Metformin enhances antidepressant response rate to ketamine in a rodent model of antidepressant treatment resistance

**Authors: *J. B. PRICE**[1], C. HE[3], S. K. ERHARDT[4], L. SCHWIELER[3], W. BOBO[1], M. A. FRYE[1], S. J. TYE[2]
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**Abstract:** Background: The mechanisms mediating the response rate for ketamine's antidepressant effect in treatment-resistant depression are not well understood. However, emerging evidence is pointing towards a neuroendocrine link between stress, depression, and metabolic dysfunction. In depression, chronic HPA-axis activation and allostatic overload ultimately impacts cellular energy regulation through impairment of insulin sensitivity, glucose homeostasis, mitochondrial function, and downstream cellular metabolic pathways. As a consequence, an individual's capacity to function and respond to challenges at the cellular level is impaired. We have previously shown that activation of the insulin signaling pathway directly correlates with antidepressant response to ketamine in antidepressant-resistant rats. The main aim of this project was to determine the synergistic therapeutic effect of metformin, a common treatment for type 2 diabetes, on ketamine's antidepressant response rate in these animals' behavioral responses and peripheral levels of glucose and insulin.
Methods: Rats were administered ACTH (100ug/day, 14 days) to establish a treatment resistant phenotype. Rats were administered control vehicle saline or ketamine (10mg/kg) and/or metformin (200mg/kg). Rats were then subjected to forced swim testing. Before and after behavioral testing, blood was collected for measurement of glucose levels.
Results: Results demonstrate that metformin+ketamine significantly reduced immobility during the forced swim test in rats pretreated with ACTH ($p<0.001$). This reduction matched the antidepressant effect of solo-ketamine treatment. However, the response rate for metformin with ketamine was significantly higher than for ketamine alone ($p<0.05$). These animals also had significantly higher glucose levels relative to ketamine responders ($p<0.01$), ACTH controls ($p<0.05$), and naïve controls ($p<0.001$). Regressional analyses revealed a significant negative relationship between glucose and insulin following metformin and ketamine administration ($p<0.01$).
Conclusions: These results suggest an important relationship between stress, insulin, and glucose homeostasis, which may be a critical mediator of ketamine's antidepressant action. This presents the possibility that metformin may be a useful co-treatment to improve response rates to ketamine.

**Disclosures:** **J.B. Price:** A. Employment/Salary (full or part-time):; Deakin University. **C. He:** None. **S.K. Erhardt:** None. **L. Schwieler:** None. **W. Bobo:** None. **M.A. Frye:** None. **S.J. Tye:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.01/RR19

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Central infusion of beta-hydroxybutyrate produces antidepressant like effects in a rodent model of depression

**Authors: *N. KAJITANI**[1], M. IWATA[1], T. YAMANASHI[1], A. MIURA[1], K. TSUNETOMI[1], S. FUKUDA[1], R. MASTUO[1], T. NISHIGUCHI[1], T. YAMAUCHI[2], R. S. DUMAN[3], K. KANEKO[1]
[1]Devision of Neuropsychiatry, Tottori Univ., Yonago-shi, Japan; [2]Dept. of Neuropsychiatry, Nara Med. Univ., Kashihara-shi, Japan; [3]Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

**Abstract:** Although the pathology of psychiatric disorders such as depression is unclear, recent studies demonstrate a role for the involvement of intracerebral inflammation, including evidence that the inflammatory cytokine interleukin-1β (IL-1β) reduces neurogenesis in adult rodent hippocampus and causes depressive behavior. Previously we had reported that stress increases ATP, which activates the Nucleotide-binding protein, Leucine-rich repeat, Pyrin domain containing 3 (NLRP3) inflammasome, which in turn increases IL-1β in the rat hippocampus. Thus, we propose a hypothesis that the inhibition of the NLRP3 inflammasome will produce an antidepressant effect by preventing IL-1β production caused by stress. Recently it has been reported that beta hydroxybutyrate (BHB), a ketone body that supports mammalian cell metabolism during states of energy deficits, such as fasting or exercise, reduces NLRP3 inflammasome-mediated production of IL-1β. We have found that peripheral BHB administration reduced inflammatory cytokines such as IL-1β and tumor necrosis factor α, and improved depressive and anxiolytic behaviors in the chronic unpredictable stress (CUS), a rodent model of depression. Also, BHB suppressed the activation of NLRP3 inflammasome. Although BHB has antidepressant effects, we don't know whether it acts in directly on brain or on peripheral inflammatory NLRP3 responses. Here, we evaluated the direct influence of BHB in brain by intracerebral administration of BHB into the lateral ventricle. The results demonstrate that direct i.c.v. infusions of BHB prevented depressive and anxiolytic behaviors caused by chronic unpredictable stress, indicating that BHB is capable of producing antidepressant effects by direct actions on the central nervous system. Further studies will be needed to test the contribution of peripheral blockade of NLRP3 on stress-induced antidepressant responses. Nevertheless, these findings are consistent with the hypothesis that inhibiting NLRP3 inflammasome in brain is a novel effective approach for the treatment of depression.

**Disclosures:** **N. Kajitani:** None. **M. Iwata:** None. **T. Yamanashi:** None. **A. Miura:** None. **K. Tsunetomi:** None. **S. Fukuda:** None. **R. Mastuo:** None. **T. Nishiguchi:** None. **T. Yamauchi:** None. **R.S. Duman:** None. **K. Kaneko:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.02/RR20

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Oral administration of medium chain triglyceride produces antidepressant effects in rats via the elevation of beta-hydroxybutyrate

**Authors: *A. MIURA**[1], M. IWATA[1], T. YAMANASHI[1], N. KAJITANI[1], K. TSUNETOMI[1], S. FUKUDA[1], R. MATSUO[1], T. NISHIGUCHI[1], T. YAMAUCHI[2], R. S. DUMAN[3], K. KANEKO[1]
[1]Tottori Univ., Yonago/Tottori, Japan; [2]Neuropsychiatry, Nara Med. Univ., Kashihara/Nara, Japan; [3]Yale Univ. Sch. Med., New Haven, CT

**Abstract:** Pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) are known to play a critical role in the development of depression via disruption of synaptic signaling and decreased neurogenesis in the adult rodent hippocampus. The key molecule that senses stress and controls the processing and release of pro-inflammatory cytokines is the Nucleotide-binding protein, Leucine-rich repeat, Pyrin domain containing 3 (NLRP3), a cytosolic pattern recognition receptor (PRR). We have demonstrated that psychological stress releases ATP, which activates the NLRP3 inflammasome and release of IL-1β and TNFα in the rat hippocampus, which produces anhedonic and anxiety behaviors in rats. Thus, inhibiting NLRP3 is a novel strategy for the treatment of stress-related depression. Beta-hydroxybutyrate (BHB), which is a ketone body that supports mammalian cell metabolism during states of energy deficits, has recently been reported to reduce NLRP3 inflammasome-mediated production of IL-1β. We have confirmed that peripheral BHB administration suppresses the activation of the NLRP3 inflammasome and reduces inflammatory cytokines such as IL-1β and TNFα, and improves depressive and anxiety behaviors in the chronic unpredictable stress (CUS) model of depression. However, administrating BHB orally is inefficient due to its rapid metabolism. Medium chain triglycerides (MCT), which make up a ketogenic diet, are known to cause rapid and sustained elevation of blood BHB levels, thus we administered MCT orally to rats and evaluated behaviors. Here we show that MCT treated animals showed anxiolytic effects in the elevated plus maze test and the open field test as previously reported, but also showed antidepressant effects in the forced swim test. Also, the MCT group showed reduced pro-inflammatory cytokines. Taking MCT orally is easy to apply in a clinical setting, and based

on the current findings would be expected to produce antidepressant effects. These results indicate that oral administration of MCT may be an easy and effective treatment strategy for stress-related depression.

**Disclosures: A. Miura:** None. **M. Iwata:** None. **T. Yamanashi:** None. **N. Kajitani:** None. **K. Tsunetomi:** None. **S. Fukuda:** None. **R. Matsuo:** None. **T. Nishiguchi:** None. **T. Yamauchi:** None. **R.S. Duman:** None. **K. Kaneko:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.03/RR21

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Beta-hydroxybutyrate, an endogenous NLRP3 inhibitor, ameliorates the activation of astrocyte in the rat hippocampus caused by immobilization stress

**Authors: *K. TSUNETOMI**[1], M. IWATA[2], T. YAMANASHI[2], N. KAJITANI[2], A. MIURA[2], N. KAMIYA[2], T. NISHIGUCHI[2], R. MATSUO[2], N. FUKUMOTO[2], A. SUZUKI[2], S. FUKUDA[2], K. KANEKO[2]
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**Abstract:** Stress is known to decrease neurogenesis and synaptogenesis in the rodent hippocampus and induces depressive-like behavior, however the mechanisms by which stress causes such neuroplasticity is still unclear. Previously, we had reported that a proinflammatory cytokine such as interleukin-1β (IL-1β) is an essential mediator of the anti-neurogenic effects of stress. Recently we have focused on the Nucleotide-binding protein, Leucine-rich repeat, Pyrin domain containing 3 (NLRP3), a cytosolic pattern recognition receptor (PRR), which is considered a key molecule for the stress reaction. NLRP3 recognizes various materials such as Damage Associated Molecular Patterns (DAMPs) and Pathogen Associated Molecular Patterns (PAMPs), and activated NLRP3 inflammasome cleaves immature IL-1β to the active form. Thus, we hypothesized that NLRP3 is a novel target for inhibiting stress-related neuroinflammation, which provides anti-depressive effects. To inhibit NLRP3, we applied beta-hydroxybutyrate (BHB), which has recently been reported to reduce the NLRP3 inflammasome-mediated production of IL-1β. We had confirmed that peripheral BHB administration suppressed the activation of the NLRP3 inflammasome, and reduced inflammatory cytokines such as IL-1β and tumor necrosis factor α (TNFα), and improved depressive and anxiety behaviors in the chronic unpredictable stress (CUS), a rodent model of depression. Interestingly, we also found that acute immobilization stress increased the number of astrocyte in the hilus and CA1 of the hippocampus, however the peripheral BHB administration suppresses the increase. Astrocyte is one of the key players which regulates neuroplasticity in the brain. Astrocyte is thought to play

two different roles; neuroprotective actions such as providing trophic support for neurons, and neuropathic actions such as promoting inflammatory processes. In this study, the number of astrocytes might be increased in response to the neural inflammation, or to inhibit neuroinflammation. However, the exact mechanism of this change is still unknown, and further studies will be needed, because depression is related to neuroplasticity including the interaction between astrocytes and neurons, astrocyte mediated by BHB might be implicated in the pathology of depression.

**Disclosures: K. Tsunetomi:** None. **M. Iwata:** None. **T. Yamanashi:** None. **N. Kajitani:** None. **A. Miura:** None. **N. Kamiya:** None. **T. Nishiguchi:** None. **R. Matsuo:** None. **N. Fukumoto:** None. **A. Suzuki:** None. **S. Fukuda:** None. **K. Kaneko:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.04/RR22

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** F31 MH109208

R01 MH101477

**Title:** The Rho-kinase inhibitor, fasudil, has antidepressant-like efficacy in adolescent mice

**Authors: *L. P. SHAPIRO**[1], S. L. GOURLEY[2]
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**Abstract:** Adolescence represents a critical period of neurodevelopment, defined by structural reorganization and synaptic maturation within the prefrontal cortex. Although these processes are critical for the transition to adulthood, structural instability may open a window of vulnerability to neuropsychiatric disorders including depression. Interventions that facilitate activity-dependent neural remodeling, as occurs during adolescence, may be advantageous. Here we evaluated the structural and behavioral effects of Rho-kinase (ROCK) inhibition, which can expedite activity-dependent dendritic spine plasticity. Within the adolescent ventromedial prefrontal cortex (vmPFC), the brain-penetrant ROCK inhibitor, fasudil, increased levels of the post-synaptic marker PSD-95, while pruning dendritic spines, resulting in adult-like spine densities. Further, fasudil had antidepressant-like effects in the forced swim test in adolescent mice and was comparable to ketamine and fluoxetine. Fasudil also decreased the latency to approach a palatable food in the novelty suppressed feeding task, a rapid antidepressant-like effect. Fasudil did not alter prefrontal-dependent cognition as measured by a reversal learning task. Immunoblotting revealed that fasudil stimulated several neurotrophin-related signaling

factors in the vmPFC, including increasing the ratio of full-length:truncated tyrosine kinase receptor B (TrkB). Nevertheless, experiments utilizing viral vectors indicated that the antidepressant-like effects of fasudil in the forced swim test are dependent on the neuronal ROCK2 isoform and not TrkB signaling. Together these findings suggest that inhibition of ROCK2 in the vmPFC may have therapeutic potential for the treatment of adolescent-onset depression.

**Disclosures: L.P. Shapiro:** None. **S.L. Gourley:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.05/RR23

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NSERC

**Title:** The effects of TNF-alpha inhibition on $GABA_A$ and GluA1-ir cells across the rodent hippocampus and cingulate cortex in an animal model of depression

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**Abstract:** Chronic stress produces morphological changes to the rodent hippocampus and prefrontal cortex and impairs memory dependent on these areas. Importantly, chronic stress disrupts GABAergic and glutamatergic activity within these same regions. Furthermore, chronic exposure to stress promotes the release of cytokines, which in turn exacerbate the stress response and disrupt cognition. Prominent among these is the cytokine TNF-alpha, which is preferentially expressed in the hippocampus and prefrontal cortex. TNF-alpha is also known to influence the activity of both GABA and glutamate. We hypothesized that treating chronically-stressed rats with the TNF-alpha inhibitor etanercept could have antidepressant effects, and could reverse stress-induced deficits in cognition. We further hypothesized that etanercept would modulate the stress-induced alterations in GABAergic and glutamatergic activity. To test this hypothesis, we examined the effect of repeated corticosterone (CORT) treatment and concurrent TNF-alpha inhibition (i.e., with etanercept) on forced-swim test (FST) behavior. Additionally, we examined object-location and object-in-place memory--tasks that are dependent on the hippocampus and pre-frontal cortex, respectively. Finally, we examined $GABA_A$ –ir neurons within the hippocampus and GluA1-ir neurons within the hippocampus and prefrontal cortex using immunohistochemistry. Rats received either 21 days of daily CORT injections (40 mg/kg) or vehicle injections. Rats also received semi-weekly injections of etanercept (0.8 mg/kg).

Behavioral testing began on day 22. CORT increased depression-like behavior and impaired both object-location and object-in-place memory. Importantly, CORT rats treated with etanercept behaved like vehicle rats. CORT decreased the number of $GABA_A$-ir neurons within the subgranular zone (SGZ), and etanercept restored this to control levels. CORT also decreased the number of GluA1-ir neurons within both the SGZ and the cingulate cortex, but etanercept restored both to control levels. These novel results clearly demonstrate that etanercept has antidepressant effects, which are accompanied by a normalization of GABA and glutamate and a restoration of hippocampal-dependent and prefrontal-cortex-dependent memory. These results highlight an important role for the immune system in the pathogenesis of depression.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.06/RR24

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CAPES

CNPq/408517/2016-6

**Title:** Cannabidiol treatment induces antidepressant-like effect in streptozotocin-induced diabetic rats

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**Abstract:** Depression is highly incident in the diabetic patients. It is known that several antidepressant drugs used to treat depression may alter the blood glucose or even interact with hypoglycemic drugs. Thus, the search for a more effective treatment for the treatment of depression associated with diabetes is of utmost importance. In that way, the endocannabinoid system has been pointed as a promising system for the treatment of several diseases, among them depression and diabetes. The aim of this study was to evaluate the behavioral response of acute and sub-chronic treatment with cannabidiol (CBD) in diabetic animals (DBT) submitted to the modified forced swimming test (mFST). Accordingly, male *Wistar* rats (180-250 g; n=7-12) were treated with citrate buffer (10mM, pH 4.5, i.p.; normoglycemic group-NGL) or streptozotocin (60 mg/kg; i.p; diabetic group-DBT). Four weeks after, a group of animals was treated with an acute injection of CBD (0, 0.3, 3, 30 mg/Kg i.p.) 1 hour before the mFST.

Another independent group of animals was submitted to the regimen of 3 injections of CBD (0, 0.3, 3, 30 mg/Kg i.p.), 24, 5 and 1 hour before the mFST. The procedures were approved by the UFPR's Committee for the Ethical Use of Animals (#749). When compared to the NGL rats, DBT animals showed hyperglycemia, reduced weight gain and a pronounced depression-like behavior (increased frequency of immobility and reduced frequency of swimming and climbing). The acute treatment with CBD was not able to change the depressive-like behavior of these DBT animals and did not affect blood glucose or the reduced weight gain of these animals. However, the sub-chronic treatment with CBD (30 mg/kg i.p.) was able to reduce the increased frequency of immobility and to improve the reduced frequency of swimming of DBT animals, demonstrating an antidepressant-like effect. This same treatment did not affect blood glucose or reduced weight gain of DBT animals. In conclusion, our data indicate that CBD may represent a promising drug for the treatment of depression associated with diabetes.

**Disclosures: A.P.F. Waltrick:** None. **H. Morais:** None. **K. Genaro:** None. **J.A. Crippa:** None. **J.M. Cunha:** None. **J.M. Zanoveli:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.07/RR25

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CONACYT 256448

**Title:** Analgesic and antidepressant-like effects of rutin in the reserpine-induced fibromyalgia in ovariectomized rats

**Authors: *A. HERNÁNDEZ LEÓN**[1,2], A. FERNANDEZ-GUASTI[1], M. GONZÁLEZ-TRUJANO[2]
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**Abstract:** Fibromyalgia (FM) is a musculoskeletal syndrome characterized by chronic widespread pain, tenderness to palpation, and various concomitant symptoms, including affective disorders like depression. The prevalence of FM in women is much higher than in men and it has been reported that FM symptoms are aggravated during sex hormone-related events, such as the menopause period. There are several pharmacological treatments for FM, such as pregabalin, fluoxetine, and duloxetine; however, in the majority of patients their adverse effects and relatively poor response to treatment have promoted its discontinuation. Therefore, it is necessary to search for new therapeutic alternatives. The antinociceptive and anti-inflammatory effects of flavonoids are well known. In this study, we investigated the analgesic and

antidepressant-like effect of rutin (RUT) (quercetin-3-O-rutinoside, a glycoside of the aglycon quercetin) by using the reserpine-induced myalgia in rats, a model of FM. Ten groups (n=8) of female Wistar rats (200-250 g) were ovariectomized (OVX) and divided in two block (5 groups per block), the first block was built to evaluate analgesic effect of RUT and the second block was used to analyze antidepressant-like response. Each block consisted in OVX group (negative control group of FM), reserpine group (positive control group of FM) and three groups of reserpinized rats receiving vehicle (solution saline/tween 80), RUT (562 mg/Kg i.p.) or reference drug Fluoxetine (FLX, 10 mg/Kg s.c.). All drugs were evaluated on the $5^{th}$ day after the last reserpine injection. The muscle pressure, tactile response, and cold allodynia thresholds were measured before drug administration, as well as at 30, 60,120, 150, 180, 210 and 240 min after treatments. Depressive-like behaviors were evaluated during 5 minutes in forced swim test (FST). Reserpine produce significant diminution in the muscle pressure and tactile response thresholds in 48% and 70%, respectively. Whereas, in the cold allodynia test, reserpine increased 8-folds the time response in acetone spray test and increased 1.5-folds the number of counts of immobility in FST. RUT produced a recovery partial in the three nociceptive thresholds resembling the effect of FLX. The number of counts of immobility was decreased in presence of RUT or FLX showing antidepressant-like effect in FST, this effect was not related to motor alteration as measured in an actimeter and in the rotarod test. In conclusion, these results give evidence that RUT produces analgesic and antidepressive-like effects in an experimental model of FM supporting its potential for the FM therapy.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.08/RR26

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** SUVN-911(nAChR $\alpha_4\beta_2$ ) antagonist addresses most of the limitations of current antidepressant therapies

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Suven Life Sci., Hyderabad, India

**Abstract:** Depression is a major contributor to the overall global burden of disease and also the leading cause of disability worldwide. Globally, more than 300 million people of all ages suffer from depression. The currently available antidepressants have a number of limitations. Some of

these include low response and remission rates, delayed onset of action, sleep disturbances, cognitive dulling and sexual dysfunction. SUVN-911 is a potent and selective $\alpha_4\beta_2$ antagonist. It showed good brain penetration and receptor occupancy following oral administration. SUVN-911 showed good oral bioavailability in all preclinical species. It demonstrated antidepressant like effects in animal models of depression like the DRL-72s and forced swim assay. SUVN-911 showed faster onset of action and also procognition property. SUVN-911 did not cause sexual dysfunction in animal models. At behaviorally effective doses, SUVN-911 produced a significant increase in cortical serotonin and norepinephrine levels. SUVN-911 showed good margin of safety in toxicity studies and is non-mutagenic. The pharmacology, pharmacokinetic, metabolic, biopharmaceutical and toxicity profiles provide strong support to develop SUVN-911 for the management of major depressive disorders. Currently, a single-center, double-blind, placebo-controlled, randomized, phase 1 study to evaluate the safety, tolerability, and pharmacokinetics of SUVN-911 after single ascending doses and multiple ascending doses in healthy male subjects, under US IND is in progress.

**Disclosures:** **R. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Kallepalli:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Goura:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **J. Thentu:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Kamuju:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S.M. Irupannanavar:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R.C. Palacharla:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S. Pandey:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **B. Narasimha:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S. Ravella:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **A. Mohammed:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.09/RR27

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** University of Ottawa Institute of Mental Health Research

NeuroQore Inc.

**Title:** The differential effects of monophasic and biphasic repetitive transcranial magnetic stimulation on attentional processing in major depressive disorder

**Authors: *M. HYDE**[1,2], P. BLIER[1,2], L. MCMURRAY[3], A. ROSTOM[4], A. KHAN[3], V. KNOTT[1,2]
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**Abstract:** In addition to mood dysfunction, patients with major depressive disorder (MDD) exhibit cognitive impairments that interfere with daily functioning and resist improvement with conventional pharmacotherapies. Repetitive transcranial magnetic stimulation (rTMS) holds promise as an MDD intervention strategy; it involves the induction of a transient magnetic field and neuronal current flow. Presently, rTMS practice involves sinusoidal biphasic pulses; however, monophasic magnetic pulses may be more effective as they are associated with more homogenous neuronal activation. MDD has been associated with attenuated P300 event-related potential (ERP) amplitudes and prolonged P300 latencies, indexing decreased attention-related cortical resource allocation and slower cortical processing speed, respectively. This study aims to investigate the impact of monophasic and biphasic rTMS treatment on clinical outcomes (MADRS-assessed), as well as the P300 ERP. This randomized, double-blind trial involves 40 treatment-resistant MDD patients stratified equally to receive either monophasic or biphasic stimulation for six weeks (daily, 30 sessions total). P300 measurements are obtained pre- and post-rTMS treatment during an auditory task whereby infrequent, low-pitched target tones and distractor sounds (e.g., baby crying) are embedded among frequently presented high-pitched tones. Distractor sounds elicit a P300a, reflective of involuntary attention to novelty, while the target tones elicit a P300b indicative of voluntary attention. Preliminary analyses ($N$=11) revealed a reduction in MADRS symptoms ($p$<.001) following rTMS, though, no treatment-specific effects were found ($p$>.05). Interestingly, while greater P300a amplitudes were observed following monophasic treatment vs. baseline ($p$=.01), this was not observed with biphasic treatment ($p$>.05). These preliminary analyses suggest that the two rTMS treatments may differentially affect auditory novelty processing, though this effect will be verified with a larger sample as data collection is ongoing. Regardless, these data are encouraging with respect to the identification of cognitive changes associated with rTMS response and targeted MDD treatment.

**Disclosures:** **M. Hyde:** 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.; NeuroQore Inc.. **P. Blier:** None. **L. McMurray:** None. **A. Rostom:** None. **A. Khan:** None. **V. Knott:** 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.; NeuroQore.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

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**Program#/Poster#:** 610.10/RR28

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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The Åhlen Foundation

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**Title:** Selective positive allosteric modulators of α7 nicotinic receptors have antidepressant-like activity

**Authors: *M. CRADDOCK**[1], H. ARIAS[2], K. TARGOWSKA-DUDA[3], B. BUDZYńSKA[4], A. MICHALAK[4], C. J. LØLAND[5], K. JOZWIAK[4], G. BIALA[4]
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**Abstract:** α7 Nicotinic acetylcholine receptors (AChRs) are widespread in the brain and involved in sensory response, memory, cognition, and mood regulation. The enhancement in activity of these receptors by positive allosteric mediators (PAMs) is of therapeutic importance in a number of cognitive deficits. Until recently, no research had considered the potential of α7-PAMs as treatments for depression. Recent results demonstrated that one of these compounds, PAM-2, has antidepressant-like activity in mice (Targowska-Duda et al., 2014, Neurosci. Lett. 569:126). To demonstrate whether other α7-PAMs have similar antidepressant-like activity, type I (NS-1738) and type II (PNU-120596 and PAM-2) PAMs were assessed using both the forced swim test (FST) and tail suspension test (TST). The FST results showed antidepressant-like activity in all α7-PAMs after subchronic treatment, and particularly in PAM-2 after chronic treatment. In general, the TST results confirmed the FST results, except for NS1738. Methyllycaconitine, an α7-antagonist, inhibited the observed antidepressant-like activity, demonstrating that α7 AChRs are involved in this activity. PAM-2, -3, -and -4 do not inhibit the human serotonin (hSERT), dopamine (hDAT), and norepinephrine (hNET) transporters at clinical concentrations, ruling out involvement of these neurotransmitter systems in the observed activity. Synergistic effects were shown when PAM-2 was co-administrated with the antidepressant bupropion, but not with the α7-agonist DMXBA. This enhancement elicited by α7-PAMs could pave the way for new adjunctive therapies in existing antidepressants since the clinical activity of many antidepressants is only around 10% better than the placebo effect. The

results show that PAM-induced α7 AChR potentiation produces antidepressant-like activity, which opens the door to a new area of therapeutic possibilities for PAMs in mood disorders.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.11/RR29

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** SMRI 03T-484

SMRI 06T-797

NIH R21MH095644

HHSN-271-2013-00017-C (NIMH-PDSP)

**Title:** CXCR4 and CXCR7 agonists are present in plant species used for treatment of mental disorders in Peruvian traditional medicine

**Authors: *C. GALLO**, G. POLETTI, R. ROJAS, A. VAISBERG
Univ. Peruana Cayetano Heredia, Lima, Peru

**Abstract:** Rescuing traditional medicine practices is one of the possible avenues to discover novel pharmacological approaches for clinical conditions like mental disorders, for which pharmacotherapies with better adherence, long-term outcome and patient functionality are strongly needed. Previous studies have led us to collect information on the traditional use of plants for the treatment of mental disorders in several Peruvian localities and geographical regions. The species of interest were sampled, dried, grinded and extracted with ethanol. A total of 477 extracts from plant collections corresponding to 265 species from 87 different plant families were tested at CXCR4 and CXCR7 receptors for agonists by beta-arrestin/Tango assays in the NIMH Psychoactive Drug Screening Program (PDSP) - University of North Carolina, Chapel Hill (UNC). We identified 10 species with greater than 1.3 fold of average basal (basal with medium alone) for the two receptors. These extracts belong to 7 different plant families.The involvement of the chemokine receptors CXCR4 and CXCR7 in mental disorders has been reported and started to be discussed only in the past few years. Our data support this possible role.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH R01 MH92429,

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NIH T34 MH14654

**Title:** BU10119, a buprenorphine analog, produces robust antidepressant-like effects in mice

**Authors:** ***C. A. BROWNE**[1,2], S. A. ROBINSON[2], E. FALCON[2], S. M. HUSBANDS[3], I. LUCKI[1]
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**Abstract:** Buprenorphine is a potent kappa opioid receptor (KOR) antagonist and mu opioid receptor (MOR) partial agonist with equimolar affinity, that is FDA approved for the treatment of opioid addiction and chronic pain. Accumulating evidence from clinical studies highlights the antidepressant potential of buprenorphine following only one week of administration, a timepoint at which conventional antidepressants are ineffective. Moreover, buprenorphine produces rapid and effective alleviation of depressive symptoms in treatment-resistant major depressed patients. Previously, our laboratory demonstrated that low-dose buprenorphine reversed behavioral deficits induced in two rodent models of depression, chronic mild stress and chronic social defeat. Furthermore, we determined that KOR antagonism mediated buprenorphine's antidepressant effects, whereas blockade of MORs facilitated buprenorphine's anxiolytic action. However, potential abuse liability associated with MOR agonist activity may impede the development of buprenorphine as a novel treatment for major depression. We show that mice exposed to chronic mild stress exhibit conditioned place preference for buprenorphine, but that this response was blunted in comparison he preference displayed by non-stressed controls. Reducing efficacy of buprenorphine at MORs without altering affinity and efficacy at other receptors, can enhance the therapeutic profile of buprenorphine. Here we evaluated the behavioral effects of a novel buprenorphine analog, BU10119 in male C57BL/6J mice using the forced swimming test (FST) at 1 and 24 h post administration and in novelty induced hypophagia (NIH) 24 h post treatment. The effects of BU10119 were compared with those of buprenorphine, the selective KOR antagonist CERC-501 and the selective MOR antagonist cyprodime.

BU10119 produced a similar inverted U-shape dose response curve to that of buprenorphine, reducing immobility in the FST and latency scores in the NIH, in the absence of MOR agonist induced hyperactivity, 1 and 24 h post treatment. Cyprodime was only effective at 1 h. CERC-501 reduced immobility at both timepoints, but reduced latency scores only at the highest dose 24 h post injection. Overall, these data show that BU10119 retains the beneficial behavioral profile of buprenorphine.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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Funded by Promentis Pharmaceuticals

**Title:** SXC-2023: Preclinical characterization of a novel activator of the cystine glutamate antiporter (System $x_c^-$) and potential therapeutic for CNS disorders

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**Abstract:** Alterations in glutamate neurotransmission represent a common phenotype of several central nervous system (CNS) disorders. Effective targets for the development of novel treatment approaches can be discovered by evaluating molecular mechanisms that modulate glutamatergic neurotransmission in key circuits within the brain. We have designed SXC-2023 to activate the cystine-glutamate antiporter (also known as System $x_c^-$), a primarily astrocytic target described as an effective means to modulate extrasynaptic glutamate signaling as well as increase the synthesis of glutathione, an important intracellular antioxidant. In addition to possessing a favorable nonclinical profile, SXC-2023 was found to be active in a variety of rodent models. SXC-2023, with a minimal effective dose of 10 mg/kg, PO, was found to improve measures of anxiety and sensorimotor gating as measured using elevated plus maze and prepulse inhibition (PPI), respectively. To validate the mechanism of action of SXC-2023, we utilized a novel transgenic rat in which the gene encoding the primary protein for System $x_c^-$ was mutated, which eliminated cystine-glutamate exchange by this transporter. In wild-type rats, SXC-2023 (10

mg/kg, PO) significantly reversed sensorimotor deficits in the PPI model. However, this response of SXC-2023 was completely abolished in transgenic rats lacking a functional cystine-glutamate antiporter. Collectively, this nonclinical profile suggests that SXC-2023 is well tolerated and that targeting System $x_c^-$ with SXC-2023 may represent a novel treatment approach to reverse CNS symptoms routinely associated with glutamatergic dysfunction and/or oxidative stress.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.14/RR32

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Partner's in Research Funding

**Title:** Noradrenergic signaling enhances MMP release from cultured hippocampal neurons; relevance to antidepressant efficacy

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**Abstract:** Drugs that target monoaminergic neurotransmission represent a first line treatment for major depressive disorder, a debilitating condition affecting approximately 12-17 % of the U.S. population at some point during an individual’s lifetime. Though a full understanding of the mechanisms that underlie antidepressant efficacy in responsive individuals is not yet fully appreciated, responsiveness correlates with increased volume of select brain regions, particularly the hippocampus. Animal models suggest that regional increases in cortical volume reflect the ability of antidepressants to enhance dendritic branching, dendritic spine formation and neurogenesis. Indeed, increased dendritic branching occurs in adult animals and, along with formation of new dendritic spines, it can positively influence excitatory neurotransmission.

Mechanisms by which antidepressant monoamine reuptake inhibitors enhance brain volume-related endpoints are unknown but likely involve indirect and slowly evolving effects of increased monoamine levels. Herein we show that the monoamine norepinephrine can significantly increase the release of matrix metalloproteinase (MMP)-3 and -9 from cultured murine hippocampal neurons. These MMPs have been previously shown to enhance long-term potentiation and/or dendritic spine formation. In related experiments we observe that MMP inhibition reduces dendritic arborization of DIV 14 hippocampal cultures. We also show that the non-selective β-adrenergic agonist isoproterenol stimulates enhanced release of MMP-3 and -9, while the β-antagonist sotalol substantially reduces norepinephrine-stimulated release of these MMPs. Ongoing experiments are focused on the potential for norepinephrine to stimulate MMP-dependent changes in dendritic complexity and spine number. In terms of related *in vivo* work, we observe that desipramine reduces norepinephrine reuptake in C57/Bl6J mice. Ongoing *in vivo* studies are evaluating the effects of norepinephrine modulators, including desipramine and venlaflaxine, and the MMP dependence of each on hippocampal structure (spine number/dendritic arbor) and function (behavior and neurophysiology).

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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NIH/NICHD (DC-IDDRC) 1U54HD090257

PRODEP-NPTC-472

**Title:** Antidepressant effects of C-terminal fragment of tetanus toxin in a rat model of depression

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**Abstract:** The C-terminal domain of the heavy chain of tetanus toxin (Hc-TeTx) is a peptide fragment with demonstrated in-vitro and in-vivo neuroprotective effects against nigrostriatal dopaminergic damage, suggesting its possible therapeutic potential in Parkinson disease (PD).

Since depression is a common co-morbid condition with PD, and recently it has been suggested that neuroprotectants are likely to have antidepressant effects, we undertook this study to determine whether Hc-TeTx might also show antidepressant-like properties. For this purpose, Wistar-Kyoto rats, a putative and non-induced animal model of depression, were treated with a single injection of Hc-TeTx (40 µg/kg) administered intramuscularly. Controls received saline. The open field locomotor activity (OFLA) as well as performance in the forced swim test (FST) of these rats was evaluated 24 h after the injection. OFLA was measured in an automated monitoring cage for 5 min and immediately following this, the FST was performed for 5 min where the animals were videotaped and their immobility score, reflective of helplessness, was counted. Animals treated with Hc-TeTx showed significant decrease (about 2.5 fold) in their immobility score compared to control. The OFLA was not significantly affected. Interestingly, when the rats were tested in the same paradigms after one week of rest, the immobility score in Hc-TeTx treated rats was still significantly lower than the control (about 2 fold), whereas the OFLA score was approximately 60% higher than the control. Following 2 weeks of rest the immobility score in Hc-TeTx treated rats was approximately 25% lower than control, which was not statistically significant. The OFLA score was also not significantly different between the 2 groups. These results indicate a fast and long lasting antidepressant effect, at least for one week, of a single Hc-TeTx injection in WKY rats. Taken together, it may be suggested that Hc-TeTx may be of therapeutic potential in both PD and depression and particularly of benefit in PD-depression co-morbidity. Supported by: NIH/NIAAA R03AA022479 and NIH/NICHD (DC-IDDRC) 1U54HD090257 (YT); PRODEP-NPTC-472(LM)

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH MH093320

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**Title:** Comparative analysis of decynium-22 analogs as novel antidepressants via inhibition at the low-affinity, high capacity biogenic amine transporters

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**Abstract:** The low-affinity, high-capacity uptake-2 transporter family includes plasma membrane monoamine transporter (PMAT) and organic cation transporter isoforms (OCT2 & OCT3) that take up neurotransmitters from the extracellular space in the brain. We have shown uptake-2 transporters limit the effectiveness of the selective serotonin reuptake inhibitor (SSRI) fluvoxamine. Discerning the uptake-2 transporter type(s) involved is restricted by the unavailability of highly selective ligands. This project examines the pharmacological characteristics of seven novel uptake-2 compounds to block low-affinity/high-capacity (PMAT, OCT2, & OCT3) versus the high-affinity/low capacity monoamine transporters (dopamine (DAT), norepinephrine (NET), & serotonin (SERT) transporters). We tested the activity of ANSTO analogs, structurally based on the non-selective inhibitor decynium-22 (D22), in HEK cell lines expressing human OCT2, OCT3, or PMAT. Ligand competitions of [$^3$H]MPP$^+$ uptake were measured in whole, attached cells. Compared to OCT2 and PMAT, dose-responses of ANSTO compounds shifted 1-log leftward, indicating they are more potent inhibitors of [$^3$H]MPP$^+$ uptake through OCT3. ANSTO analogs displayed similar potencies to corticosterone, a potent blocker of OCT3. Binding displacement of selective radioligands in mouse brain preparations from striatum ([$^3$H]WIN35428, DAT) or hippocampus ([$^3$H]Nisoxetine, NET & [$^3$H]Citalopram, SERT) revealed most ANSTO analogs to have similar inhibition potencies as D22. However, two of the compounds were significantly more potent inhibitors at the SERT. Analyses of analogs and SSRI competitions measured in brain preparations from SERT, OCT3 & PMAT knockout mice are ongoing to further develop the specificity of the new compounds. These studies will reveal more about the pharmacological profile of these novel compounds for their potential therapeutic application to treat disorders with neurotransmitter dysregulation, such as depression and drug abuse.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.17/RR35

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Brain and Behavior Foundation

Quinnipiac University

**Title:** Chronic unpredictable stress modifies the quantity and morphology of microglia and neuronal health in cortico-limbic circuitry

**Authors: C. QUAILEY**[1], C. FLYNN[1], C. WHITELOCK[1], B. DALENA[2], K. JONES[1], K. RONALTER[1], M. M. MIRRIONE[3], *A. J. BETZ[2]
[1]Hlth. Sci., [2]Psychology, [3]Biomed. Sci. Dept., Quinnipiac Univ., Hamden, CT

**Abstract:** Chronic unpredictable stress (CUS) is an animal model of major depressive disorder (MDD) The hippocampus has been shown to be a neuroanatomical substrate altered in MDD models. Microglia are essential CNS immune cells that undergo morphological modifications in response to stimuli and have been found to regulate hippocampal neurogenesis and neurodegeneration via inflammatory pathways. The main objective of this study was to dissociate the role of microglia activation, neuronal dysfunction and neurogenesis in regions of the hippocampus (HIPP) and prefrontal cortex (PFC) in adult male rats exposed to CUS (n= 5) versus control (n=5). Using immunofluorescence HIPP tissue was stained for Iba-1, NeuN, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and Ki67 and quantified using MATLAB and ImageJ. We found a significant increase in ameboid microglia ($p<0.020$) in the CUS molecular dentate gyrus and CA1. PFKFB3 is present in the molecular layer of the DG and may indicate neuronal excitotoxicity for stress exposure. This data suggests that modifications in microglia of the hippocampus and PFC may differentially regulate depressive-like behavior. Overall, our results may provide insight to the molecular mechanisms responsible for atrophy and neuronal dysfunction in cortico-limbic circuits related to MDD.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.18/RR36

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Brain and Behavior Foundation

Quinnipiac University

**Title:** Oral riluzole alters inflammatory markers and glutamate transporters in rodent hippocampus following chronic unpredictable stress

**Authors: *I. SCHIANO**[1,2], L. TELISKA[3], L. FRUEHAUF[3], A. ROSELUND[3], M. SZAHAJ[3], A. NAJJAR[3], T. MEDWID[4], J. DEMURO[4], A. J. BETZ[3]
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**Abstract:** Major depressive disorder (MDD) is among the most frequently diagnosed psychiatric disorders in the United States and adversely affects children, adolescents and adults. Evidence suggests a close relationship between emotional stress and vulnerability to MDD. MDD is characterized by behavioral and neurochemical adaptations, such as dysfunctional cognitive-affective processes and elevated hypothalamic-pituitary-adrenaocortical (HPA) activity. The hippocampus has been shown to be a neuroanatomical substrate altered in MDD models. Two regions of the hippocampus, the dorsal and the ventral regions, are suggested to be functionally different while the dorsal hippocampus is involved in cognitive function and the ventral hippocampus is involved in emotion and stress. Animal studies examining how treatments might ameliorate MDD have shown increased neurogenesis in the hippocampus after antidepressant treatments but less is known about the role of glutamate and antidepressant activity. Chronic unpredictable stress (CUS) has been used as a rodent model of MDD. We aimed to examine the expression of dorsal and ventral hippocampal glutamate activity in rats that experienced CUS and CUS + riluzole. Riluzole is a glutamate antagonist that modulates glutamate release and uptake. In the present study, male Sprague Dawley rats were exposed to CUS for 22 days or CUS + riluzole and control conditions were maintained. First, we examined behavioral tasks to demonstrate the antidepressant actions of oral riluzole. We found glutamatergic markers correlating with behavior. Second, we found altered protein levels of CD11b, GFAP, EAAT3, EAAT2, EAAT1 and VGLUT1 suggesting that chronic stress has effects on neuronal and glial metabolism and function. Further, riluzole may modulate glutamate through these mechanisms. Our data may provide insight as to the molecular mechanisms responsible for MDD vulnerability, pathogenesis and possible therapeutic remedies.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.19/SS1

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** DA07315

DA035316

T32DA07268

**Title:** The non-selective opioid diprenorphine produces delta-opioid receptor-mediated rapid antidepressant-like effects in the mouse

**Authors: *T. M. HILLHOUSE**[1], J. E. HALLAHAN[2], N. GRIGGS[2], E. SCHRAMM[2], J. R. TRAYNOR[2]
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**Abstract:** Major depressive disorder (MDD) is the most common mood disorder worldwide with a lifetime prevalence of ~15% (Kessler et al., 2012). Although patients suffering from MDD have access to a number of FDA approved medications including typical and atypical antidepressant drugs, there are two major issues with these drugs. First, there is a delay of 4-12 weeks from the start of treatment until symptom remission is attained. Second, there is a subpopulation of treatment-resistant patients that do not achieve adequate symptom remission following chronic antidepressant drug treatment. There is preclinical and clinical evidence that opioid receptors, particularly the delta-opioid receptors (DOPR) and kappa-opioid receptors (KOPR), play a role in the neurobiology of MDD. As such DOPR agonists and KOPR antagonists are viable targets for rapid-acting antidepressant drugs. The present study sought to extend findings on the opioid system and depressive-like behaviors using the non-selective opioid ligand diprenorphine which has been described as a mu-opioid receptor antagonist, but a DOPR and KOPR partial agonist. In the mouse, diprenorphine over the range 1 to 10 mg/kg produced a dose-dependent antidepressant-like effect in the tail suspension test and an anxiolytic-like effect in the novelty-induced hypophagia assay. The effects of diprenorphine in these two behaviors were completely prevented by treatment with 3.2 mg/kg of the selective DOPR antagonist naltrindole confirming that diprenorphine was functioning as a DOPR agonist rather than a KOPr antagonist. Functional ([35S]GTPgammaS) *in vitro* assays confirmed the partial DOPR agonist nature of diprenorphine. A major concern with DOPR agonists is the induction of convulsions. Importantly, diprenorphine up to 32 mg/kg did not produce convulsions in mice, and blocked convulsions caused by the full DOPR agonist SNC80. Taken together, these results provide further evidence that the DOPR system is a target for rapid antidepressant-like effects. Moreover, partial agonism of DOPR may provide a safer profile as compared to full DOPR agonists.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

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**Program#/Poster#:** 610.20/SS2

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH087583

Seed Grant Program - FSU - College of Medicine

**Title:** CX614, a cognitive enhancing AMPAkine, as fast onset antidepressant

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**Abstract:** Although fast-onset antidepressants, such as ketamine, an antagonist of NMDA-type glutamate receptors (NMDAR) and psilocybin, a partial agonist of various serotonergic receptors, provide important benefits in treating major depressive disorder (MDD), they produce multiple undesirable physical and mental side effects. Recent evidence indicates that blockade of AMPA-type glutamate receptors (AMPAR) abrogates the antidepressant effects of ketamine, that a ketamine metabolite [(*2R,6R*)-HNK; (*2R, 6R*)-hydroxynorketamine] interacts directly with AMPAR, and that the antidepressant effects of (*2R,6R*)-HNK are independent of NMDAR inhibition. Here, we provide strong evidence that a single dose of CX614 (4 and 6 mg/kg b.w.), elicits fast onset antidepressant effects that are at least as potent and long lasting as those elicited by a single ketamine dose (10 mg/kg b.w.) in the forced swim and sucrose preference tests (FST and SPT, respectively). Both CX614 doses and ketamine produced a statistically significant reduction in immobility in the FST and counteracted the reduction in sucrose consumption in the SPT. In vivo CX614 and ketamine treatments activate/phosphorylate TrkB, mTOR, ERK and AKT/PKB with CX614's effects on the phosphorylation of these molecules being dose-dependent. In addition, hippocampal tissue collected from ketamine- and CX614-treated rats and ketamine- and CX614-treated acute hippocampal slices were used to further elucidate the signaling pathways activated by these drugs. Our results indicate that both ketamine and CX614 treatments increased activation/phosphorylation of mTOR, ERK, AKT/PKB, and phosphorylation of 4EBP1. These results imply activation of the protein translation machinery, as was confirmed with increased expression of ARC. CX614 treatment of acute hippocampal slices increases the phosphorylation of TrkB, SSH1, cofilin, LIMK and GluR1. Taken together, our results infer coupling between protein synthesis, actin polymerization and GluR1 trafficking following CX614 treatment. Our results have implications for the use of CX614 as a new fast-onset antidepressant. Considering the highly significant correlation between depression and

cognitive impairment, our results afford added value in the treatment of cognitively impaired and/or depressed patients.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.21/SS3

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Antidepressant effect of transcranial direct current stimulation of the rat medial prefrontal cortex

**Authors: *J. CONWAY**[1,2], L. WALTERS[1], R. J. RAYMOND[2], J. NOBREGA[2], F. R. BAMBICO[1,2]
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**Abstract:** Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation procedure hypothesized to influence neuronal excitability and plasticity in a region-targeted manner. Recent investigations have highlighted its benefits in improving cognitive performance and psychiatric symptoms (Mondino et al., *BP* 2014), although its precise therapeutic mechanisms remain largely unclarified. We developed a rat model of tDCS and tested its antidepressant/anxiolytic activity using the forced swim (FST), novelty-suppressed feeding (NSFT) and elevated plus maze (EPMT) tests. We also examined its effects in rats that have undergone olfactory bulbectomy (OBX), a well-established animal model of depression. We targeted the medial prefrontal cortex (mPFC) because electrical stimulation of this region has been previously shown to result in an antidepressant response (Hamani and Nobrega, *EJN* 2010). Animals were given sham or tDCS current twice on day 1 and once on day 2 (30 min before testing). The current generator delivered low power current (0.05 to 0.1 mA) onto a conducting metal plate (1.5±0.25 x 2.5±0.25mm) fixed on the skull surface over the mPFC (AP+4.7 to +2.2mm; Paxinos and Watson, 2005). In the non-OBX group, stimulated animals receiving 0.1 mA exhibited significantly lower immobility episodes ($p<0.05$) in the FST. Locomotor activity was increased by stimulation but reverted to normal levels upon cessation of the current delivery. At 0.1 mA, animals also exhibited a non-significant increase in anxiety-like behaviour in the NSFT and EPMT. Post-mortem brain analyses using in situ hybridization imaging of the immediate early gene *zif268*, a marker of cell activation, revealed significant activation patterns in target regions, including the cingulate, prelimbic and orbital cortices. Behavioural analyses in OBX animals are underway. These data suggest that tDCS can be effectively modeled in the rat,

yielding highly selective patterns of brain activation, and could therefore serve to elucidate potential antidepressant mechanisms.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Program#/Poster#:** 610.22/SS4

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH AT009169-02

VA BX001149

**Title:** N-3 poly-unsaturated fatty acids facilitate the differentiation of patient-derived induced neural progenitor cells into glia

**Authors: *J.-Z. YU**[1], J. WANG[2], R. PERLIS[3], M. RASENICK[4]
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**Abstract:** Major depressive disorder affects nearly 7% of adults in the US in any given 12 month period, and is predicted to be the number two cause of disability worldwide by the year 2020. While effective interventions exist, at least 1/3 of individuals do not achieve symptomatic remission despite treatment. The evidence from epidemiological, laboratory, and randomized placebo-controlled trials suggests deficiency of dietary n-3 poly-unsaturated fatty acids (n-3 PUFAs) may contribute to development of mood disorders, and supplementation with n-3 PUFAs (alone or in conjunction with SSRIs) may provide a new treatment option. The mechanisms of the antidepressant effects of n-3 PUFAs are unknown. Recent studies found that the density and number of glial cells are reduced in fronto-limbic brain regions in major depression. The purpose of the present investigation was to evaluate the impact of n-3 PUFA on the differentiation of neural progenitor cells. Neural progenitor cells (NPCs) were generated from iPSCs from fibroblasts of subjects with depression that were either sensitive or resistant to SSRI therapy, as well as from healthy control subjects. NPCs were treated with n-3 PUFAs (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), omega-6 PUFAs (arachidonic acid (AA) and linoleic acid (LA) and saturated fatty acid (steric acid (SA)) in glial cell-induction

medium. After 3 days treatment the GFAP positive cells were increased from 8% in control to ~35% in DHA and EPA group ($\tau$ test $p<0.05$). AA and LA group showed a trend of increasing GFAP positive cells without reaching statistical significance. SA treated cells were no different from control. Furthermore, real time quantitative PCR showed a twofold increase of GFAP mRNA expression with DHA and EPA treatment ($\tau$ test $p<0.05$), but not with SA treatment. Expression of neural (MAP2a) and oligodendrocyte (OLIG2) markers were not affected by any fatty acid treatment, These data suggest that n-3 PUFAs may alter downstream cell types differentiated from the NPCs, and may be one of mechanisms underlying n-3 PUFAs anti-depressant effect in the generation of new astrocytes.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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VA facilities

**Title:** Methanandamide stimulates release of TRH and TRH-like peptides throughout male rat brain

**Authors: *A. E. PEKARY**, A. SATTIN
Res., VA Greater Los Angeles Hlthcare Syst, Los Angeles, CA

**Abstract:** There are currently no effective treatments for traumatic brain injury (TBI) or posttraumatic Stress Disorder (PTSD). As a result, many combat veterans resort to self-treatment with cannabis. The principal components of cannabis include delta1-tetrahydro-cannabinol (THC) which binds to both of the endocannabinoid receptors, CB1 and CB2. CB1 receptors predominate in the central nervous system (CNS) while CB2 receptors are concentrated in immune cells. CB1 receptors are part of the endocannabinoid system which in the CNS provides feedback regulation of the glutamatergic neurons which are the predominant excitatory nerves. These neurons require retrograde feedback inhibition of electrical activity by the endocannabinoid, anandamide, and co-release of TRH and TRH-like peptides to protect against the neurotoxicity of glutamate.
We wish to know whether endogenous TRH and TRH-like peptides, which have potent antidepressant, anti-epileptic, anxiolytic, analeptic, neuroprotective and anti-aging properties, and lack undesirable psychotropic side effects, are downstream mediators of the therapeutic

actions of the exogenous cannabinoids, including THC, and endocannabinoids, such as anandamide. Male Sprague-Dawley rats were given methanandamide(MA) ip and decapitated at 0, 2, 4 and 6 h later. TRH and TRH-like peptides were then measured in 12 brain regions: Medulla oblongata (MED), cerebellum (CBL), piriform cortex (PIR), hippocampus (HC), nucleus accumbens (NA), striatum (STR), amygdala (AY), entorhinal cortex (ENT), frontal cortex (FCX), hypothalamus (HY), anterior cingulate (ACNG), posterior cingulate (PCNG) by a combination of HPLC and RIA. The predominant effect observed was a sustain decrease (↓) in TRH and/or TRH-like peptide levels throughout the brain during the 2-6 h interval following ip MA. For example, TRH and 5 TRH-like peptide levels in PIR fell by 2 h consistent with MA-induced release of these peptides. We abbreviate this result as PIR(6↓,2h). The corresponding changes at 4 and 6 h were PIR(8↓,4h) and PIR(8↓,6h). Result for the 2-6 h interval we summarize as PIR(22↓). The cumulative result for the other brain regions were CBL(17↓), STR(14↓,2↑), MED(13↓,2↑), HC(13↓,2↑), HY(7↓,8↑), AY(6↓,5↑), NA(10↓), ENT(9↓,1↑), FCX(1↓,5↑), ACNG(2↓,3↑), PCNG(3↓). MA can release TRH and TRH-like peptides throughout the endocannabinoid-responsive regions of rat brain. We conclude that these peptides may have therapeutic potential for the treatment of traumatic brain injury and posttraumatic stress disorder.

**Disclosures: A.E. Pekary:** None. **A. Sattin:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.24/SS6

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** IBRO (International Brain Research Organization)

CNPq (308723/2013-9 and 449436/2014-4)

CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior)

NENASC Project (PRONEX-FAPESC/CNPq) Grant 1262/2012-9.

**Title:** A single administration of agmatine, similar to ketamine, reverses depressive-like behavior induced by chronic administration of corticosterone in mice

**Authors: *V. B. NEIS**[1], L. E. B. BETTIO[2,1], M. MORETTI[1], P. B. ROSA[1], G. OLESCOWICZ[1], D. B. FRAGA[1], F. M. GONÇALVES[1], A. E. FREITAS[1], I. A. HEINRICH[1], M. W. LOPES[1], R. B. LEAL[1], A. L. S. RODRIGUES[1]
[1]Federal Univ. of Santa Catarina, Florianopolis, Brazil; [2]Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada

**Abstract:** Agmatine has been shown to exert antidepressant-like properties in several clinical and preclinical studies, and recent evidence has indicated that at least some of the mechanisms underlying its antidepressant effect are similar to ketamine. Therefore, the present study investigated the effects of a single administration of agmatine on the depressive-like behavior induced by chronic administration of corticosterone in mice, comparing its effects with those presented by fluoxetine and ketamine in the tail suspension test (TST) in mice. For this purpose, adult female Swiss mice were submitted to administration of corticosterone (20 mg/kg, p.o.) for 21 days. In the 21$^{st}$ day, mice received a single administration of agmatine (0.1 mg/kg, p.o.), fluoxetine (10 mg/kg, p.o.) or ketamine (1 mg/kg, i.p.), and were submitted to the tail suspension test (TST) and open-field test after 24 h. Mice were immediately euthanized after behavioral tests and the hippocampi dissected for Western blot analysis of synaptic proteins (i.e. GluA1, PSD-95 and synapsin I). It was demonstrated that chronic administration of corticosterone induced an increase in the immobility time in the TST, an effect reversed by a single administration of agmatine or ketamine, but not fluoxetine. None of the drugs, alone or in combination, produced significant effects in the locomotor activity of mice. Western blot analysis demonstrated a corticosterone-induced decrease in the immunocontent of synapsin I, an effect that was reversed only by fluoxetine. In addition, an increased immunocontent of GluA1 was observed in vehicle-treated mice that received a single administration of agmatine, ketamine or fluoxetine, but not in animals submitted to chronic corticosterone treatment. However, no alterations in GluA1 phosphorylation (at both Ser831 and Ser845 phosphorylation sites) and in the immunocontent of PSD-95 were observed in any experimental group. Altogether our results reinforce the hypothesis that agmatine may be a novel therapeutic strategy for the treatment of depression and suggest that this compound may have fast-acting antidepressant properties. Further studies are warranted to elucidate whether this compound is able to induce an increase in the levels of synaptic proteins at different time points.

**Disclosures: V.B. Neis:** None. **L.E.B. Bettio:** None. **M. Moretti:** None. **P.B. Rosa:** None. **G. Olescowicz:** None. **D.B. Fraga:** None. **F.M. Gonçalves:** None. **A.E. Freitas:** None. **I.A. Heinrich:** None. **M.W. Lopes:** None. **R.B. Leal:** None. **A.L.S. Rodrigues:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.25/SS7

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** KAKENHI JP (24223004)

Grants-in-Aid for Scientific Research on Innovative Areas “Adaptive Circuit Shift” (26112009)

the Strategic Research Program for Brain Sciences (SRPBS)

**Title:** Impact of low-frequency repetitive transcranial magnetic stimulation (rTMS) to the lower part of the medial frontal cortex on behavioral activity, sociability and motivation in monkeys

**Authors: *S. NAKAMURA**, T. IIJIMA, K.-I. TSUTSUI
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**Abstract:** The medial frontal cortex (MFC) has long been considered to be involved in emotional, social and motivational behavior. Particularly, the lower part of the ACC is of special interest because the abnormal functioning of this brain region has consistently been reported in patients with major depressive disorder. In this study, we examined how the change of activity in this part of the MFC affects monkey behavior by applying low-frequency repetitive transcranial magnetic stimulation (LF-rTMS). Two Japanese monkeys were used in this study. In order to evaluate their mood and emotional state, we measured their within-cage spontaneous activity. Furthermore, we introduced a modified version of the Brinkman board test, which is originally developed to test manual dexterity, to quantify their motivational level. The test required monkeys to grasp pieces of food placed in vertically and horizontally oriented slots on the board. The food pieces were randomly placed in 12 out of 50 slots per session. Two types of boards were used to change the difficulty of the test: one had narrow (difficult) and the other had wide (easy) slots. We let the monkeys perform multiple sessions until it spontaneously stopped performing. We quantified two parameters before and after the LF-rTMS treatment: the number of sessions the monkey performed and the average time the monkey spent to finish a single session. As the LF-rTMS, TMS pulses were continuously given for 20 min at 1 Hz (1200 pulses in total), which is considered to have an inhibitory effect on the stimulated brain region. The LF-rTMS targeting the anterior region of the MFC including the lower part of the ACC induced significant decrease in the within-cage spontaneous activity, which is accompanied by a drastic decrease in sociability and an increase in cortisol level. Moreover, we observed significant reduction of the number of sessions in the difficult condition, but not in the easy condition. In contrast, we did not observe those effects following sham stimulation, the LF-rTMS to the posterior region of the MFC, or the LF-rTMS to the superficial region of the anterior MFC. The average time consumption per session in the behavioral test did not change before and after the treatment in both difficulties in any target regions. These results indicate that the lower part of the ACC and the surrounding MFC may be critically involved in the emotional, social and motivational control.

**Disclosures: S. Nakamura:** None. **T. Iijima:** None. **K. Tsutsui:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.26/SS8

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NSERC

**Title:** Reelin as a novel antidepressant: Behavior and neurobiological correlates

**Authors: *L. E. KALYNCHUK**[1], K. J. BRYMER[2], J. J. BOTTERILL[5], M. A. MITCHELL[2], H. J. KIM[3], H. J. CARUNCHO[4]
[2]Psychology, [3]Col. of Physiol. and Pharmacol., [4]Col. of Pharm. and Nutr., [1]Univ. of Saskatchewan, Saskatoon, SK, Canada; [5]The Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY

**Abstract:** The extracellular matrix protein reelin is highly expressed in the adult hippocampus and is known to participate in the regulation of synaptogenesis, dendritic spine plasticity, learning and memory, and glutamatergic and GABAergic transmission. Previous work in our lab has demonstrated that repeated corticosterone (CORT) injections reduce hippocampal reelin levels, increase depression-like behavior, impair objection-location memory, and disrupts the balance between glutamatergic and GABAergic signaling. Here, we hypothesized that infusing reelin into the hippocampus of CORT-treated rats could reverse the behavioral deficits through a restoration of the balance between glutamate and GABA. To test this hypothesis, we examined the effect of repeated CORT injections and concurrent hippocampal infusions of reelin on object-location memory, forced-swim test (FST) behavior, GluA1, GluN2B, and $GABA_A$ immunohistochemistry. Rats underwent stereotaxic surgery to implant an indwelling cannula into the dorsal hippocampus, and received either 21 days of daily CORT injections (40 mg/kg) or vehicle injections. Rats also received infusions of reelin (1µl/ µg) on days 7, 14, and 21 (repeated reelin) or only on day 21 (acute reelin) of the CORT injections. Behavioral testing began on day 22. As expected, CORT increased depression-like behavior and impaired object-location memory. Importantly, these effects were reversed in rats treated with reelin either once or three times. CORT upregulated GluN2B immunoreactivity, and reelin infusions brought this down to control levels, whereas CORT decreased GluA1-ir cells, and infusions of reelin increased this to control levels. Finally, CORT decreased the number of $GABA_A$-ir cells, and infusions of reelin restored this to control levels. These novel results demonstrate that a single infusion of reelin into the dorsal hippocampus has fast acting antidepressant effects, which are accompanied by restoration of the balance between glutamate and GABA.

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**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.27/SS9

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** MH082933

MH090067

Center for Biomedical Neuroscience

**Title:** Novel mechanisms contributing to the antidepressant-like effect of pharmacological hippocampal activation

**Authors: *F. R. CARRENO**[1,2], L. D. ARROYO[1], D. J. LODGE[1,2], A. FRAZER[1,3,2]
[1]Pharmacol., UT Hlth. San Antonio, San Antonio, TX; [2]Ctr. for Biomed. Neurosci., San Antonio, TX; [3]South Texas Veterans Hlth. Care Syst., San Antonio, TX

**Abstract:** We recently demonstrated that selective modulation of hippocampal transmission by systemic administration of an α5-containing-GABAA receptor negative allosteric modulator, namely L-655,708, is capable of producing a hippocampal-dependent sustained antidepressant (AD)-like effect in the absence of any psychotomimetic or abuse-related effects as seen with ketamine. Although the AD-like effect of ketamine is dependent on BDNF-TrkB signaling in the hippocampus, that of L-655,708 does not seem to be. L-655,708 did not cause an increase in the phosphorylation of TrkB in the ventral hippocampus (vHipp) 30 or 60 min after its administration nor did intra-hippocampal administration of TrkB inhibitor, K252a, block the sustained antidepressant-like effect of L-655,708. To begin to examine alternative molecular mechanisms associated with the effects of L-655,708, we took advantage of a PathScan approach, which consists of an array kit that allows for the simultaneous detection of proteins, including 28 receptor tyrosine kinases and 11 important signaling nodes when phosphorylated. Using such an approach, in rats given L-655,708 one hour prior to tissue collection, we not only confirmed that TrKB was not involved, but that other receptor tyrosine kinases were not either. Of all the downstream markers analyzed, only ERK activation was increased in the vHipp, as measured by its phosphorylation. We confirmed this result using western blot analysis from vHipp total lysate collected after L-655,708 administration. We hypothesized that the CamKII pathway could be involved in the ERK activation via voltage gated calcium channels activating CamKII in the vHipp. Using western blot analysis, CamKII phosphorylation in the vHipp was found to be increased following L-655,708 administration. By identifying the mechanisms by which systemic administration of α5-GABAA receptor negative allosteric modulators recapitulate the therapeutic effects of ketamine without its psychotomimetic and abuse-related

effects, it should be possible to provide novel, safe, and effective approaches for treating patients suffering from refractory depression.

**Disclosures: F.R. Carreno:** None. **L.D. Arroyo:** None. **D.J. Lodge:** None. **A. Frazer:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.28/SS10

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CNPq (# 308723/2013-9 and # 449436/2014-4)

CAPES (Coordenação de aperfeiçoamento de pessoal de nível superior)

NENASC Project (PRONEX-FAPESC/CNPq) # 1262/2012-9.

**Title:** Agmatine exhibits antidepressant and pro-neurogenic effects in mice submitted to chronic exposure to corticosterone

**Authors: *A. S. RODRIGUES**[1], G. OLESCOWICZ[1], V. B. NEIS[2], D. B. FRAGA[2], P. B. ROSA[2], F. F. MELLEU[2], P. S. BROCARDO[2], J. GIL-MOHAPEL[3]
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**Abstract:** Depression is a high prevalent psychiatric disorder with severe socioeconomic consequences. Adult hippocampal neurogenesis has been shown to be reduced in depressive patients, and the behavioral responses to antidepressant drugs are associated with pro-neurogenic effects. However, current available antidepressants have serious limitations and efforts have been done to find some new strategies as alternative antidepressant therapy. In this context, emerges agmatine, an endogenous polyamine, that has been shown to exhibit antidepressant effects in preclinical and clinical studies. The present study investigated the antidepressant-like effect of chronic treatment with agmatine in an animal model of stress induced by chronic administration of corticosterone to mice, as well as its effects on hippocampal cell proliferation in the ventral and dorsal subregions of the subgranular zone (SGZ) of the dentate gyrus. Moreover, the dendritic complexity (length and arborization in the dentate gyrus of the hippocampus) in mice exposed to corticosterone and/or agmatine or fluoxetine was investigated by Sholl analysis. Chronic administration of corticosterone (20 mg/kg, p.o., 21 days) increased the immobility time in the tail suspension test (TST), an effect reversed by the co-administration of agmatine (0.1 mg/kg, p.o., 21 days) or fluoxetine (10 mg/kg, p.o., 21 days, positive control). Moreover, corticosterone administration induced a decreased hippocampal cell proliferation in the whole dentate gyrus as shown by the decreased number of Ki-67 and PCNA positive cells, as well as in

the ventral and dorsal aspects of the hippocampus (as shown by Ki-67 staining). Treatments with agmatine or fluoxetine were able to reverse these corticosterone-induced alterations on cell proliferation parameters. Agmatine and fluoxetine also enhanced both the dendritic length and arborization in the dentate gyrus of the hippocampus. Altogether, our results show that the increase in hippocampal proliferation induced by agmatine may contribute, at least in part, to the antidepressant-like response of this compound in this mouse model of stress induced by chronic exposure to corticosterone.

**Disclosures:** **A.S. Rodrigues:** None. **G. Olescowicz:** None. **V.B. Neis:** None. **D.B. Fraga:** None. **P.B. Rosa:** None. **F.F. Melleu:** None. **P.S. Brocardo:** None. **J. Gil-Mohapel:** None.

**Poster**

**610. Novel Drugs and Treatments for Affective Disorders**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 610.29/SS11

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Grants-in-Aid for Scientific Research (KAKENHI)

**Title:** Chronic treatment with a selective serotonin reuptake inhibitor increases spontaneous activity of dorsal raphe nucleus serotonergic neurons through activating L-type voltage dependent $Ca^{2+}$ channels

**Authors: *N. ASAOKA**[1], N. NISHITANI[1], H. KINOSHITA[1], H. KAWAI[1], N. SHIBUI[1], K. NAGAYASU[1], H. SHIRAKAWA[1], T. NAKAGAWA[2], S. KANEKO[1]
[1]Dept. of Mol. Pharmacol., Kyoto Univ., Kyoto-shi, Japan; [2]Dept. of Clin. Pharmacol. and Therapeut., Kyoto Univ. Hosp., Kyoto-shi, Japan

**Abstract:** Decrease in the activity of dorsal raphe nucleus (DRN) serotonergic neurons is implicated in various mental disorders. One of the main strategies for treating psychiatric disorders is to activate serotonergic system, while the precise action mechanisms of clinically used drugs, such as selective serotonin reuptake inhibitors (SSRIs), remain to be elucidated. It is widely accepted that raphe serotonergic neurons show tonic firing, while little is known how serotonergic neurons control their firing activity. In this study, we examined control mechanisms for serotonergic activity and effects of chronic treatment with an SSRI on serotonergic firing activity. By using modified *ex vivo* electrophysiological recording methods, DRN serotonergic neurons showed spontaneous tonic firing in drug-free condition. Furthermore, even in the absence of excitatory and inhibitory ionotropic inputs, serotonergic neurons still generate spontaneous action potentials. The spontaneous firing activity was positively and negatively modulated by bath application of activator and blocker, respectively, for L-type voltage-dependent $Ca^{2+}$ channel (VDCC). Blockage of $GABA_B$ receptor increased both VDCC current

and spontaneous activity of serotonergic neurons through PKA-dependent mechanism. Additionally, chronic administration of an SSRI, citalopram increased both VDCC current and spontaneous firing activity of serotonergic neurons. Either bath application of L-type VDCC blocker, $GABA_B$ receptor antagonist or PKA inhibitor blocked the increasing effects of citalopram on VDCC current and spontaneous firing activity. Taken together, these results suggest that the serotonergic spontaneous firing activity is maintained by L-type VDCC, which is continuously inhibited by $GABA_B$ receptor-mediated signaling, and that chronic citalopram disinhibits serotonergic neuronal activity by weakening the continuous GABA inhibition.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.01/SS12

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** JSPS KAKENHI 26670542

JSPS KAKENHI 16K10189

JSPS KAKENHI 15K09832

the “Integrated Research on Neuropsychiatric Disorders” conducted under the Strategic Research Program for Brain Sciences from the MEXT and AMED

GSK Japan Grant

**Title:** Altered plasma protein glycosylation in depression

**Authors: *H. YAMAGATA**[1], S. UCHIDA[3], K. MATSUO[4], K. HARADA[5], Y. WATANABE[2]
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**Abstract:** Glycosylation, the addition of a carbohydrate moiety to a protein during biosynthesis, is a common post-translational modification involved in several disease states. Specific protein-glycan structures have been reported to be useful as biomarkers for cancer and some neuropsychiatric disorders; however, the relationship between glycosylation of plasma proteins

and major depressive disorder (MDD) has not been studied. This study aimed to determine whether the plasma protein-glycan structure would differ in MDD from typical values, using both a stress-based mouse model and samples from patients with MDD. As a mouse model of depression and remission, we used chronically ultra-mildly stressed mice that were not treated or treated with imipramine, respectively. We also made comparisons between samples from patients with MDD who were actively depressed and those who were remitted. Protein glycosylation was analyzed using a lectin microarray that included 45 lectins with binding affinities for various glycan structures. The glycan structure profile of plasma proteins might represent candidate biomarkers for MDD.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.02/SS13

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** dmPFC-rTMS as a treatment for major depression: Preliminary results of a comparison of 1 Hz, 20 Hz and sham rTMS

**Authors: *K. DUNLOP**[1], J. SHEEN[2], B. WOODSIDE[3], P. COLTON[3], M. OLMSTED[3], F. FEFFER[2], D. BLUMBERGER[3,4], Z. J. DASKALAKIS[3,4], J. DOWNAR[3]
[1]Univ. of Toronto, Etobicoke, ON, Canada; [2]Krembil Res. Inst., Toronto, ON, Canada; [3]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [4]CAMH, Toronto, ON, Canada

**Abstract:** Introduction: Bilateral dorsomedial prefrontal cortex (DMPFC) rTMS has previously been used in open-label settings for a variety of psychiatric disorders, including treatment-resistant depression (TRD). However, its clinical efficacy is heterogeneous, and requires testing under placebo-controlled conditions. The aim of this study is to assess the efficacy of two protocols for DMPFC-rTMS in TRD against sham stimulation, and identify neuroimaging, psychometric or behavioral predictors of treatment response. Here we report preliminary results of the first 30 subjects enrolled. Methods: rTMS-naive TRD patients between 18-65 years were enrolled and randomized to one of three treatment arms: 1 Hz active, 20 Hz active, or sham rTMS to the bilateral DMPFC. All treatments were performed with a custom MagStim active-placebo coil to allow blinding of both patients and technicians. Treatments occurred twice daily for a total of 30 sessions. Patients completed the Beck Depression Inventory (BDI), at baseline, weekly throughout treatment, and at follow-up. A repeated-measures ANOVA was performed to

determine the effects of treatment group on severity. Patients also undergo an anatomical T1 and 10-minute resting-state fMRI, as well as a suite of psychometric scales (NEO Personality Inventory; impulsivity scales [BIS/BAS; BIS-11; UPPS-P; Monetary Choice Questionnaire]; emotion regulation scales [DERS]; and the Rumination Responses Scale), and a behavioral measure (Probabilistic Reward Task). Neuroimaging, psychometric and behavioral measures will be used to identify predictors of clinical response to dmPFC-rTMS. Results: To date, 30 participants (n=20 females, range 19-58 years) have completed treatment. We found a trend in the interaction between BDI severity and treatment arm (F=1.516, p=0.164), and a significant main effect of time on BDI (F=9.105, p=0.007). Both Group A (n=11) (p=0.021) and Group B (n=11) improved significantly (p=0.026). Group C (n=8), however, had a non-significant reduction on BDI (p=0.421). Discussion: Although preliminary, it appears that two treatment arms (treatment arms A and B) achieve a significant reduction of depressive symptoms relative to treatment arm C. Further work will enrol more patients in this study to confirm the effects of DMPFC-rTMS in TRD. As indicated above, we are collecting a battery of psychometric, behavioural and neuroimaging measures to identify predictors of treatment response and measures that change in association with treatment response. Additionally, we are also collecting a cohort of healthy individuals to use as a comparator group for our TRD patients.

**Disclosures: K. Dunlop:** A. Employment/Salary (full or part-time):; University of Toronto. 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.; Vanier Scholar, CIHR. **J. Sheen:** None. **B. Woodside:** None. **P. Colton:** None. **M. Olmsted:** None. **F. Feffer:** None. **D. Blumberger:** None. **Z.J. Daskalakis:** None. **J. Downar:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.03/SS14

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Reduced serum and cerebrospinal fluid levels of autotaxin in major depressive disorder

**Authors: K. ITAGAKI**[1], H. ABE[1], C. SHIBASAKI[2], W. OMORI[1], N. KAJITANI[1], M. OKADA-TSUCHIOKA[1], K. HATTORI[3], S. YOSHIDA[3], H. KUNUGI[3], *M. TAKEBAYASHI[1]
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**Abstract:** [Background] Autotaxin (ATX) is a major enzyme that is secreted to generate lysophosphatidic acid (LPA) which has diverse biological effects on neurodevelopment, the immune system, and inflammation. We previously reported the possible involvement of LPA1

receptor in the action of antidepressants (Kajitani et al., JBC 2016). However, the possible association between the ATX/LPA axis and the pathophysiology of major depressive disorder (MDD) remains to be elucidated. [Methods] Serum levels of ATX were measured in patients with MDD (N = 37) who underwent electroconvulsive therapy (ECT), and compared with those of healthy controls (N = 53). Serum levels of ATX before and after a course of ECT were then compared in the patients. In another sample set without ECT treatment, cerebrospinal fluid (CSF) levels of ATX were examined between patients with MDD (N = 26) and healthy controls (N = 27). An analysis was also conducted to determine whether there was an association between serum and CSF levels of ATX and clinical symptoms using the Hamilton Depression Rating Scale score. Serum and CSF ATX concentrations were measured by ELISA. Effects of antidepressants on ATX expression were investigated using rat brain samples. The ethics committees of NHO Kure Medical Center and the National Center of Neurology and Psychiatry approved the study protocol. All participants provided written consent. [Results] In the total subjects, both serum and CSF ATX levels for females were significantly higher than those of males ($P < .001$, $P = .006$, respectively), which indicates a gender difference for ATX. Prior to ECT, both serum and CSF ATX levels with MDD were significantly lower than those of healthy controls ($P = .001$, $P = .028$, respectively). After ECT, serum ATX levels in MDD were significantly increased ($P = .001$). There was a significant negative correlation between depressive symptoms and serum ATX levels in MDD at Pre-ECT and Post-ECT. Chronic antidepressant treatment did not change any mRNA and protein levels of ATX in rat hippocampus. [Conclusion] Both serum and CSF levels of ATX were significantly decreased in MDD patients, and serum ATX levels were increased following a course of ECT, which indicates a state-dependent manner. Antidepressants appear not to be directly associated with ATX expression. Our findings suggest that alteration of lysophospholipids such as the ATX/LPA axis could be related to the pathophysiology of MDD.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.04/SS15

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH RO1 AT009169

VA BX001149

American Heart Association Postdoctoral Award

**Title:** Dynamic relationship between α-tubulin acetylation and $G\alpha_s$ localization in lipid-rafts: Potential basis of depression and antidepressant mechanism of HDAC6 inhibitors

**Authors:** *H. SINGH[1], ***H. SINGH**[1], J. SCHAPPI[1], *G. PANDEY[2], M. M. RASENICK[3]
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**Abstract:** Major depressive disorder (MDD) is a debilitating illness lacking clear molecular and cellular correlates as well as consistently effective treatments. Several studies have demonstrated that sustained antidepressant treatment moves $G\alpha_s$ out of lipid rafts, where it appears associated with tubulin. Histone deacetylase-6 (HDAC-6) enzymes deacetylate α-tubulin and are upregulated in neuropsychiatric disorders. HDAC6 knockout or HDAC6 inhibitors also displayed an antidepressant profile in animal models. While, a possible role for HDAC6 inhibitors in treatment of depression exists, the potential mechanism for this remains elusive. Previously, we demonstrated that treatment of rats or C6 glioma cells with several classes of antidepressants translocates $G\alpha_s$, from lipid-rafts, inducing a sustained elevation in cAMP production. Once freed from lipid-raft domains, $G\alpha_s$, couples more effectively with adenylyl cyclase-6 (AC6). Although $G\alpha_s$ interacts directly with tubulin to modify microtubule dynamics, tubulin also acts as an anchor for $G\alpha_s$ in lipid-rafts. Based on HDAC-6 roles in tubulin deacetylation and our data showing $G\alpha_s$ complexes with tubulin in lipid-rafts, we hypothesized that acetylation of α-tubulin disrupts tubulin-$G\alpha_s$ anchoring, rendering $G\alpha_s$ free to activate AC6. To test this, C6 glial cells were treated with the HDAC-6 inhibitor, tubastatin-A. Three-day treatment not only increased acetylation of α-tubulin but also caused translocation of $G\alpha_s$ out of lipid-rafts. Reciprocally, depletion of α-tubulin acetyl transferase-1 (ATAT-1), ablated this phenomenon. Fluorescence Recovery After Photobleaching (FRAP) on C6 cells stably expressing GFP-$G\alpha_s$, after tubastatin-A treatment showed an “antidepressant signature”. Finally, tubastatin-induced sustained elevation of cAMP was revealed by increased cAMP response element binding protein (CREB) phosphorylation and increased expression of brain derived neurotrophic factor (BDNF). Also, an increase in acetylated α-tubulin prevented the formation of tubulin-$G\alpha_s$-complexes in lipid rafts. Although other classes of antidepressants also reduced the association between tubulin and $G\alpha_s$, they had no effect on the acetylation of tubulin. Postmortem human brain tissue analysis revealed decreased membrane tubulin acetylation in subjects with depression even as the total acetylated tubulin content was comparable in depressed and control subjects. Thus, it is possible that compounds that decrease tubulin-$G\alpha_s$ interactions by increasing acetylation of α-tubulin may show promise for antidepressant therapy.

**Disclosures: H. Singh:** A. Employment/Salary (full or part-time):; Full time. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; American Heart Association. **J. Schappi:** None. **G. Pandey:** None. **M.M. Rasenick:** A. Employment/Salary (full or part-time):; Full-time, University of Illinois at Chicago. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH RO1

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.05/SS16

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH R01AT009169

NIH T32 MH067631

VA BX001149

**Title:** Several antidepressants increase expression and activity of fatty acid desaturases

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**Abstract:** We have demonstrated that all classes of antidepressants translocate $G\alpha_s$ from plasma membrane lipid raft domains to non-raft domains, where $G\alpha_s$ is more likely to couple with its second effector adenylyl cyclase. This phenomenon has been demonstrated in both rats and cultured neural or glial cells, and cells respond to drug treatment within 3 days (15 minutes for ketamine). These drugs also accumulate, slowly, in lipid rafts. We have also shown that in post-mortem brain tissue from depressed subjects $G\alpha_s$ is localized in lipid rafts. These data are consistent with a clinically relevant /non-canonical action of antidepressants. One extant question concerns the possibility that some genetic program is elicited as a response of antidepressant treatment. To test this, we carried out an RNA sequencing study analyzing changes in mRNA expression. After three days escitalopram or imipramine treatment of primary rat astrocytes, fatty acid desaturase 1 (FADS1), fatty acid desaturase 2 (FADS2) and Stearoyl-CoA desaturase-1 (SCD1), showed significant increases. Western blotting confirmed increased protein expression levels of all desaturating enzymes in C6 glioma cells after three days treatment of escitalopram, imipramine, and phenelzine. Matrix-assisted laser desorption/ionization (MALDI) mass spec analysis of the plasma membrane derived from C6 cells revealed an antidepressant-induced increase in wide array of polyunsaturated fatty acids (PUFAs). Furthermore, probing for membrane fluidity using laurdan indicates an overall increase in membrane fluidity reflecting an increase in PUFAs present in the plasma membrane of antidepressant treated C6 cells. These changes in the plasma membrane may be responsible for the redistribution of $G\alpha_s$ to non-raft

micro-domains after antidepressant treatment and are consistent with antidepressant effects of n-3 PUFA.

**Disclosures:** **N. Wray:** None. **M.M. Rasenick:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience. F. Consulting Fees (e.g., advisory boards); Otsuka.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.06/SS17

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** T32 MH067631

R01 AT009169

VA BX001149

**Title:** Adenylyl cyclase VI is required for SERT-independent antidepressant effects in glial cells: A potential screen for novel antidepressants

**Authors: *J. SCHAPPI**[1], A. H. CZYSZ[2], S. J. ERB[3], M. M. RASENICK[4]
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**Abstract:** Depression is a major public health problem and antidepressants are in widespread use throughout the world. Despite decades of investigation, the molecular mechanism for their action is unknown. Especially confusing is the long lag period (6-8 weeks) between the initiation of therapy and clinical effect. Antidepressants of different chemical classes promote sustained increases in cAMP, due, at least in part to the redistribution of $G_{\alpha}s$ from lipid rafts into non-raft membrane fractions resulting in increased $G\alpha_s$ functional coupling with adenylyl cyclase (AC). This has been demonstrated in both rats and cultured neural and glial cells by a number of techniques, including cell fractionation, functional assays, and imaging studies such as Fluorescence Recovery After Photobleaching (FRAP). Although monoamine transporters have been assumed to be a target for many antidepressants, C6 cells lack these, yet still show a "signature" antidepressant response. Furthermore, while these neural and glial cells showed an "antidepressant response", kidney epithelial cells like COS7 and HEK293 (or kidney and liver from rat) were unchanged by antidepressant treatment. In this study we sought to determine whether cellular antidepressant response, with respect to increased cAMP signaling and $G\alpha_s$ localization, is dependent on the type of AC isoform expression. Cell lines "insensitive" to

antidepressant treatment, such as HEK293, become responsive after transfection with AC6 (but not AC2 or AC3), showing translocation of $G\alpha_s$ from lipid rafts, both by cell fractionation and by FRAP. Thus, it is suggested that AC6 performs an anchoring function for $G\alpha_s$ outside of rafts, affixing the translocated $G\alpha_s$ into the non-raft domain, or perhaps even creates an antidepressant binding site, facilitating an antidepressant-induced increase in adenylyl cyclase activity.

**Disclosures: J. Schappi:** None. **A.H. Czysz:** None. **S.J. Erb:** None. **M.M. Rasenick:** A. Employment/Salary (full or part-time):; University of Illinois at Chicago Departments of Physiology & Biophysics and Psychiatry, Jesse Brown VAMC. 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.; R01 AT009169, T32 MH067631, VA BX001149. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pax Neuroscience. F. Consulting Fees (e.g., advisory boards); Otsuka Pharmaceutical.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.07/SS18

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** RAS L.R. 7/2007-CRP10810/2012

**Title:** Antidepressant drug signalling through $LPA_1$ in human fibroblasts

**Authors: *P. ONALI**, S. DEDONI, M. C. OLIANAS
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**Abstract:** Lysophosphatidic acid (LPA) is a major regulator of fibroblast function and drugs that block LPA receptors have been proposed to be effective agents in controlling abnormal fibroblast activity in distinct fibrotic diseases. We have recently reported that in Chinese hamster ovary fibroblasts different classes of antidepressants stimulate intracellular signalling by activating endogenous $LPA_1$, thus providing the first evidence that this receptor is a molecular target of certain antidepressants. We have also shown that these drugs activate the human $LPA_1$ over-expressed in HEK-293 cells. However, whether antidepressants are capable to signal through the human $LPA_1$ expressed and operative under native conditions remains unknown. In the present study we examined the effects of tricyclic and tetracyclic antidepressants on ERK1/2 signalling and DNA synthesis in cultured human dermal fibroblasts. We found that in these cells LPA potently induced ERK1/2 phosphorylation and stimulated [$^3$H]-thymidine incorporation into DNA. These effects were mimicked by the antidepressants amitriptyline, clomipramine and

mianserin. AM966, a selective $LPA_1$ antagonist, and Ki16425, a $LPA_{1/3}$ blocker, antagonized LPA stimulation of ERK1/2 phosphorylation in a concentration-dependent manner with nanomolar potencies. Blockade of $LPA_1$ also prevented the stimulatory effects of amitriptyline and mianserin on ERK1/2 phosphorylation and DNA synthesis. These data demonstrate for the first time that different antidepressants can act through the human $LPA_1$ expressed in fibroblasts to induce ERK1/2 activation and mitogenesis.

**Disclosures: P. Onali:** None. **S. Dedoni:** None. **M.C. Olianas:** None.

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**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.08/SS19

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** DARPA/ARO Contract #W911NF-14-2-0043

**Title:** Real-time decoding of mood from human large-scale ECoG activity

**Authors: *O. G. SANI**[1], Y. YANG[2], E. F. CHANG[4], M. M. SHANECHI[3]
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**Abstract:** Identifying reliable neural biomarkers that are predictive of mood dynamics in an individual is important both for personalized treatment of mood disorders and for understanding the neural processes underlying mood regulation. Here, based on semi-chronic, high-resolution, multi-site ECoG recordings obtained from epilepsy patients and simultaneous self-reports of mood (Immediate Mood Scaler), we identify network-level neural biomarkers that are predictive of mood dynamics over time in individual patients. To identify the biomarker, we first use a data-driven linear state-space modelling framework to describe neural network dynamics. Fitting the state-space model using neural features extracted from ECoG recordings, we find that a low-dimensional neural state is sufficient to describe the dynamics of large-scale ECoG. We estimate this low-dimensional neural state in real time using a Kalman filter. We then build a regression model that predicts mood variations over time from the estimated low-dimensional neural state. We find that using network ECoG activity and our modelling framework, we can decode mood variations over time in individual subjects. We further use the identified dynamical models to characterize the spatial and temporal characteristics of the mood biomarkers. Our analyses reveal subnetworks in the limbic system that are highly predictive of an individual's mood variations over time. We also identify the time-scales of these mood biomarkers. These dynamical mood biomarkers and mood decoding algorithms can provide insight into brain processes underlying

mood regulation and also enable the development of personalized stimulation therapies for depression based on feedback of the estimated mood.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.09/SS20

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** DARPA/ARO Contract # W911NF-14-2-0043

**Title:** Modeling dynamic brain-network responses to electrical stimulation

**Authors: *Y. YANG**[1], K. K. SELLERS[2], E. F. CHANG[2], M. M. SHANECHI[1]
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**Abstract:** Modeling the effects of electrical stimulation on brain network dynamics plays a key role in developing automatic closed-loop stimulation therapies for neurological disorders such as depression. Our prior work has proposed to use a multivariate linear state-space model (LSSM) to describe brain network dynamics in response to stimulation input (input-output model). In addition, we have designed a binary noise (BN) modulated electrical stimulation pattern---a pulse train modulated by stochastic BN parameters such as pulse frequency and amplitude---to collect optimal input-output datasets for model identification. Here, we develop and validate the input-output brain network model by performing stimulation experiments in epilepsy patients. In these experiments, we apply the BN-modulated electrical stimulation pattern and concurrently record the high-dimensional electrocorticography (ECoG) response. As the input in our models, we use the pulse frequency and amplitude. As the output in our models, we use ECoG power features. We use a template-subtraction artifact removal algorithm to remove stimulation artifacts from ECoG activity during stimulation. Based on the collected input BN stimulation and output ECoG datasets, we fit a LSSM. We first evaluate how well the fitted model predicts the dynamic response of ECoG features. To do this, we use the fitted model to feed-forward predict the evolution of neural features in response to BN stimulation. We compare the predicted evolution to the observed evolution. Further, we evaluate how well the model predicts the steady-state neural response by performing a separate continuous stimulation experiment on the same patient. In this continuous stimulation experiment, we stimulate from the same location with pulse trains of fixed amplitudes. We then compare the observed continuous stimulation response to the steady-state response predicted by the LSSM. We find that the identified LSSM from BN stimulation experiments 1) achieved good prediction of the neural dynamics in cross

validation and 2) was significantly predictive of the steady-state responses measured in the continuous stimulation experiments. We also find that both stimulation pulse frequency and amplitude play important roles in regulating the output neural responses. Our results provide insight into the effect of electrical stimulation on human ECoG dynamics, and have important implications for devising novel model-based closed-loop therapies for treatment of various neuropsychiatric disorders.

**Disclosures: Y. Yang:** None. **K.K. Sellers:** None. **E.F. Chang:** None. **M.M. Shanechi:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.10/SS21

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** CA1 and dentate gyrus synaptic transmission and plasticity after physical exercise in an animal model for depression

**Authors: E. DAHLIN**, E. HANSE, *H. SETH
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**Abstract:** Physical exercise has long been regarded as beneficial for wellbeing, physical as well as mental. However, it is not until more recently that detailed studies on the effects of physical exercise has been conducted. Among the beneficial effects, physical exercise has proven to be anti-depressant and is now prescribed by medical doctors in several countries. The mechanisms behind the anti-depressive effect are, however, not well known.
Several reports indicate an altered hippocampal function in depressed humans as well as in animal models. For example, depression is associated with a decreased synaptogenesis and neurogenesis in hippocampus leading to reductions in synaptic transmission and plasticity, most strikingly CA3-CA1 and dentate gyrus long-term potentiation (LTP).
Importantly, it is also known that running increases neurogenesis and facilitates LTP in the dentate gyrus. Therefore, it is not surprising that cognitive functions associated with hippocampus-dependent tasks are restored when some patients with depression are exercising. However, a detailed study of the events that leads to a restored cognitive function after physical exercise in an animal model for depression is lacking. We examine this by extracellular field recordings to study baseline synaptic transmission and plasticity at Schaffer collateral CA3 to radiatum CA1 synapses as well as medial perforant path to dentate gyrus synapses in an animal model for depression.
We used two age groups of male Wistar rats, P30-P40 or P60-P70, from either dexamethasone or saline treated dams. Dexamethasone (150ug/kg) or saline was injected daily for five consecutive days during the last trimester to create offspring with a depressive-like phenotype. Animals at

P30 or P60 were then allowed to run voluntarily for 10 days prior to electrophysiological recordings. Hippocampal samples are also stored to allowed analysis of proliferation and neurogenesis in the dentate gyrus. Later experiments will include patch-clamp recordings of evoked current in immature as well as mature neurons.
Experiments are ongoing but preliminary data show a limited effect of running on synaptic transmission and plasticity in exercising young P30 control anoimals. Whether this holds true also in older or depressed rats are under investigation. A longer running regime might also be needed in order to reveal the true effects of exercise on cognition.

**Disclosures: E. Dahlin:** None. **E. Hanse:** None. **H. Seth:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.11/SS22

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** KAKENHI

JST-CREST

**Title:** Altered expression of long, noncoding RNAs in patients with major depression

**Authors: *T. SEKI**, H. YAMAGATA, S. UCHIDA, K. HARADA, K. MATSUO, Y. WATANABE
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**Abstract:** Although depression is the leading cause of disability worldwide, its pathophysiology is poorly understood. There is growing evidence suggesting that aberrant transcription regulation play an important role in the pathophysiology of depression. Although long noncoding RNAs (lncRNAs) have important functions in chromatin structure, gene expression, and the subsequent manifestation of various biological processes in the central nervous system, it is still unclear whether the aberrant expression of lncRNAs is associated with the pathophysiology of major depressive disorder (MDD). We therefore examined the expression of lncRNAs in peripheral blood leukocytes as predictive biomarkers for MDD. The study sample included 39 patients with MDD under a current depressive state and 40 healthy subjects, who were matched for age and sex. This study was approved by the Institutional Review Board of Yamaguchi University Hospital. Written informed consent was obtained from all participants after providing them with a complete description of the study. We measured the expression levels of 84 lncRNAs in the peripheral blood leukocytes of patients with MDD and healthy subjects using a quantitative real-time PCR analysis. We found that patients with MDD exhibited distinct expression signatures.

Several lncRNAs were differentially expressed in patients with MDD versus healthy subjects. Thus our data suggest that peripheral lncRNAs may serve as potential biomarkers for MDD. The study also suggests the contribution of lncRNAs in the pathophysiology of MDD. Further study would be necessary to clarify the role of lncRNAs in an animal model of depression.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** MEXT/JSPS KAKENHI Grants

Grant-in-Aid from the Japanese Ministry of Health and Labor

**Title:** The relationship between circulating mitochondrial DNA and inflammatory cytokines in patients with major depression

**Authors: *Y. KAGEYAMA**[1,2], T. KASAHARA[2], M. KATO[3], S. SAKAI[3], Y. DEGUCHI[1], M. TANI[4], K. KURODA[5], K. HATTORI[6], S. YOSHIDA[7], Y. GOTO[6], T. KINOSHITA[3], K. INOUE[1], T. KATO[2]
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**Abstract:** Although inflammatory cytokines are established biomarkers of mood disorders, their molecular mechanism is not known. We hypothesized that circulating mitochondrial DNA (mtDNA) contributes to inflammation and could be used as biomarkers. We investigated if circulating mtDNA level is associated with inflammatory cytokines and can be used as a biomarker of mood disorders. This study was approved by the ethics committees of Hannan Hospital, Osaka City University, Kansai Medical University, RIKEN, and National Center of Neurology and Psychiatry and conducted in accordance with the Declaration of Helsinki. Plasma mtDNA level was measured with real-time quantitative PCR targeting two regions of the mtDNA and plasma level of four cytokines (GM-CSF, IL-2, IL-4, and IL-6) were measured with a multiplex immunoassay method in 109 patients with major depressive disorder (MDD). The most significantly correlated cytokine was verified with an enzyme-linked immunosorbent assay

(ELISA). The data from 28 patients with bipolar disorder (BD), 17 patients with schizophrenia, and 29 healthy controls were compared. MtDNA levels showed a nominal positive correlation with GM-CSF, IL-2, and IL-4 in patients with MDD. The most significant correlation with IL-4 ($\rho$ = 0.38, $P$ < 0.00005) was verified with an ELISA ($\rho$ = 0.19, $P$ = 0.0049). Unexpectedly, patients with MDD and BD showed significantly lower plasma mtDNA levels than controls. MtDNA levels were lower in the depressive state than in the remitted state in patients with MDD. Plasma mtDNA is reportedly affected by exercise (Lim *el al*., 2000) possibly due to the release from muscles. Thus, decreased physical activity during the depressive state could decrease the circulating mtDNA. In this regard, plasma mtDNA might be a candidate biomarker reflecting psychomotor retardation.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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Otsuka Pharmaceutical Ltd.

**Title:** Alterations in the amino acids in the mouse brain regions after adjunctive treatment of brexpiprazole with fluoxetine: Comparison with (R)-ketamine

**Authors: *Q. REN**[1], M. MA[1], Y. FUJITA[1], C. YANG[1], C. DONG[1], Y. OHGI[2], T. FUTAMURA[2], K. HASHIMOTO[1]
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**Abstract:** Brexpiprazole, a serotonin-dopamine activity modulator, is approved in the USA as an adjunctive therapy to antidepressants for the treatment of major depressive disorder (MDD). Similar to the *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine, the combination of brexipiprazole and fluoxetine has shown antidepressant-like effects in animal models of depression. The present study was undertaken to examine whether the combination of brexpiprazole and fluoxetine could affect tissue levels of amino acids (glutamate, glutamine, GABA, D-serine, L-serine, glycine) related with NMDAR neurotransmission. A single injection of the combination of fluoxetine and brexpiprazole significantly increased GABA levels in the

striatum, and it significantly increased the D-serine/L-serine ratio in the frontal cortex and glycine/L-serine ratio in the hippocampus. Repeated administration of combination of two drugs significantly altered tissue levels of amino acids in the all regions. Interestingly, repeated administration of combination of two drugs significantly decreased D-serine/L-serine ratio in the frontal cortex striatum and hippocampus. In contrast, a single administration of (*R*)-ketamine significantly increased D-serine/L-serine ratio in the frontal cortex. In conclusion, these results suggest that alterations in these amino acids may be involved in the antidepressant-like effects of the combination of brexpiprazole and fluoxetine.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Otsuka Pharmaceutical Ltd

The Nurture of Creative Research Leaders in Immune System Regulation and Innovative Therapeutics Program of Chiba University

JSPS Fellowship

**Title:** Adjunctive treatment of brexpiprazole with fluoxetine shows a rapid antidepressant effect in social defeat stress model: Role of BDNF-TrkB signaling

**Authors: M. MA**[1], Q. REN[3], C. YANG[2], J.-C. ZHANG[2], W. YAO[2], C. DONG[2], Y. OHGI[4], T. FUTAMURA[4], *K. HASHIMOTO[5]
[1]Clin. Neurosci, [2]Chiba Univ. Ctr. for Forensic Mental Hlth., Chiba, Japan; [3]Chiba Univ. Ctr. Forensic Mental Hlth., Chiba, Japan; [4]Otsuka Pharmaceut. Ltd, Tokushima, Japan; [5]Chiba Univ. Ctr. Forensic Men Hlth., Chiba, Japan

**Abstract:** Brexpiprazole, a serotonin-dopamine activity modulator, is approved in the USA as an adjunctive therapy to antidepressants for the treatment of major depressive disorder (MDD). Addition of low doses of the atypical antipsychotic drug brexpiprazole with selective serotonin reuptake inhibitors (SSRIs) could promote antidepressant effect in patients with MDD although

the precise mechanisms underlying the action of the combination are unknown. Combination of low dose of brexpiprazole (0.1 mg/kg) and SSRI fluoxetine (10 mg/kg) could promote a rapid antidepressant effect in social defeat stress model although brexpiprazole or fluoxetine alone did not show antidepressant effect. Furthermore, the combination of brexpiprazole and fluoxetine significantly improved alterations in the brain-derived neurotrophic factor (BDNF) - TrkB signaling and dendritic spine density in the prefrontal cortex, hippocampus, and nucleus accumbens in the susceptible mice after social defeat stress. Interestingly, TrkB antagonist ANA-12 significantly blocked beneficial effects of combination of brexpiprazole and fluoxetine on depression-like phenotype. These results suggest that BDNF-TrkB signaling plays a role in the rapid antidepressant action of the combination of brexpiprazole and fluoxetine.

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**Poster**

**611. Depression and Antidepressants: Mechanism**

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**Support:** Japan Society for the Promotion of Science KAKENHI Grants

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**Title:** Identification of lysophosphatidic acid receptor 1 in astrocytes as a target for glial cell line-derived neurotrophic factor expression induced by antidepressants

**Authors: *N. KAJITANI**[1], K. MIYANO[3], M. OKADA-TSUCHIOKA[1], H. ABE[1], W. OMORI[1,2], K. ITAGAKI[1,2], Y. UEZONO[3,4], M. TAKEBAYASHI[1,2]
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**Abstract:** [Background] Preclinical and clinical evidence suggests that glial cell line-derived neurotrophic factor (GDNF) is important in the therapeutic effect of antidepressants. A previous study demonstrated that the tricyclic antidepressant amitriptyline induces Gi/o activation, which leads to GDNF expression in astrocytes. However, the specific target expressed in astrocytes that mediates the antidepressant-evoked Gi/o activation has yet to be identified. Thus, the current study examined the possibility that antidepressant-induced Gi/o activation depends on

lysophosphatidic acid receptor 1 (LPAR1), a Gi/o-coupled receptor. [Method] Rat C6 astroglial cells (C6 cells), primary cultured astrocytes, and primary cultured neurons were used in the following experiments. GDNF mRNA expression was examined using real-time PCR, Gi/o activation was examined using the cell-based receptor assay system CellKey™, and intracellular signaling cascades were examined using immunoblotting. [Results] AM966 (a selective LPAR1 antagonist) and Ki16425 (a selective LPAR1/3 antagonist), but not H2L5186303 (a selective LPAR2 antagonist), blocked GDNF mRNA expression and Gi/o activation evoked by various classes of antidepressants (amitriptyline, nortriptyline, mianserin, and fluoxetine) in C6 cells. In addition, deletion of LPAR1 by RNAi suppressed amitriptyline-evoked GDNF mRNA expression. Treatment of astroglial cells with the endogenous LPAR agonist LPA increased GDNF mRNA expression and GDNF protein release through LPAR1, whereas treatment of primary cultured neurons with LPA failed to affect the GDNF mRNA expression. The potential involvement of the transactivation cascade (FGFR, FRS2α and ERK1/2) related to GDNF expression in LPAR1-mediated signaling was examined. Pertussis toxin (a specific Gi/o inhibitor), SU5402 (an FGFR inhibitor), or U0126 (a MEK/ERK inhibitor) completely blocked LPA-evoked GDNF mRNA expression in C6 cells. Amitriptyline-induced phosphorylation of ERK1/2 and phosphorylation of FRS2α, a surrogate of FGFR activation, were significantly inhibited by AM966 and Ki16425, but not H2L5186303. LPA-induced phosphorylation of both ERK1/2 and FRS2α were also significantly inhibited by AM966 and Ki16425. [Conclusion] The current data are the first to clearly demonstrate that Gi/o-coupled LPAR1 in astrocytes mediates GDNF expression evoked by various antidepressants. It is suggested that LPAR1 is a novel, specific target of antidepressants which leads to GDNF expression in astrocytes.

**Disclosures:** **N. Kajitani:** None. **K. Miyano:** None. **M. Okada-Tsuchioka:** None. **H. Abe:** None. **W. Omori:** None. **K. Itagaki:** None. **Y. Uezono:** None. **M. Takebayashi:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.16/SS27

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** RAS L.R.7/2007-CRP10810/2012

**Title:** Inhibition of cytokine-induced intracellular signalling and neuronal cell death by antidepressants acting through $LPA_1$

**Authors: *M. C. OLIANAS**, S. DEDONI, P. ONALI
Biomed. Sciences, Sect. Neurosci., Univ. of Cagliari, Monserrato, Italy

**Abstract:** Pre-clinical and clinical studies have suggested that pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1a, IL-6 and interferons (IFN), play a role in the pathogenesis of major depression. A cellular mechanism by which these cytokines may exert a depressogenic effect is the impairment of hippocampal neural progenitor cell development and survival. However, how antidepressants can counteract the neurotoxic effects of pro-inflammatory cytokines is still not completely understood. We have recently discovered that distinct classes of antidepressants act through the lysophosphatidic acid (LPA) receptor $LPA_1$ to trigger growth factor receptor transactivation, cell proliferation and protection of glial cells from oxidative stress. In the present study we report that in mouse HT22 immortalized hippocampal cells, a neuroblast-like cell line, TNF-α-induced apoptotic cell death is inhibited by the tetracyclic antidepressants mianserin and mirtazapine and that this protection is mediated by $LPA_1$. TNF-α (10 ng/ml) enhanced the activity of caspases 8, 7 and 3, induced the cleavage of poly ADP-ribose polymerase (PARP) and increased DNA fragmentation. Cell exposure to either mouse IL-1a, IFN-β, IFN-γ or IL-6 failed to affect per se PARP cleavage, but enhanced the stimulatory effect of TNF-α, indicating the occurrence of a synergistic interaction between the cytokines in inducing apoptosis. Pre-treatment with the antidepressants failed to affect the stimulation of caspase 8 by TNF-α but markedly inhibited the induction of cytochrome c release from mitochondria, the activation of caspases 9 and 3, the stimulation of PARP cleavage, and the increase of DNA fragmentation elicited by the cytokine. Mianserin also counteracted the enhanced apoptotic response triggered by TNFα in association with either IL-1a or IFN-β. Pharmacological blockade of $LPA_1$ with the selective antagonist AM966 inhibited the anti-apoptotic effects of the antidepressants. These data show for the first time that certain antidepressants protect neuronal cells from cytokine-induced apoptotic cell death through activation of $LPA_1$.

**Disclosures: M.C. Olianas:** None. **S. Dedoni:** None. **P. Onali:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.17/SS28

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** The effect of Deep Brain Stimulation (DBS) on stress-induced changes in electrical activity of Locus Coeruleus (LC) in awake, freely moving rats

**Authors: *S. TORRES-SANCHEZ**[1,2,3], E. BERROCOSO[1,2,3], R. J. VALENTINO[4], J. A. MICO[1,2,3], A. L. CURTIS[4]
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Investigación e Innovación en Ciencias Biomédicas de Cádiz (INiBICA), Cadiz, Spain; [4]Dept Anesthesiol., Children's Hosp. Philadelphia, Philadelphia, PA

**Abstract:** Deep Brain Stimulation (DBS) of the subgenual cingulated cortex (SCC) is an innovative therapeutic approach that has reported promising results in resistant major depressive patients. Preclinical studies using DBS of the ventromedial prefrontal cortex (vmPFC), the rodent correlate to human SCC, demonstrated its antidepressant effect along with consistent changes in the activity of locus coeruleus (LC)-noradrenergic system and in the rhythm of pontine-cortical network oscillations. Given LC-noradrenergic system is modified after stressful conditions and by antidepressants treatment, the aim of this study was to evaluate the effect of vmPFC DBS on stress-induced changes in electrical activity of LC neurons in unanaesthetized rats. For this purpose, the tonic and auditory-evoked activity of LC neurons were recorded before and after acute social stress exposition in control and vmPFC DBS treated rats.The results showed a robust inhibitory effect of DBS on tonic discharge rate of LC neurons after social stress, in contrast to the increased firing rate observed in control animals. Auditory stimulation generated an evoked response that was reduced after social stress in control and DBS rats, measured as lower number of evoked spikes discharged. However, the duration of the evoked response was significantly shorter in control animals after social stress than before stress exposition. By contrast, the duration of the evoked response in DBS animals remained similar after and before social stress exposure. Interestingly, stress tended to reduce the signal to noise ratio but DBS enhanced it.These results suggest that DBS promotes an inhibitory mechanism on LC induced by stress, inhibiting the tonic activity of LC neurons that might represent the more rapid LC-noradrenergic system recovery after stress. Furthermore, the modifications of vmPFC DBS on stress-induced changes on LC activity pattern might be to lead an optimal behavioral state as a strategy to combat stress. Supporting by: MH093981, CIBERSAM G18, Spain’s Ministerio de Economía y Competitividad SAF2015-68647-R, FIS (PI12/00915), CTS-510, CTS-7748, 2011-145-FPI fellowship.

**Disclosures:** **S. Torres-Sanchez:** None. **E. Berrocoso:** None. **R.J. Valentino:** None. **J.A. Mico:** None. **A.L. Curtis:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.18/SS29

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH grant MH108043

IOER funds

PhRMA Foundation Starter Grant

**Title:** Synaptic potentiation, cytoskeletal remodeling, and rapid-acting antidepressant actions

**Authors: Z. REHMAN**[1], S. SAICHELLAPPA[1], L. HERRING[3], L. SEMKE[1], *N. A. O'CONNELL[2], E. WAUSON[1], L. GRAVES[3], V. DURIC[1], L.-I. YUAN[1]
[1]Physiol. and Pharmacol., [2]Des Moines Univ., Des Moines, IA; [3]Pharmacol., Univ. of North Carolina, Chapel Hill, NC

**Abstract:** Ketamine at sub-anesthetic doses has shown promising results as a potent and fast-acting antidepressant. Evidence from animal model studies suggests that the functional restoration by ketamine is associated with activation of MAPK and mTOR signaling cascades in the prefrontal cortex (PFC) within a few hours of ketamine administration, followed by a second wave of synaptic protein upregulation. Together, these molecular events lead to rapid synaptogenesis and reversal of neural atrophy. Furthermore, (2*R*, 6*R*)-hydroxynorketamine (HNK) has recently been identified as the main active component of ketamine metabolism. HNK is believed to be responsible for the antidepressant actions of ketamine, but with minimal side effects, and potentially through NMDAR-independent mechanism of action. However, critical gaps exist in understanding the exact molecular mechanisms of ketamine actions. Thus, it would be of great importance and interest to delineate the molecular mechanisms that translate mTOR activation into synaptogenesis-based structural remodeling. Cofilin and its upstream pathways, ideally positioned to bridge the gap between mTOR activation and spine formation, have emerged from an unbiased kinome/phosphoproteome screening for targets of ketamine treatment. As the major cytoskeletal component of dendritic spines, actin controls spine morphology and formation through changes in its polymerization state. Actin polymerization is negatively regulated by activation of cofilin protein. Further studies are currently underway to address the significance of cofilin phosphorylation and actin polymerization in synaptic potentiation induced by ketamine and HNK.

**Disclosures: Z. Rehman:** None. **S. Saichellappa:** None. **L. Herring:** None. **L. Semke:** None. **N.A. O'Connell:** None. **E. Wauson:** None. **L. Graves:** None. **V. Duric:** None. **L. Yuan:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.19/SS30

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NI Centre for Stratified Medicine: Centre Grant

**Title:** Methylome profiling of young adults with depression supports link with immune response

**Authors: *C. R. LAPSLEY**[1], R. IRWIN[2], M. MCLAFFERTY[3], S.-J. THURSBY[2], S. O'NEILL[3], A. BJOURSON[1], C. WALSH[2], E. MURRAY[1]
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**Abstract:** Depression is the leading cause of global disability, affecting over 300 million people of all ages worldwide, with alarming rates of psychopathology recently identified among young people. Mental health problems typically develop in adolescence, and suicide is the second leading cause of death in young people aged 5-24. Despite its prevalence, the biology of depression and risk factors for suicide are poorly understood, hence there is a critical need to develop biomarkers that can be used to assist in diagnosis and identify individuals at risk. Genetic and environmental factors have been linked to increased risk for depression and suicide and their interaction may lead to development of mental illness. The aim of this study was to compare the DNA methylome of individuals with depression and matched controls to determine differences in methylation associated with the disorder. Participants recruited to the Ulster University student wellbeing study provided a saliva sample and completed a clinically validated online mental health survey (DSM-IV criteria). Cases of severe depression with comorbid 12 month and life-time self-harm and suicide attempt and age and gender matched controls were selected (n=32; 16 cases and 16 controls). DNA was prepared and quantified using the Illumina Infinium Methylation EPIC Kit, and validated with pyrosequencing. Raw data from the scanner (idat files) were processed using the RnBeads pipeline in the R statistical and programming environment. Samples separate out by gender, confirming the known difference in methylation between the sexes. There is a separation of depressed and healthy individuals in the female group, suggesting that there are differences in methylation between participants with depression and those without. Smoking status and age cofounders did not significantly contribute to the clustering associated with depression. Analysis of the top 1000 ranked sites shows that many of these are CpG gaining methylation in the depression group, and gene ontology analysis of the promoters showing largest change in methylation from this group indicated highly significant enrichment for immune response. Depression is associated with significant effects on DNA methylation, and the genes most affected are related to immune function in females. This data suggests an immune component to the aetiology of depression, consistent with the accumulating evidence supporting a relationship between inflammation and depression

**Disclosures: C.R. Lapsley:** None. **R. Irwin:** None. **M. McLafferty:** None. **S. Thursby:** None. **S. O'Neill:** None. **A. Bjourson:** None. **C. Walsh:** None. **E. Murray:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.20/SS31

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Nucleus accumbens deep brain stimulation increases mammalian target of rapamycin and glycogen synthase kinase 3 phosphorylation in ventral hippocampus

**Authors: *T. L. NGUYEN**[1], R. P. KALE[2], S. J. TYE[3]
[1]Mol. Pharmacol. & Exptl. Therapeut., [3]Psychiatry & Psychology, [2]Mayo Clin., Rochester, MN

**Abstract: Background:** While antidepressant effects of deep brain stimulation (DBS) are associated with progressive neuroadaptations within the mood network, their underlying molecular mechanisms are poorly understood. Glycogen synthase kinase 3 (GSK3) and mammalian target of rapamycin (mTOR) are important regulators of neuroplasticity. In this study, we investigated the effect of chronic nucleus accumbens (NAc) DBS on GSK3 and mTOR protein expressions within the dorsal (dHIP) and ventral hippocampus (vHIP) in an antidepressant-resistant rat model.
**Method:** Antidepressant-resistance was induced by daily injection of adrenocorticotropic-hormone (ACTH; 100 μg/day; 15 days). Portable microdevices provided continuous bilateral NAc DBS (130Hz, 100μA, 90μs) for 7 days. Four conditions were set up: ACTH (n=10), saline control (n=10), ACTH DBS (n=15), and ACTH sham control (n=11). Antidepressant efficacy was assessed using the forced swim test. Total and phosphorylated levels of GSK3 and mTOR within the dHIP and vHIP were quantified via Western Blot.
**Results:** Within the vHIP, we detected increased level of phospho-GSK3β in DBS compared to ACTH animals ($p<0.01$), and increased level of phospho-mTOR in DBS and sham compared to ACTH animals ($p<0.05$). No significant differences were detected within the dHIP. These vHIP increases of phospho-GSK3β and phospho-mTOR occurred concurrent with DBS antidepressant efficacy ($p<0.001$ relative to ACTH).
**Conclusion:** In an antidepressant-resistant rat model, chronic NAc DBS upregulated phospho-GSK3β and phospho-mTOR within the vHIP of the mood network and elicited antidepressant-like behavior.

**Disclosures: T.L. Nguyen:** None. **R.P. Kale:** None. **S.J. Tye:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.21/SS32

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** 5P50MH106428-03

**Title:** Which Lateral Habenula output pathway mediates the antidepressant effect of inhibitory DREADDs?

**Authors: *K. COFFEY**, J. NEUMAIER, P. BARTLETT
Univ. of Washington, Seattle, WA

**Abstract:** The lateral habenula (LHb) is small epithalamic region that acts as a key gateway between limbic forebrain neurons and the midbrain monoaminergic systems. Over the past decade there has been resurgence of interest in the LHb as evidence has emerged that it plays a central role in encoding rewarding and aversive stimuli and in neuropsychiatric diseases, including mood disorders. Recently, we found that expressing an inhibitory DREADD (hM4Di) in the LHb of rats and administering the otherwise inert ligand, clozapine-N-oxide (CNO) produced an antidepressant-like effect in the modified forced swim test (mFST) but the downstream target of LHb neurons mediating this effect is not known. There are three important targets of glutamatergic outputs from LHb: ventral tegmental area (VTA), rostral medial tegmental nucleus (RMTG), and dorsal raphe nucleus (DRN). Isolating the pathway responsible for this effect could provide an excellent target for developing novel antidepressant treatments with fewer off-target effects. In order to study the effect of inhibiting these pathways in isolation we utilized an intersectional viral vector strategy: AAV-EF1 α-DIO-$HM_4Di$ injected into LHb, and CAV2-Cre (a retrograde viral vector) injected into one of the three target areas in 16 rats per pathway. We utilized the mFST in a 3x2 design to determine if inhibiting any (or all) of the three pathways are sufficient to confer an antidepressant-like effect. We developed a novel automated activity scoring algorithm to provide unbiased behavioral output which compared well to conventional time sampling analysis of the mSFT. A small number of “anatomical misses”, that showed no LHb DREADD expression, generated a useful negative control group. The only treatment difference between groups was injection of either vehicle or CNO. Our results suggest that inhibiting the LHb to DRN pathway provides an anti-depressant effect, while inhibition of the other pathways does not. Animals with $hM_4Di$ in the LHb to DRN pathway that were treated with CNO showed less inactivity during the mFST, as well as increased swimming and climbing. These results supports the idea that inhibiting the LHb to DRN pathway provides animals with resilience to the behavioral despair normally induced by the mFST, and also opens up the LHb to DRN pathway to manipulation by highly specific therapeutics for the treatment of depression.

**Disclosures:** **K. Coffey:** None. **J. Neumaier:** None. **P. Bartlett:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.22/SS33

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** MH14654

DA041180

**Title:** Identification of genes under the transcriptional regulation of CREB or CREM in the hippocampus

**Authors: *M. T. MANNERS**[1], J. K. BRYNILDSEN[2], J. A. BLENDY[2]
[2]Systems Pharmacol. and Translational Therapeut., [1]Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Major Depressive Disorder (MDD) is a serious medical issue in the United States with over 16 million adults suffering from one or more major depressive episodes at any given time. Current antidepressant therapy is inadequate; with delayed efficacy and undesirable side effects, there is a clear unmet medical need for those requiring fast acting and continuous antidepressant therapy. The transcription factor cAMP response element-binding protein (CREB) regulates expression of many downstream genes that have been implicated in depression and the response to antidepressant treatment. Our lab has previously shown that reductions of CREB throughout the brain results in an antidepressant phenotype[1] as well as a rapid response to antidepressant treatment[2]. Further, localized deletion of CREB in the hippocampus is associated with an accelerated response to antidepressants[3]. However, in both constitutive and conditional CREB deletion models, the related Cre-binding protein CREM is upregulated. Overexpression of CREM activator isoform, $CREM_T$ in the hippocampus also resulted in an accelerated response to antidepressants[3]. While CREB and CREMτ can both bind to and activate CRE-target genes, it is not known if there are distinct genes that are differentially regulated by these proteins.
To further understand the individual roles of hippocampal CREB and CREM in depression, we constructed a mouse line with $Creb^{loxP/loxP}$ +/- Crem and an inducible enhanced GFP fused ribosomal L10a subunit (EGFP-L10a). Injection of adeno-associated virus expressing Cre recombinase into the hippocampus localized deletion of Creb and expression of EGFP-L10a to hippocampal neurons. Translating ribosome affinity purification (TRAP) was conducted by immunoprecipitation of the ribosomal subunit, and isolation of the actively translating mRNA. Comparative analysis of mRNA from wildtype and $Creb^{loxP/loxP;}$ +/- Crem enabled us to identify a unique population of CREB or CREM target mRNAs in the hippocampus. Identification of genes under the transcriptional regulation of CREB or CREM in the hippocampus will allow us to

better understand the role of CRE- binding proteins in depression and may ultimately result in identifying targets for future pharmacological therapy in the treatment of depression.

**Disclosures: M.T. Manners:** None. **J.K. Brynildsen:** None. **J.A. Blendy:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 611.23/SS34

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CNPq (Brazil)

Royal Society (UK)

**Title:** Excess significance bias in basic antidepressant research: The case of the forced swimming test

**Authors: *C. LINO DE OLIVEIRA**[1], M. R. MACLEOD[2]
[1]CFS-CCB-UFSC, Florianopolis, Brazil; [2]Univ. Edinburgh, Edinburgh, United Kingdom

**Abstract:** Our studies aim to apply the principles of 3R (reducing, refining and replacing) to animal models used in the basic antidepressant. Currently, our main strategy is to modify the experimental design and analysis of existent animal models, mainly the forced swimming test (FST). Regardless of the efforts to follow protocols previously published, the FST provided negative results in our laboratory [1] [2]. A systematic review of the literature revealed that quality of our work was similar to other publications and suggested the unlikely possibility that our negative results were the first of a kind in the literature. Alternatively, we may be facing consequences of excess significance which may inflate the expectation of positive results leading to unexpected, underpowered studies and negative results [3]. Therefore, the main objective in this work is to investigate the prevalence of positive results or, excess significance, in the studies using FST. For that, a comprehensive systematic review of the literature was performed retrieving 4355 publications organized in a database [4]. Sixty two studies randomly selected from the database were analyzed using quality scale [3]. Quality evaluation revealed that sex, strains and ages of animals, type of drug and via of administration, treatment schedule and protocols of FST seemed source of heterogeneity. Additionally, actions to avoid bias such as “random allocation to a treatment”, “concealment of treatment allocation” and “sample size calculation” were neglected for most of the publications evaluated. Independent of the quality score of the publication, 88% of the experiments rejected the null hypothesis for the primary outcome while 92% of the studies reported non-significant results for secondary outcomes. Moreover, 100% percent of the studies reported results agreeing with the primary hypothesis.

These data suggest that FST studies may excess significance bias to the field of basic antidepressant research. Further analyses are necessary to identify the origin and the impact of the excess significance in the field of basic antidepressant research. Indeed, the abundance of "positive results" in the basics contrasts with the partial success of antidepressant treatments in clinical trials or in therapeutics [5]. [1]Domingues, K. (2015) https://repositorio.ufsc.br/xmlui/handle/123456789/159865. [2]Summan, P. R. (2016) https://repositorio.ufsc.br/xmlui/handle/123456789/168078.[3]Sena, Emily S., et al. PLoS Biol 8.3 (2010): e1000344.[4]https://drive.google.com/file/d/0B30wfjnG6aEQRTdBRnFPRWlOcFU/view. [5]R. E. Becker, N. H. Greig, Sci. Transl. Med. 2, 61rv6, 2010.

**Disclosures: C. Lino De Oliveira:** None. **M.R. Macleod:** None.

**Poster**

**611. Depression and Antidepressants: Mechanism**

**Location:** Halls A-C

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**Program#/Poster#:** 611.24/SS35

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH Grant K99 MH108719-01

NIH Grant R37 MH068542

HDRF MPPN8883

NYSTEM

**Title:** Imaging fluoxetine modulation of dentate gyrus function

**Authors: *E. CARAZO**[1], C. ANACKER[1], R. HEN[2]
[1]Integrative Neurosci., [2]Neurosci. and Psychiatry, Columbia Univ., New York, NY

**Abstract:** The molecular effects of Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants, such as fluoxetine, have been studied extensively. However, despite being widely used in the clinic, only one-third of patients experience remission after up to four months of treatment with SSRIs. The lack of efficacy and delayed onset of action emphasizes the need to develop a more comprehensive understanding of the underlying mechanisms and neural circuits involved in anxiety regulation and antidepressant action. At the neural circuit level, it remains elusive how SSRIs modulate cellular networks implicated in mood- and anxiety-like behavior. We have previously shown that granule neurons of the dentate gyrus are crucial mediators of antidepressant effects on behavior. Here, we used miniature microscopes to perform in vivo $Ca^{2+}$ imaging in the ventral dentate gyrus of freely-moving mice, to investigate granule cell activity

during anxiogenic behavioral tasks before and after chronic fluoxetine treatment. We find that $Ca^{2+}$ activity of granule cells in the ventral dentate gyrus is increased during exploration of anxiogenic environments. Granule cell activity in the open arms of the elevated plus maze is increased by ~20% as compared to activity in the closed arms ($p<0.05$). These activity response patterns are modulated by chronic treatment with fluoxetine. Our data suggest that activity changes of granule cells in the ventral dentate gyrus may mediate anxiety-like behavioral responses.

**Disclosures: E. Carazo:** None. **C. Anacker:** None. **R. Hen:** None.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

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**Program#/Poster#:** 612.01/SS36

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NSF Grant 1547693

NSF Grant 1632891

**Title:** Use of a novel degradable biocomposite material as a delivery system for brain cell treatment and cell growth modification in 2D and 3D spheroid cultures

**Authors: *N. H. NGUYEN**, Z. NORCROSS, U. KANSAKAR, M. A. DECOSTER
Biomed. Engin., Louisiana Tech. Univ., Ruston, LA

**Abstract:** The three-dimensional (3D) cell spheroid model has emerged in recent years as a new approach in cell sciences in contrast to traditional 2D model with several advantages. Cell proliferation and morphology in 3D model better mimics the tissue micro-environment with potentially three layers of cells (proliferating, quiescent and necrotic), growing at different rates, instead of more even rate in 2D model. Furthermore, *in vitro* 3D model can give a more accurate response for drug treatment compared to 2D monolayers due to diffusion considerations. However, 3D model takes longer time to form and for the drug to take effect due to diffusion rate differences between the two models. Also, 3D spheroids data needs to be normalized (area and volume) to interpret the results. We here investigated the use of a novel degradable biocomposite material developed by our group called Copper High Aspect Ratio Structure (CuHARS), combined with 2D cells and 3D cell spheroid models as potential delivery system for cancer drug and cell growth modification. Two types of brain cells, brain tumor cells (CRL-2303 - American Type Culture Collection) and primary astrocytes (derived from rat cortical brain cells) were successfully grown in suspension with a density of 200,000 cells per well and transformed into 3D cell spheroids using a matrix-free system (Nanogaia: Ruston, LA). After developing for 7

days in vitro (DIV), biomaterials were added to the fully formed spheroids at 5, 10, and 25 ug per mL. Three types of materials were used for comparison: copper nanoparticles (CuNP), halloysite nanotubes (HNTs), and Cu-HARS. Spheroid area was measured every two days with microscopy and Image Pro Plus 7.0 and Matlab 2015b software, and viability measured using resazurin assay with fluorescent plate reader, at 3,5,7 and 9 DIV, post-treatment (n=96 total spheroids). Biomaterials were also added to 2D model to study the effect versus 3D model. Compared to the same brain tumor cell density, astrocyte spheroids compacted faster and grew slowly. Results showed that in both brain tumor cells and primary astrocytes, CuHARS material was internalized by the outer layer of the spheroid and caused less toxicity compared to the CuNP at the same concentrations. Viability of brain tumor cells with CuNP dropped initially to 36% at 3 DIV and then stabilized to 47% (5-9 DIV). In contrast, with CuHARS, viability was initially 80% and then stabilized to 74% at the same timepoints. These data for a copper biocomposite material indicate that the CuHARS may be functionalized by coating with anticancer drug or growth factor to use as a drug delivery system for cancer treatment as well as cell growth modification in normal brain cells.

**Disclosures:** **N.H. Nguyen:** None. **Z. Norcross:** None. **U. Kansakar:** None. **M.A. DeCoster:** None.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

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**Program#/Poster#:** 612.02/SS37

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NIH/NIMH R43MH108481

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**Title:** Clathrin nanoparticles efficiently deliver brain-derived neurotrophic factor to the hippocampus, reverse BDNF deficits and enhance neurogenesis and memory in a HIV-tat mouse model

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**Abstract: Background:** Advances in treatment of neurodegenerative disorders have been made by administering brain derived neurotrophic factor (BDNF) directly to the brain, or by using drugs that increase BDNF indirectly. BDNF promotes neuroregeneration and restores brain functions, but cannot easily cross an intact blood brain barrier (BBB) or diffuse within the brain. Our goal was to produce BDNF-clathrin nanoparticles (NPs) that can efficiently target brain TrkB receptors, and reverse neurotoxic effects of HIV transactivator of transcription (tat) protein. **Methods:** GT-tg bigenic mice were treated daily for 7 days with saline (tat-) or doxycycline (100mg/kg/d i.p.) that induces tat expression (tat+). Concurrently, animals received daily intranasally either NPs (0.3mg/kg of BDNF with 2.4mg/kg of clathrin); BDNF; clathrin; or saline (40µl). Subsequently, NP/saline treated tat+ mice were tested with Barnes maze and Novel Object Recognition tests. For immunohistochemistry, mice also received Bromodeoxyuridine (BrdU 50mg/kg, Q12h i.p.) on days 1 and 2, and were sacrificed on days 7 or 14 of dox/saline administration. Neurogenesis and newborn cell proliferation and survival were determined with Doublecortin (DCX), Ki67 and BrdU antibodies respectively. For Western Blot analyses, hippocampi were removed and processed on the 4th day of dox/saline administration, and BDNF concentrations and signaling were analyzed. **Results:** BDNF-clathrin NPs significantly improved cell survival and proliferation, and doubled the density of young neurons in the hippocampus. BrdU+ ($p<0.0002$), Ki67+ ($p<0.002$) and DCX+ ($p<0.001$) cell densities significantly increased in the granule cell layer of dentate gyrus in NP treated tat+ mice, compared to saline treated tat+ or tat- mice. Hippocampal-based memory acquisition ($p<0.04$) and flexibility ($p<0.004$), and novel object recognition ($p<0.016$) significantly improved in NP vs. saline treated tat+ mice. NPs reversed BDNF signaling deficits in tat + animals by significantly increasing hippocampal levels of mature-BDNF ($p<0.007$), pro-BDNF ($p<0.01$), pAKT ($p<0.01$) and Akt ($p<0.002$) in NP treated mice, compared to mice that didn't receive NPs. **Conclusions:** NPs bypassed the BBB, doubled BDNF levels, enhanced hippocampal cell proliferation, survival and neurogenesis, improved memory and reversed neurodegenerative effects of tat protein in GT-tg mice. Hence, clathrin provides a highly efficient nanoplatform for delivery of BDNF to the brain. This noninvasive nanotechnology may be able to enhance neuronal regeneration and plasticity, and restore brain functions more quickly and completely than existing treatment methods.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.03/SS38

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** The effect of blood-brain barrier (BBB)-specific laminin isoforms for barrier function with the new *In vitro* BBB model incorporating multi-culturing system of BBB components

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**Abstract:** Background The blood-brain barrier (BBB) is the brain-specific capillary barrier that is critical for preventing toxic substances. The BBB is primarily formed by microvascular endothelial cells, pericytes, astrocytes and basement membranes. The basement membranes are composed by collagen I, IV and laminins. Recently, we revealed that BBB composed laminin α4 and 5, β1, γ1 (Takeshita et al., 2017 Clin. Exp Neuroimmunol). However, the effect of this laminin isoforms at the BBB is still unknown because of the lack of the *in vitro* models that are specialized to interaction between laminin and other BBB components.
Aim We construct the new BBB model incorporating multi-cultured system of BBB specific laminin isoform (α4 and 5 β1, γ1) and conditionally immortalized human BBB cell lines [endothelial cells (hEC), pericytes (hPCT) and astrocytes (hAST)] by the Nunc UpCell technology. Then, we evaluate whether the barrier function is influenced by the BBB specific laminin.
Method hPCT were co-cultured on luminal side of insert (3 μm pores) with hAST on abluminal side. hEC were cultured on Upcell dish, which can achieve sheet-like detachment of confluent cells and extra-cellular matrix by temperature-shifting to 20 degree Celsius, after coating laminin (α4 and 5, β1, γ1), or blood-nerve barrier specific laminin isoform(α4, β1, γ1). Then sheet-like detachment of confluent hEC and laminin were transferred onto the hPCT, which were co-cultured with hAST on the insert. Solute permeability with 10k dextran was measured.
Result The values of permeability in laminin (α4 and 5, β1, γ1) were significantly lower than laminin (α4 β1, γ1).
Conclusion Our model is the first *in vitro* BBB model to evaluate the crosstalk between BBB specific laminin and other BBB components. This result suggested that BBB specific laminin regulated the barrier function at the BBB.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.04/SS39

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Therapeutic chips: Prolonged release of NGF for neurodegenerative treatment

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**Abstract:** Nerve growth factor (NGF) is a well characterized protein and an essential contributor to neuronal survival and maintenance. NGF has shown high pharmacological potential in clinical trials with patients with mild Alzheimer's disease, where human NGF expressing cells were injected into their forebrain. However, biological treatments hold unanticipated risks and may cause serious adverse effects. In addition, free growth factors endure rapid degradation which leads to a short biological half-life, limiting the effectiveness in therapeutics. In previous works which were done in our group; we have shown that conjugation of growth factors to nanoparticles stabilized the protein and extended its biological effectiveness. Here, we have designed and examined high porous chips made of silicon as the drug carriers. The delivery system we have developed, composed of porous silicon (PSi) chips and growth factor, allows sustained and controlled release of NGF. Different PSi nanostructures (vary in size and depth) were fabricated by anodic electrochemical etching of single-crystalline Si wafers and the synthesis conditions were adjusted to allow efficient loading of NGF by physical adsorption. Remarkably, the NGF release profile demonstrates a sustained release for a prolong period of one month. Bio-functionality was examined on PC12 cell culture and *ex-vivo* DRG neuronal culture, common model systems. We have shown that the prolonged release of the bioactive NGF promoted differentiation and neurites outgrowth. Our work demonstrates that NGF entrapment within the PSi chips allows for its sustained delivery constantly over time without any need of external supplement. Currently we examine the therapeutic effects of our novel system on neurodegenerative diseases. We have designed and characterized a model of differentiated SH-SY5Y neuroblastoma cells with Amyloid-β peptide (Aβ), a main component of Alzheimer disease senile plaques. Our novel methodology addresses the challenge of distribution of NGF in a controlled and targeted manner. This system holds a promise for a new strategy of treatment for neurodegenerative diseases.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.05/SS40

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Monoamine oxidase (MAO-A and MAO-B) enzyme occupancy assay - a non-radiolabelled method in rat

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**Abstract:** Monoamine oxidase (MAO) enzymes (subtype A and B) are important to human health because of their regulatory and protective effects in controlling the concentrations of neurotransmitters and drugs. MAO inhibitors are known for their effects in the treatment of depression, Alzheimer's and Parkinson's diseases. Development of new MAO inhibitors can be accelerated by considering the receptor occupancy data as a major threshold measure. Thus in the process of setting up the occupancy assay, selective PET tracers (Harmine for MAO-A and R (-) Deprenyl for MAO-B) were identified and optimized with their non-radiolabelled form (using mass spectrometry, LC-MS/MS) in rat. Tracer suitability was tested by brain regional distribution at different dose and time points. Dose dependent decrease (Harmine) or increase (Deprenyl) of tracer binding in selective brain regions of pre-treated (standard inhibitors) rats reflected gradient MAO-A or MAO-B occupancy, respectively. The ratio of occupancy effective doses (MAO-B $ED_{50}$/ MAO-A $ED_{50}$) of inhibitor drug highlights its in-vivo selectivity and associated enzyme mediated effects. This factor would broaden the application of inhibitors in treatment where the enzyme selectivity is the major factor. Further by using this LC-MS/MS based method, new chemical entities can be screened for MAO occupancy at a faster rate and a correlation can be drawn against the brain or plasma exposures from the same experiments.

**Disclosures:** **J.B. Thentu:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **R. Dyavarashetty:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **K. Bandaru:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **R. Boggavarapu:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **H. Pantangi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **N. Padala:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.06/SS41

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Lu AA41178: A novel, brain penetrant, pan-selective KCNQ/Kv7 potassium channel opener with efficacy in preclinical models of neurological and psychiatric disorders

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**Abstract:** The voltage-gated Kv7.2-Kv7.5 channels are broadly expressed in the central nervous system, where they, among other functions, control the excitability of neurons by acting as a 'brake' on repeated action potential discharges. Thus, pharmacological activation of neuronal Kv7 channels has proven to be a successful therapeutic strategy within partial-onset seizures by use of the Kv7 activator retigabine (Gunthorpe *et al*, 2012). Interestingly, activation of Kv7 channels also holds promise as a therapeutic strategy within psychiatric disorders such as schizophrenia (Sotty *et al*, 2009)) and depression (Friedman *et al*, 2016). In the present study we have characterized the pharmacological profile of the novel, pan-selective Kv7.2-7.5 opener Lu AA41178, using both *in vitro* assays and a broad range of *in vivo* assays related to epilepsy, schizophrenia, and depression. Using two-electrode voltage clamp in *Xenopus* oocytes expressing human Kv7.2-Kv7.5 Lu AA41178 was confirmed to be a pan-selective activator of Kv7 channels. When applying a voltage ramp protocol the activation threshold of Kv7.2+Kv7.3, Kv7.4 and Kv7.5 was significantly left-shifted in the presence of increasing concentrations of Lu AA41178 (0.3-30 µM). Importantly, in a $Rb^+$ efflux assay Lu AA41178 did not display inhibitory effects on human Kv7.1 channels stably expressed in CHO cells, suggesting no impact on cardiac repolarization safety. Next, we tested Lu AA41178 in preclinical *in vivo* models of neurological and psychiatric disorders. In the maximum electroshock threshold test (MEST) subcutaneous pre-treatment of mice with Lu AA4178 significantly increased the electroconvulsive shock threshold, thus demonstrating an anticonvulsant effect. In the mouse forced swim test, a model of behavioral despair with antidepressant predictive validity, AA41178 significantly reduced immobility time to the same extent as the positive control, imipramine. In anesthetized rats we performed *in vivo* electrophysiology recordings of VTA activity, a brain region demonstrated to be hyperactive in animal models of schizophrenia (Lodge and Grace, 2007) and depression (Friedman *et al*, 2016). Here, AA41178 potently reduced both spontaneous firing and burst firing upon IV administration. Behavioral testing of AA41178 was accompanied

by plasma and brain exposure sampling, revealing minimum effective plasma levels below 1000 ng/ml. In summary, Lu AA41178 is a potent activator of neuronal Kv7 channels demonstrating efficacy in animal models of epilepsy, schizophrenia and depression. Thus, it may serve as a valuable tool for exploring the role of Kv7 channels in both neurological and psychiatric disorders.

**Disclosures:** **M. Grupe:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **M. Grunnet:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **T. Benned-Jensen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Frisch Herrik:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **B. Hjorth Bentzen:** 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.; H. Lundbeck A/S. **K. Frederiksen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **A. Graven Sams:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J. Frank Bastlund:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.07/SS42

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** SIP20171379

**Title:** Acupuncture reverses behavioral disturbances and testosterone levels in social isolated rats

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**Abstract:** INTRODUCTION: A growing number of human and animal studies suggest a modulatory role of testosterone in the regulation of emotionality, stress response, and psychiatric disorders, including depressive-like disorders. Acupuncture has been demonstrated to have antidepressant-like effect in humans and ameliorate depression-related behavior in depression models. However, the effect of acupuncture on testosterone and depressive-like behavior remains unknown. OBJECTIVE: To explore a possible mechanism of acupuncture for relieving depression, the effects of embedding thread in acupoints on depressive symptoms and testosterone serum levels were evaluated in an animal depression model. METHODS: On postnatal day 21, forty Sprague-Dawley male rats were weaned and randomly divided into:

social isolation and acu-catgut embedding (SI+AC); social isolation and sham acu-catgut embedding (SI+Sham); group housing and acu-catgut embedding (GH+AC); group housing and sham acu-catgut embedding (GH+Sham) with 10 animals in each group. Depression model was established by post-weaning social isolation for 8 weeks. In AC groups, surgical suture (acu-catgut) was embedded in Baihui (DU 20), Yintang (Ex 3), Baihui (DM20), Shenshu (BL 23), Pishu (BL 20), Ganshu (BL 18) and Xinshu (BL 15). In Sham groups, the acupoints were punctioned without the embedding thread. Depression-like behavior was evaluated through the Forced swimming test (FST). Testosterone levels in the serum were examined by ELISA. RESULTS: Social isolation increased immobility and reduced active behaviors in the FST respect to rats raised in a group ($P<0.05$). False acu-catgut embedded (Sham group) was ineffective to reverse immobility behavior in isolated rats. However, in the isolated group acu-catgut significantly ($P<0.05$) reduced the immobility time and increased the time that rats spent on active behaviors, reaching the level of the group housed. Social isolation induced a decrease in serum testosterone ($P<0.05$) and treatment with acu-catgut partially restored the peripheral levels of this steroid in socially isolated rats. CONCLUSION: Acupuncture as embedded thread in acupoints, reversed the effects of social isolation on depressive behavior which could be related to its effects in up-regulating serum testosterone.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.08/SS43

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Research in Depression: Endocrinology, Epigenetics and neuroiMaging: the REDEEM study is funded through a Health Research Award granted by the Health Research Board of Ireland

**Title:** Modifying the kynurenine pathway as a potential therapy to treat patients with depression

**Authors: K. D. BORNEMANN**[1], M. WEILAND[1], K. DOOLIN[2,3], V. O'KEANE[2,3], A. HARKIN[2], B. HENGERER[1], *K. A. ALLERS[1]
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**Abstract:** The kynurenine (KYN) pathway involves several enzymes, which catabolize the amino acid tryptophan (TRP), via the Kynurenine 3-monooxygenase (KMO) branch, into

quinolinic acid (QUIN) and, through the kynurenine aminotransferase II (KAT II) branch, into kynurenic acid (KYNA). Several TRP catabolites (TRYCATS) have been shown to be altered in CNS disorders such as major depressive disorder (MDD). The KYN pathway is activated by proinflammatory cytokines, which are also elevated in neuropsychiatric diseases. Indoleamine 2, 3-dioxygenase 1 (IDO1) and Kynurenine 3-monooxygenase (KMO) are cytokine-inducible enzymes, which use TRP and KYN, respectively, as their substrate for further degradation. We hypothesized that patients with MDD differ from one another in their TRYCAT levels and that selective manipulation of these pathways can rectify these disturbances. We have identified subgroups of patients with different MDD profiles, which show altered plasma TRYCAT levels compared to other patients within their groups (samples measured within the REDEEM study, Trinity College). One subpopulation of patients that experienced a first depressive episode, revealed very low plasma KYNA levels, whereas some patients with recurrent MDD showed high levels of plasma QUIN. It may be possible that these subgroups respond to different types of TRYCAT modulation. It is proposed that KMO inhibitors shift the TRP catabolism into the direction of neuroprotective KYNA production and also reduce neurotoxic QUIN levels, whereas IDO1 inhibitors lower QUIN levels. Preclinically, we could demonstrate differential manipulation of either the QUIN pathway or the KYNA pathway, using such selective enzyme inhibitors. For this purpose, we have treated mice with a single dose of Lipopolysaccharide (LPS) to induce inflammation-mediated TRYCAT activity. Twenty-four hours were allowed for enzyme induction, at which time a substantial increase in TRYCAT activity could be measured. We could demonstrate very distinguished effects on serum TRYCAT levels by IDO1 and KMO enzyme inhibitors. These data led to our hypothesis that selective modulation of the TRYCAT pathway may display a realistic and ‘personalized treatment’ option for subgroups of MDD patients.

**Disclosures:** **K.D. Bornemann:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma. **M. Weiland:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma. **K. Doolin:** None. **V. O'Keane:** None. **A. Harkin:** None. **B. Hengerer:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma. **K.A. Allers:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.09/SS44

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** N.G. is an Intermediate Fellow of Wellcome DBT-India Alliance, IA/I/15/2/502091

**Title:** An adaptive and interactive software for automated delivery of cognitive behavioral therapy for depression

**Authors: *N. GUPTA**[1], A. GHOSH[1], S. WAGLE[1], K. RAMIAAH[1], P. SHARMA[2], B. BHUSHAN[3], A. BAJPAI[4]
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**Abstract:** According to WHO, depression will be the second leading cause of disability by 2020. Even though depression is treatable with medications and psychotherapy such as the Cognitive Behavioral Therapy (CBT), many patients never seek professional help because of high costs, poor accessibility, and social stigma associated with visiting a therapist. Computerized Cognitive Behavioral Therapy (CCBT), i.e. delivering CBT automatically though a computer, is a relatively new approach that has the potential to bridge this gap. However, the existing CCBT tools suffer from multiple drawbacks including lack of personalization and high dropout rates. We have developed a fully stand-alone and freely accessible internet-based tool, TreadWill (treadwill.org), for treating depression. TreadWill is based on evidence-based CBT and educates the user through interactive and multimedia elements. The user interacts with the automated program that adapts to the user's dysfunctional thoughts, intermediate and core beliefs, and profession. To increase adherence to the program, TreadWill also includes social networking features where the users can post their problems in a support group and get help from other users. To test the effectiveness of TreadWill, we plan a double-blinded randomized controlled trial. The results will be compared to an active control: a limited, non-adaptive version of the program in which the users learn CBT through plain text-based material and do not have access to various interactive and social networking features. Both the full-featured and the limited versions of the program are designed to be completed in six weeks. In the trial, about a hundred participants in the age group of 16-35 years will be randomly assigned to the experimental or the control group. The participants will be administered Patient Health Questionnaire-9 (PHQ-9) to assess baseline depression level. Thereafter, self-administered PHQ-9 will be used to measure reduction in the severity of depression after completion of the program and at 90 days follow-up. The total time spent by the users in both the versions will serve as an indicator of user engagement.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.10/SS45

**Topic:** I.05. Biomarker and Drug Discovery

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ICID-UAEM, 3789

**Title:** Astrocityc & microglial response after gold nanoparticles administration

**Authors: *E. LIRA**[1,2], M. G. GONZALEZ-PEDROZA[3], T. V. CAMPOS-ORDONEZ[1,2], V. N. MADRIGAL-SAUCEDO[1], N. MOY-LOPEZ[1], J. GUZMAN-MUNIZ[1], R. A. MORALES-LUCKIE[3], O. GONZALEZ-PEREZ[1]
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**Abstract: Abstract**
**Background**: Gold nanoparticles are a novel device of nanometric scale (1 – 100 nm) that possess physicochemical characteristics, which allow them to be introduced into an organism with very few secondary effects. Nanoparticles can deliver drugs, DNA, proteins, cytokines and other peptides in several systems. To date, the effects of nanoparticles in neural tissues are not well known and limited evidence is currently available. Astrogliosis and Microglial reactivity comprise a complex glial response against external insults, neuroinflammation or neurodegeneration. These glial reaction limits neural damage and activates brain repair. **Objective:** To evaluate the astrocytic and microglial response after an intracerebral injection of gold nanoparticle into the brain parenchyma. **Methods:** Male CD1 mice (P60) were divided in three groups: Gold nanoparticles (AuNP), control-vehicle and control-sham. The AuNP group received a single intracerebral injection of PEGylated AuNP [85 X $10^6$/nl] dissolved in 100 nl of 0.9% NaCl solution in the striatum. The control-vehicle group received 100 nl of 0.9% NaCl solution, whereas the control-sham group was submitted to craniotomy without meningeal disruption. All animals were sacrificed by transcardial perfusion at 7 and 14 days after administration with paraformaldehyde as fixative. The brain was processed and sliced in 30 µm thick sections. We performed GFAP-immunohistochemical detection for astrocytes and Iba1 immunohistochemistry for microglia detection. GFAP+ and Iba1+ cells were counted in the striatum. **Results:** At day 7, we observed that the number of GFAP+ astrocytes and Iba1+ cells was higher in the control-vehicle group when compared to the AuNP group. We did not find statistical significant differences between both AuNP groups when compared astrocytic and microglial reactivity at day-7 vs. day-14 time points. **Conclusions:** This preliminary analysis showed that PEGylated gold nanoparticles do not produce significant or progressive astrogliosis and microgliosis, which suggest that gold nanoparticles produces a limited glial response.

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**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.11/SS46

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Method for screening neuronal tumor cell surface markers for high specificity and rapid internalization as potential oncologic treatments

**Authors: *L. ANCHETA**[1], P. A. SHRAMM[2], D. A. LAPPI[3]
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**Abstract:** Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in the growth, progression, and spread of cancer. These therapies are often cytostatic; they block tumor cell proliferation as opposed to chemotherapy that kills tumor cells. A primary approach to identifying potential targets is the ability to compromise a ligand-receptor relationship that causes tumor cell proliferation. There are now many examples of the use of antibodies in tumor therapy to cause a breakdown in that relationship, including the recently approved bevacizumab for glioblastoma. Others in clinical use against brain tumors are antibodies to cell-surface EGFR, VEGFR, PDGFR, and c-kit (Hernandez-Pedro *et al.* http://dx.doi.org/10.1155/2013/716813). These work by down-regulation of the receptor by antibody-mediated internalization. It is crucial to have a method to determine the suitability of an antibody for development as a targeted therapy to cause internalization rapidly and completely, often referred to as “rational design.” Here we describe a method for the efficient determination of internalization of cell surface molecules by antibodies: a cytotoxicity assay utilizing an antibody labeling method to streamline the process of multiple candidate screening. Cells are chosen that have significant levels of expression of the desired marker and the assay readout is definitive: evidence of cell death is demonstrated in 72 hours. This method is designed for the rapid screening of multiple antibodies for specificity and internalization in neuronal tumor cells.

**Disclosures: L. Ancheta:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **P.A. Shramm:** A. Employment/Salary (full or part-time):; Advanced Targeting Systems. **D.A. Lappi:** F. Consulting Fees (e.g., advisory boards); Advanced Targeting Systems.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.12/SS47

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Optimization of MRI-guided AAV delivery into de CSF for CNS gene therapy in NHP

**Authors: L. SAMARANCH**[1], P. HADACZEK[1], *J. BRINGAS[1], P. C. ALLEN[2], D. STOCKINGER[2], Y. YU[2], V. SUDHAKAR[1], M. CAMPAGNA[2], W. SAN SEBASTIAN[1], E. A. SALEGIO[2], G. C. HWA[2], K. S. BANKIEWICZ[1]
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**Abstract: Background:** Therapies based on adeno-associated virus (AAV) technology have demonstrated significant potential to correct CNS pathologies. Although parenchymal delivery has been by far the favored route, the potential advantages of CSF infusion of AAV have been recognized particularly for infants, too young for skull-mounted neuronavigation devices. In a number of studies in nonhuman primates (NHP), injections into CSF space have yielded impressive but variable transduction of spinal cord, cortex and cerebellum. **Objective:** Analyze patterns of distribution of different routes of CSF delivery by magnetic resonance imaging (MRI) to understand the fluid dynamics of the AAV infusion in the CSF space. In addition, levels of circulating AAV9 were analyzed to determine the clearance rate of injected vector over time. **Methods**: Thirteen non-human primates (NHP) were infused under MRI control with AAV9 into the CSF via 3 routes of delivery. Animals were randomly assigned to acute cerebellomedullary cistern (CM) injection group (n=3, 6mL/min), bilateral lateral ventricle injection group (n=2, 6mL/min), lumbar injection group (n=3, 6mL/min), cisternal and lumbar combined injection group (n=2, 6mL/min) or slow CM injection group (n=3, 6mL/h). CSF fluid dynamics of the vector was analyzed by sequential acquisition of contrast agent in a MRI scanner along the infusion. Vector clearance from CSF was analyzed by qPCR at different time points (15', 30', 1h, 6h, 12h, 24h and 28 days), and percentages of remaining capsids were calculated for each of the time points. **Results:** Fluid distribution analysis revealed variability in the pattern of vector dissemination regardless route of delivery. qPCR analysis of AAV9 in the CSF established its ½ life to be approximately 3 hours. Vector was undetectable 24h after injection regardless of the delivery paradigm. Acute CM injection revealed longer ½ life of vector in the CSF, while slow CM injection showed fastest clearance rate, most likely due to a dilution effect. **Conclusion:** Our data will allows us to improve CSF delivery, optimizing parameters such as rate and duration of vector infusion and frequency of AAV administration to the brain and spinal cord.

**Disclosures:** **L. Samaranch:** None. **P. Hadaczek:** None. **J. Bringas:** None. **P.C. Allen:** None. **D. Stockinger:** None. **Y. Yu:** None. **V. Sudhakar:** None. **M. Campagna:** None. **W. San Sebastian:** None. **E.A. Salegio:** None. **G.C. Hwa:** None. **K.S. Bankiewicz:** None.

**Poster**

**612. Drug Delivery**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 612.13/SS48

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NIH 1R21DA03383

NIH R44 DA032464

College of Arts and Sciences at Lehigh University

the Department of Biological Sciences at Lehigh University

R43MH094004

41DA032464

**Title:** Targeting the nicotinic acetylcholine receptor in delivery of drugs across the blood brain barrier

**Authors: *N. I. NISSEN**[1], K. R. ANDERSON[2], S. A. EICHELBERGER[3], J. M. MIWA[2]
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**Abstract:** Several devastating neurodegenerative diseases are caused by genetic mutations resulting in degeneration and death of neurons. Unfortunately, although specific candidate genes causing disease pathology are well-known, no cure for these diseases exist, because at present efficient gene therapy technologies have not been developed. RNA interference can target and silence specific mRNA and therefore offers great therapeutic opportunities in neurodegenerative disorders. The technical limitation of gene therapy strategies lies in the design of the relevant drug and its carrier to get across the impermeable blood-brain barrier (BBB). The nicotinic acetylcholine receptor (nAChR) is expressed on brain endothelial cells denoting the impermeable BBB and widely within the brain, and it is therefore an obvious target for the delivery of drugs across the impermeable BBB.
This project involves the use of a peptide-based nAChR-mediated transport technology to deliver siRNA across the BBB and into neurons to target specific mRNA. This carrier peptide was engineered based on our understanding of nAChR binding proteins interactions. We hypothesis

that this peptide-based nAChR mediated transport is able to carry siRNA across the BBB resulting in knock down of specific mRNA in neurons. We assess mice injected with a peptide-siRNA-complex using quantitative methods to measure knock down of target mRNA, and behavioral assays to assess the functional effects of such knock down.
This technology could have the potential of being used in the delivery of therapeutics in neurological disorders.

**Disclosures: N.I. Nissen:** None. **K.R. Anderson:** None. **S.A. Eichelberger:** None. **J.M. Miwa:** Other; CEO of Ophidion, Inc..

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.01/SS49

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIGMS/NIH P20GM0103423

**Title:** Chronic exposure to and withdrawal from ethanol induces depressive-like symptoms that are reduced by prenatal choline supplementation

**Authors: N. MORRISS**, *M. J. GLENN
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**Abstract:** Major depressive disorder (MDD) is a chronic, heterogeneous mental illness characterized by somatic, neurological, and behavioral symptoms. Key hallmark features include depressed mood, anhedonia, anxiety, and cognitive deficits. Globally, MDD affects up to 350 million people and is the leading cause of disability. An array of treatment options are available, yet a large number of those afflicted start out as, or over time become, treatment resistant. Thus, there is a need to explore other avenues for intervention and symptom attenuation. Recent literature has indicated the potential for the vital amine, choline, to mitigate depressive-like symptoms in animal models. Choline has widespread beneficial effects on the brain and behavior, many of which are in opposition to MDD's effects: decreased anxiety and despair; enhanced attention and memory; increased adult hippocampal neurogenesis and neurotrophic expression. Supplemental choline during early life is particularly potent, likely through its epigenetic action as a major source of methyl groups for methylation reactions. Unfortunately, a major challenge blocking our understanding of choline and MDD is the deficiencies in animal models: few reliably reproduce hallmark MDD symptoms and none capture the breadth of the disease. Recently, attention has focused on findings in humans and animal models, that chronic ethanol exposure and withdrawal have effects that resemble depressive symptoms: impaired mood and memory, and decreased neurogenesis and neurotrophic expression. In the present

study, groups of adult female and male rats treated prenatally with choline supplementation or a standard diet received daily injections ethanol (1 mg/kg, i.p.) or saline for 21 days. On the final days, anxiety and anhedonia were assessed; in the 5 days after treatment (withdrawal), behavioral despair and spatial learning were assessed. Neural assays included H3-K14 acetylation and adult hippocampal neurogenesis. The main results were robust sexually dimorphic patterns: in standard-fed females, ethanol treatment increased anxiety and decreased H3-K14 acetylation; in standard-fed males, ethanol treatment impaired spatial learning and reduced hippocampal neurogenesis. Importantly, prenatal choline supplementation prevented ethanol's effects in females and males. Taken together, these results support chronic ethanol as a useful tool for modelling aspects of MDD and prenatal choline supplementation as a viable intervention strategy. The sexually dimorphic results demand more attention in light of long-standing deficiencies in numbers of studies that include sex as a biological variable.

**Disclosures:** **N. Morriss:** None. **M.J. Glenn:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.02/SS50

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Intracerebral transplantation of mesenchymal stem cells in Wistar Kyoto rat as an animal model of depression

**Authors: *K. KIN**[1], T. YASUHARA[1], Y. TOMITA[1], M. UMAKOSHI[1], K. KUWAHARA[1], I. KIN[1], J. MORIMOTO[1], M. OKAZAKI[1], T. SASAKI[1], M. KAMEDA[1], N. TAJIRI[1,2], I. DATE[1]
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**Abstract:** Object
Despite advances in pharmacological therapies, treatment-resistant depression is still difficult to resolve. The development of effective therapies is therefore needed. Wistar Kyoto (WKY) rats are known to be one of the most promising models of treatment-resistant depression. Mesenchymal stem cells (MSCs) are regarded as a potential candidate for treatment of neurodegenerative disorders. In this study, we evaluated the therapeutic potential of MSCs in WKY rats.
Methods
MSCs were transplanted unilaterally into the cerebral ventricle or the striatum of WKY rats (day 0). A sham operation was performed in control WKY rats. An open field test (OFT, day 14) and a forced swim test (FST, days 15 and 16) were performed to evaluate the antidepressant effect of MSCs. All rats received intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrdU, 50 mg/kg)

every 12 h over the last 3 days of the testing period.
Results and Discussion
MSC-transplanted WKY rats did not show significant improvement in the OFT or the FST, compared to control WKY rats. There was no difference in the number of cells positive for BrdU and BrdU/Doublecortin in the subventricular zone and the dentate gyrus of the hippocampus. It was previously reported that MSC transplantation increases hippocampal neurogenesis and counteracts depressive-like behavior in depression-model rats. However, MSC transplantation did not show such an effect in WKY rats. We must consider the optimal location of transplantation, the optimal dose of MSCs, and the optimal administration route in a future study.

**Disclosures: K. Kin:** None. **T. Yasuhara:** None. **Y. tomita:** None. **M. umakoshi:** None. **K. kuwahara:** None. **I. kin:** None. **J. Morimoto:** None. **M. okazaki:** None. **T. Sasaki:** None. **M. kameda:** None. **N. Tajiri:** None. **I. Date:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.03/SS51

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** RR030651

**Title:** Antidepressant-like actions of inhibitors of poly(ADP-ribose) polymerase in rodent models

**Authors: *G. A. ORDWAY**[1], A. SZEBENI[1], L. J. HERNANDEZ[1], H. WANG-KEATON[1], J. D. CRAWFORD[1], K. SZEBENI[1], M. J. CHANDLEY[2], K. C. BURGESS[1], C. C. DE PRETER[1], W. ONGTENGCO[1], R. W. BROWN[1]
[1]Biomed. Sci., East Tennessee State Univ. James H Quillen Col. of Med., Johnson City, TN; [2]Hlth. Sci., East Tennessee State Univ. Col. of Publ. Hlth., Johnson City, TN

**Abstract:** The DNA base excision repair enzyme, poly(ADP-ribose) polymerase-1 (PARP1), is a multi-functional enzyme and a member of a subfamily of three PARPs that covalently build PAR polymers onto proteins to regulate their function. Drug inhibitors of PARPs have anti-cancer, anti-inflammatory, and neuroprotective effects. Recently, we reported elevated gene expression levels of PARP1 in postmortem brain tissues from donors who had an active major depressive disorder at the time of death. Since PARP1 gene expression is positively correlated with PARP1 activity, these findings indicate that elevated PARP1 activity may contribute to brain pathology associated with depressive behavior. Therefore, we speculated that drug inhibitors of PARP1 may have antidepressant properties. To determine whether a rodent model could be used to evaluate the role of PARP1 in depressive-like behaviors, rats were exposed to

repeated psychological stressors (social defeat and chronic unpredictable stress) for 10 days. Anhedonia (estimated by sucrose preference) and brain PARP1 gene expression levels were measured. After stress exposure, rats exhibited significantly reduced sucrose preference and significantly higher levels of brain PARP1 gene expression. To examine potential antidepressant activity of PARP inhibitors, rats were administered PARP inhibitors or saline vehicle and were exposed to the Porsolt swim test or repeated social defeat and chronic unpredictable stress. Two PARP inhibitors were investigated, 3-aminobenzamide (3-AB) and 5-aminoisoquinolinone (5-AIQ). PARP inhibitors produced antidepressant-like effects in the Porsolt swim test similar to the common antidepressant fluoxetine by significantly decreasing immobility time and increasing latency to immobility. PARP1 inhibitors did not significantly affect locomotor activity or swim speeds, suggesting that antidepressant-like actions of these drugs were not secondary to a stimulant effect. Treatment of rats with a combination of 3-AB and fluoxetine, at low doses of these drugs that individually did not have antidepressant-like effects, significantly decreased immobility time and increased latency to immobility in the swim test. Finally, treatment of rats with 3-AB significantly increased sucrose preference and social interaction times relative to vehicle-treated control rats following repeated exposure to combined social defeat and unpredictable stress, exhibiting effects similar to fluoxetine treatment. These findings uncover PARP1 as a unique molecular target for the development of a novel class of antidepressants that could be used alone or in combination with existing antidepressants.

**Disclosures:** **G.A. Ordway:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-author of provisional patent for PARP inhibitors to treat depressive disorders. **A. Szebeni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-author of provisional patent for use of PARP inhibitors for depressive disorders. **L.J. Hernandez:** None. **H. Wang-Keaton:** None. **J.D. Crawford:** None. **K. Szebeni:** None. **M.J. Chandley:** None. **K.C. Burgess:** None. **C.C. De Preter:** None. **W. Ongtengco:** None. **R.W. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-author of provisional patent for PARP inhibitors to treat depressive disorders.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.04/SS52

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CIHR

FRQS

**Title:** D-lysergic acid diethylamide (LSD) reverses depressive-like behavior and serotonergic (5-HT) neurotransmission impairments in a murine model of chronic stress

**Authors: *D. DE GREGORIO**[1], Y. EL-RAHIMY[1], L. POSA[1], A. AGUILAR-VALLES[2], M. LOPEZ-CANUL[1], J. ENNS[1], S. COMAI[3], N. SONENBERG[2], G. GOBBI[1]
[1]Dept. of Psychiatry, [2]Dept. of Biochem. and Goodman Cancer Res., McGill Univ., Montreal, QC, Canada; [3]Div. of Neurosci., San Raffele Scientific Istitute, Milano, Italy

**Abstract: Background**: D-lysergic acid diethylamide (LSD) is a hallucinogenic drug that has recently generated interest due to clinical findings reporting its beneficial mood-enhancing properties (Gasser et al. 2014. J Nerv Ment Dis.). However, until now, the effects of LSD in areas of the brain associated with mood regulation have not been well examined in animal models. A previous study demonstrated that treatment with LSD (0.13 mg/kg/day, 11 days) improved active avoidance learning in an animal model of depression (olfactory bulbectomy) (Buchborn et al., 2014 J Psychopharmacol.). Recent studies have demonstrated that low doses of LSD (5-20 μg/kg) decreased the activity of serotonin (5-HT) neurons in Dorsal Raphe Nucleus (DRN), while at higher doses (60-120 μg/kg), LSD decreased the firing rate of dopaminergic neurons in the Ventral Tegmental Area, suggesting a psychotic-like effect at higher doses (De Gregorio et al. 2016. Pharmacol Res.). The aim of this study was to investigate the effect of chronic administration of low-dose LSD in a mouse model of chronic stress (CS), employing behavioural paradigms of depression and *in vivo* electrophysiological recordings. **Methods:** The CS stress paradigm was performed as previously described (Andrus et al. 2012. Mol Psychiatry): 8-week old male C57BL/6J mice were individually placed in restrainers for 2 hours per day, over 14 days. Control mice (CTL) remained undisturbed in their original cages. From the 7$^{th}$ to 14$^{th}$ day of stress, both CTL and CS mice received LSD (15 or 30 μg/kg/day, s.c.) or vehicle (veh); on the 15th day after the CS, the groups of mice were tested separately. Specifically, the Open Field Test (OFT), Forced Swim Test (FST) and Novelty Suppressed Feeding Test (NSFT) were employed. *In-vivo* single unit extracellular recordings of 5-HT DRN neurons were also performed. **Results:** CS mice showed decreased locomotion in OFT, compared to the CTL group ($p<0.05$). Treatment with LSD (30 μg/kg/day, s.c) normalized the distance travelled ($p=0.024$). The CS group showed increased immobility time compared to CTL mice in the FST ($p = 0.009$), while CS mice treated with LSD (30 μg/kg/day,s.c.) showed a decreased immobility time, vs. veh ($p = 0.006$). Moreover, the NSFT revealed that LSD (15 and 30, μg/kg/day, s.c) reduced the latency to feed in CS mice ($p<0.001$), which was increased after 14 days of restraint. Finally, CS mice showed a decreased mean firing activity of 5-HT DRN neurons compared to CTL ($p<0.05$). LSD restored firing rates to CTL group level ($p<0.05$). **Conclusion:** This study demonstrates that chronic treatment with low doses of LSD improves depressive-like behaviour and restores the low 5-HT firing activity induced by chronic stress.

**Disclosures: D. De gregorio:** None. **Y. El-Rahimy:** None. **L. Posa:** None. **A. Aguilar-Valles:** None. **M. Lopez-Canul:** None. **J. Enns:** None. **S. Comai:** None. **N. Sonenberg:** None. **G. Gobbi:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.05/SS53

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** R01 MH099085

**Title:** Sex differences in ketamine addiction-like behavior and nucleus accumbens spine morphology in chronic mild-stressed rats pre-treated with ketamine

**Authors: *K. N. WRIGHT**, C. E. STRONG, M. KABBAJ
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**Abstract:** Major depressive disorder (MDD) is a profoundly debilitating disease that affects roughly twice as many women as men. Among MDD patients who are resistant to traditional antidepressant drugs, sub-anesthetic ketamine infusions possess rapid antidepressant effects. While low doses of ketamine has a clear therapeutic benefit for MDD, chronic exposure to higher doses is well-known for its abuse liability. This is a concern for women especially, since women display a more rapid progression through addiction milestones than men, despite similar levels of drug use. Since MDD is highly comorbid with drug addiction, ketamine's therapeutic potential must be assessed alongside its abuse liability, which has not yet been assessed in MDD patients, females, or in preclinical studies. Previous work from our lab indicates that female rodents are more sensitive to ketamine's antidepressant-like effects than males. Additionally, both males and females display ketamine-seeking behavior under an extinction-reinstatement test following intermittent intravenous ketamine self-administration. The rats used in the aforementioned studies, however, were stress-naïve, and it is unknown whether rats with depression-like behavior will have a greater propensity to self-administer ketamine, and furthermore, whether administration of antidepressant ketamine will increase the subsequent likelihood to self-administer ketamine. To answer these questions, male and female rats underwent chronic mild stress (CMS) and were treated with four intermittent, intravenous infusions of sub-anesthetic ketamine. Antidepressant ketamine infusions ameliorated some of the depression-like traits as measured by the novelty-suppressed feeding test and sucrose preference test. Then, rats underwent self-administration of 0.5 mg/kg/inf ketamine under fixed-ratio 1 (FR1) and progressive ratio (PR) schedules of reinforcement, and incubation of ketamine craving at multiple time points after the last exposure. CMS females displayed greater ketamine addiction-like behaviors than non-stressed females or males of either condition. Interestingly, CMS females pre-treated with ketamine display augmented addiction-like behavior compared to CMS females without ketamine pre-treatment, an effect not observed in male counterparts. Finally, alterations in dendritic spine morphology were measured in the nucleus accumbens, an

area affected by both depression and addiction. Taken together, the integration of these two behavioral models will contribute towards a more complete understanding of ketamine's therapeutic and abusive properties in susceptible populations.

**Disclosures: K.N. Wright:** None. **C.E. Strong:** None. **M. Kabbaj:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.06/SS54

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** US NIH grant MH107615 to TDG

**Title:** Group II metabotropic glutamate receptor blockade promotes stress resilience

**Authors: *J. N. HIGHLAND**[1,2], P. ZANOS[2], P. GEORGIOU[2], T. D. GOULD[2,3,4]
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**Abstract:** Stress is one of the most widely recognized and well-studied risk factors for the development of psychiatric disorders, including depression. Enhancing stress resilience may be a therapeutic strategy to prevent the development of depression, or the exacerbation of symptoms. Group II metabotropic glutamate receptor ($mGluR_2$ and $mGluR_3$) antagonists have gained attention for their rapid-acting antidepressant action in animal models; however, the effects of genetic and pharmacological manipulation of these receptors have not been studied as extensively in regards to stress resilience. We assessed the effects of prophylactic treatment with the selective group II antagonist LY341495 and utilized knockout mice lacking either $mGluR_2$ or $mGluR_3$ to determine the role of mGluRs on stress resilience. We also assessed whether activation of group II mGluRs by the selective agonist LY379268 would promote stress susceptibility. Treatment with LY341495 prior to exposure to inescapable shock decreased the development of helpless behavior assessed in the learned helplessness paradigm. In contrast, treatment with the group II mGluR agonist LY379268 increased the number of escape failures in the same paradigm. Neither treatment affected pain threshold assessed via response to shock. Furthermore, we show that $mGluR_2$ knockout ($mGluR_2^{-/-}$) mice are more resilient to stress, as evidenced by decreased immobility time in the forced-swim test and reduced escape failures in the learned helplessness paradigm as compared to wild-type (WT) littermate controls, without altered sensitivity to shock. $mGluR_3^{-/-}$ mice were not significantly different than WT controls in the forced swim test. Following chronic social defeat stress, $mGluR_2^{-/-}$ mice also demonstrated enhanced resilience, as evidenced by a lack of stress-induced sucrose preference deficits. Using both pharmacological and genetic manipulations, our results demonstrate that group II mGluR

activity can modulate susceptibility to maladaptive stress-induced behaviors in mice. Specifically, activation of group II mGluR receptors promotes stress susceptibility, while inhibition of $mGluR_2$ prevents such effects. These data suggest that $mGluR_2$ antagonists may be protective against stress-induced neurobiological changes, which may underlie susceptibility to psychiatric disorders, including depression.

**Disclosures:** **J.N. Highland:** None. **P. Zanos:** None. **P. Georgiou:** None. **T.D. Gould:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.07/SS55

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CONACyT CB-241247

Scholarship CONACYT 337839

PhD. Cristina García Viguera - Phytochemistry Lab, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

**Title:** Antidepressant-like action of Punica granatum extract in a menopause model in Wistar rats

**Authors: *B. G. VALDÉS SUSTAITA**[1], E. M. ESTRADA[3], C. LOPEZ-RUBALCAVA[2]
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**Abstract:** The pomegranate extract has a high content of phytoestrogens and polyphenols that confer estrogenic and chemopreventive activities suggesting its potential use as an alternative for the treatment of depression associated with menopause. The aim of this work was to determine if the aqueous extract of pomegranate (AE-PG) produces antidepressant-like effects in the estrogen deprivation animal model and whether its mechanism of action involved the activation of estrogen receptors. Also, the effect of the combination of AE-PG with citalopram was explored. Thus, the antidepressant-like effect of AE-PG(0.1, 1, 10 and 100 mg/kg) was evaluated in ovariectomized female Wistar rats using the forced swimming test and its effect was compared with that produced by 17β-estradiol ($E_2$, 1.25, 2.5, 5.0 and 10$\mu$ g/rat) and citalopram (CIT; 2.5, 5.0, 10 and 20.0 mg/kg). In a second experiment, AE-PG(1 mg/kg) was evaluated in the presence of the antagonist of estrogen receptors tamoxifen (Tmx; 15 mg/kg). Finally, the combination of suboptimal doses of AE-PG(0.1 mg/kg) and CIT (2.5 mg/kg) was evaluated. All treatments were administered chronically for 14 days. AE-PG produced antidepressant-like effects at 1, 10 and 100 mg/kg decreasing immobility and increasing swimming behavior in a similar manner that ,

CIT (5.0, 10 and 20.0 mg/kg) and $E_2$(5.0 and 10.0 g / rat) Tmx blocked the antidepressant-like effect ofAE-PG confirming the participation of the estrogen receptor (ER). The combination of suboptimal doses of CIT and AE-PG synergies to produce an antidepressant-like effect in the forced swimming test. No treatment produced locomotor activity alterations. In conclusion, the results support the hypothesis that AE-PG exerts antidepressant-like effects in an animal model of menopause.

**Disclosures: B.G. Valdés Sustaita:** None. **E.M. Estrada:** None. **C. Lopez-Rubalcava:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.08/SS56

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** the National Basic Research Program of China

Natural Science Foundation of China

**Title:** Altered peptide ligands of myelin basic protein produce antidepressant-like effects via inflammatory factors and p11

**Authors: *Y. HAN**[1], C.-Y. SUN[1], S.-Q. MENG[1], K. YUAN[2], J. SHI[3], L. LU[4]
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**Abstract: Background:** Cytokine levels are generally changed in both depressed patients and animal models. Altered peptide ligand (APL) of myelin basic protein (MBP) regulates levels of various cytokines, and $MBP_{87\text{-}99}[A^{91}]$ produces an antidepressant effect in rats. **Methods:** The antidepressant effects of two APLs of $MBP_{87\text{-}99}$, $MBP_{87\text{-}99}[A^{91}, A^{96}]$ and $MBP_{87\text{-}99}[R^{91}, A^{96}]$, were evaluated using the forced swim test (FST), learned helplessness (LH) and chronic unpredictable stress (CUS) paradigms in rats. In addition, using immunohistochemistry, western blot and enzyme-linked immunosorbent assay, we assessed the role of inflammatory factors and p11 signaling in the medial prefrontal cortex (mPFC) in the behavioral responses. **Results:** A single dose of either $MBP_{87\text{-}99}$[A91, A96] or $MBP_{87\text{-}99}$[R91, A96] produced prolonged antidepressant effects by reducing the immobility in the FST, reducing the escape latency and failures in the LH, and preventing CUS-induced anhedonia. $MBP_{87\text{-}99}$[A91, A96] dose-dependently produced antidepressant and anxiolytic-like effects in CUS paradigm. However, $MBP_{87\text{-}99}[R^{91}, A^{96}]$ tended to aggravate the CUS-induced anxiety-like behaviors. $MBP_{87\text{-}99}[A^{91}, A^{96}]$ prevented CUS-induced increases in proinflammatory cytokines, and reversed CUS-induced

decreases in p11, p-CREB and BDNF expression in the mPFC. $MBP_{87-99}$[R91, A96] prevented CUS-induced decrease in BDNF in mPFC, and p11 in DG. Moreover, knockdown of p11 in the mPFC by lentivirus-mediated short-hairpin RNA blunted the antidepressant-like effects of $MBP_{87-99}[A^{91}, A^{96}]$ in rats subjected to FST and CUS. **Conclusion:** Immunization with $MBP_{87-99}[A^{91}, A^{96}]$ produced prolonged antidepressant effects in rats, and the behavioral response is mediated by inflammatory factors and p11 signaling in the mPFC.

**Disclosures: Y. Han:** None. **C. Sun:** None. **S. Meng:** None. **K. Yuan:** None. **J. Shi:** None. **L. Lu:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.09/SS57

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH Grant R01MH086828

Psi Chi Fall 2016 Undergraduate Research Grant

**Title:** Intraventricular and intrahippocampal infusions of alpha5 subunit-selective negative allosteric modulators of GABA-A receptors produce rapid antidepressant behavioral changes

**Authors: *C. KOSTELNIK**[1], M. MADDEN[1], K. ROBEY[1], S. M. THOMPSON[2], A. BAILEY[1]
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**Abstract:** Depression is a leading cause of mortality and morbidity worldwide. Current antidepressant medications, such as selective-serotonin reuptake inhibitors, require several weeks to produce behavioral effects and are not effective in treating all patients. Recent work has indicated that systemic injections of negative allosteric modulators of GABA-A receptors with alpha5 subunits (GABA-NAMs, such as L-655,708) produce rapid antidepressant effects in rodents. Their site(s) of action remains unknown however. Given the high expression of alpha5 subunits in the hippocampus, we tested the hypothesis that bilateral intrahippocampal infusions of L-655, 708 (5ng/0.2µL) would be sufficient to induce antidepressive responses in the chronic unpredictable stress (CUS) animal model of depression, and compared the results to those produced by intra-ventricular infusions (ICV). CUS produced numerous depressive-like behavioral outcomes including reduced body weight, reduced sucrose intake, increased latency to eat in a novelty suppressed feeding task (NSF) and a reduced social interaction score. Both ICV infusions of L-655, 708 (5ng/0.2µL) and intrahippocampal infusions of L-655, 708 (5ng/0.2µL) significantly improved behavior in the NSF task 24-hours after infusion. However, ICV and intrahippocampal infusions produced differing effects on sucrose intake and social

interaction. Intrahippocampal, but not ICV, infusions of L-655, 708 reversed deficits in social interaction, but improvement in sucrose intake was seen only following ICV infusions but not direct hippocampal infusions. Our data confirm the rapid antidepressant effects of GABA-NAMs and also suggest their behavioral effects on chronic stress-sensitive behaviors may result from actions on different neuroanatomical circuits.

**Disclosures: C. Kostelnik:** None. **M. Madden:** None. **K. Robey:** None. **S.M. Thompson:** None. **A. Bailey:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.10/SS58

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** R01MH086828

**Title:** Acute glucocorticoid administration rapidly modulates hippocampal local field potentials

**Authors: *A. B. COLE**[1,2], N. HESSELGRAVE[1,2], S. M. THOMPSON[1]
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**Abstract:** Glucocorticoids are the major neuroendocrine product of the mammalian stress response and are produced during acute stress. Homeostatic regulation of glucocorticoid levels by the hypothalamic-pituitary-adrenal (HPA) axis is critical, as prolonged duration of elevated glucocorticoids is detrimental to many organ systems including the brain. Dysregulation of the HPA axis is one of the most consistent biological findings in patients with major depression. This dysregulation is evident by administration of the synthetic glucocorticoid dexamethasone (DEX), which is unable to suppress cortisol production in some forms of depression. Glucocorticoid receptors are expressed in the hippocampus at high levels. Recent evidence also suggests glucocorticoid receptors can act rapidly via non-genomic pathways. We therefore tested the hypothesis that glucocorticoids modulate hippocampal neuronal activity rapidly and changes in hippocampal activity are relayed to other downstream nuclei that control corticosteroid secretion, such as the paraventricular nucleus of the hypothalamus (PVN). We have tested this hypothesis by recording local field potentials *in vivo* from the hippocampus and PVN of isoflurane-anesthetized rats. Acute administration of DEX rapidly induced a characteristic pattern of discharge, resembling burst suppression firing seen in electroencephalography, within 10 minutes and this discharge persisted for around 60 minutes. Similar firing patterns were seen in response to acute stress. Highly correlated discharge patterns were recorded simultaneously from the PVN. Our work will further explore the modulation of these firing patterns by glucocorticoids, the role of membrane glucocorticoid receptors, potential glucocorticoid

interaction with anesthetics, and the potential alteration of this signaling in chronic stress models of depression.

**Disclosures: A.B. Cole:** None. **N. Hesselgrave:** None. **S.M. Thompson:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.11/SS59

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** R01MH086828

T32NS007375

NARSAD YI Award

**Title:** Synaptic potentiation at the hippocampus-nucleus accumbens synapse modulates reward behavior

**Authors: *T. A. LEGATES**, M. D. KVARTA, S. M. THOMPSON
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**Abstract:** The nucleus accumbens (NAc) is a central component of the reward system and is responsible for integrating information from cortical and limbic brain regions to drive goal directed behavior. The ventral hippocampus provides excitatory input to the NAc shell, which is important for modulating NAc activity and providing contextual information to reward processing. This synapse has received increased attention due to emerging evidence of its critical role in mood regulation, specifically in response to reward and motivation to seek rewards. Whether these synapses display activity-dependent synaptic plasticity, and by what mechanisms, remains to be clarified. We used whole-cell electrophysiological recordings in the NAc shell examine the mechanisms of induction and expression of plasticity. Our data show that this synapse is capable of undergoing activity-dependent long-term potentiation (LTP) via postsynaptic mechanisms. The induction of LTP was N-methyl-D-aspartate (NMDA) receptor - and $Ca^{2+}$/calmodulin-dependent kinase type II (CaMKII)-dependent, and was independent of dopamine receptor signaling. We used optogenetic stimulation of this synapse to assay the behavioral consequence of *in vivo* manipulation of this synapse. We found that a brief period of 100Hz high frequency stimulation (HFS) was capable of inducing conditioned place preference, whereas the same number of stimuli delivered at low frequency (4 Hz) did not. HFS but not 4Hz caused a corresponding increase in cFos expression in the NAc shell. This suggests that potentiation of this synapse *in vivo* is important for contextual reward processing. These experiments provide the first detailed electrophysiological characterization of the excitatory

input from the hippocampus to the NAc and provide a functional role for potentiation of this synapse in regulating reward related behaviors.

**Disclosures: T.A. LeGates:** None. **M.D. Kvarta:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Asulon Therapeutics. **S.M. Thompson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Asulon Therapeutics.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.12/SS60

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH Grant R01MH086828

**Title:** The influence of alpha5 subunit-selective negative allosteric modulators of GABA-A receptors on sexual conditioned place preference in a rodent model of depression

**Authors: *A. BARRETT**[1], K. LA[1], H. STARNES[1], S. M. THOMPSON[2], A. BAILEY[1]
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**Abstract:** Human depression is characterized by significant sexual dysfunction, particularly loss of libido. Negative allosteric modulators of GABA-A receptors (GABA-NAMs) with an alpha5 subunit (such as L-655,708) produce rapid antidepressant effects in rodent models of anhedonia, typically measured as a loss of sucrose preference and a loss of social interaction following chronic stress exposure, but the effects of stress and GABA-NAMs on sexual reward are unknown. Conditioned place preference (CPP) is often used to test the rewarding properties of various stimuli and may be used as another measure of anhedonia. We used the CPP task to measure changes in sexual conditioning to examine the effects of both chronic stress and L-655, 708 on a behavior with ethological relevance. Male rats trained in a standard CPP paradigm showed a significant increase in preference for the chamber associated with a sexually receptive female following six days of conditioning. Animals were then exposed to 15 days of chronic restraint stress (CRS). CRS significantly reduced weight gain, decreased sucrose intake, and reduced sexual behavior (as measured by mounts and intromissions) compared to control animals. One injection of L-655, 708 (0.7 mg/kg; i.p.) significantly increased sucrose intake 24-hours later in animals previously exposed to CRS, replicating the rapid antidepressant effect of L-655, 708 in rodents. Examination of the sexual CPP task following CRS exposure identified a significant increase in time spent in the chamber associated with a receptive female following

injection of L-655, 708 compared to stressed animals given a vehicle injection. Similarly, L-655 increased the number of mounts and intromissions in animals previously exposed to CRS compared to CRS animals given a vehicle injection. We conclude that chronic stress induces sexual anhedonia, characterized by loss of interest and decreased mating, and that L-655, 708 rapidly (24 hrs) reverses these changes, providing additional evidence of the promise of GABA-NAMs as novel fast-acting antidepressants.

**Disclosures: A. Barrett:** None. **K. La:** None. **H. Starnes:** None. **S.M. Thompson:** None. **A. Bailey:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.13/SS61

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NC123240.1

**Title:** Differential effects of fluoxetine and citalopram treatments on seizure susceptibility and depressive-like behavior in an animal model of epilepsy and depression comorbidity

**Authors: *A. VALDÉS-CRUZ**, A. DÍAZ-JIMÉNEZ, B. A. GARAY-CORTES, D. U. GONZÁLEZ-MÉNDEZ, M. G. MARTÍNEZ-MONTALVO, P. DOMÍNGUEZ-ZÚÑIGA
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**Abstract:** Two of the selective serotonin reuptakes inhibitors (SSRIs) in clinical use for the treatment of depression are fluoxetine (FLX) and citalopram (CIT). Despite the efficacy of both drugs on the depression, the effect on comorbidity with epilepsy remains controversial. Experimental models have shown that SSRIs affects excitability in short time scales which may lead to changes in epilepsy severity. Amygdaloid kindling (AK) is a model of epileptogenesis characterized by sustained increase in seizure susceptibility. Therefore, the goal of the present study was to examine AK epileptogenesis with behavioral correlates of depression in the rat forced swim test (FST) as well as the effects of FLX and CIT on seizure susceptibility and depressive-like behavior. Male Wistar rats (280-320 g) were used. Tripolar electrodes were placed in the basolateral nucleus of left temporal lobe amygdala, and both frontal cortices. AK was induced by daily electrical stimulation (1 s train, 1 ms pulses, 60 Hz, 250-500 μA) until reach AK stage V (tonic-clonic seizure) for three consecutive days. One hour after last seizure, FST sessions were conducted by placing rats in individual glass cylinders (46 cm height; 20 cm diameter). An initial FST of 15 min (pre-test) was performed and after 24 h, was performed a 5 min test. Three FLX doses (10 mg/Kg) or three CIT (10 mg/Kg) doses were administered following a sub-acute schedule, three injections administered between pretest and test sessions

(21 h, 5 h, and 1 h before test session). Animals were assigned to ten experimental groups of seven rats each: a) Control-FST only with FST; b) Control-K only with AK; c) K-FST-FLX, in which AK, FST and FLX injections were applied; d) K-FST-CIT, in which AK, FST and CIT injections were applied; e) K-FST-Vh, AK, FST and Vh (Vehicle, 2 ml/Kg NaCl 0.9%); f) K-FLX, in which AK, and FLX injections were applied; g) K-CIT in which AK, and CIT injections were applied ; h) Sham-FST-FLX, FST and FLX; i) Sham-FST-CIT, FST and CIT; and j) Sham-Vh, FST and Vh. Immobility time in FST and seizure susceptibility on test stimulations of post stage V were assessed. We found a decrement in immobility time in the FST on KEA+PNF+FLX compared with all groups to except KEA+PNF+Vh; and between KEA+PNF+Vh compare to Control-PNF, KEA+PNF+CIT and Sham+PNF+CIT. An increase in swim on KEA+PNF+FLX compare to Control-PNF, KEA+PNF+CIT and Sham+PNF+CIT. Whereas also KEA+PNF+FLX group shows an increase on susceptibility to tonic-clonic seizure. Our results suggest that differential effects of FLX, on seizure susceptibility, and CIT, in non-antidepressive effect, could be by the pharmacodynamic differences at the level of 5-HT receptors affinity of each SSRIs.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.14/SS62

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** The Naito Foundation

**Title:** Cgrp ameliorate depression-like behavior in social defeat stress model mice

**Authors: *N. HASHIKAWA-HOBARA**, S. MISHIMA, S. MATSUUCHI, N. HASHIKAWA
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**Abstract:** Calicitonin gene-related peptide (CGRP), which is produced in both peripheral and central nervous system, is well known as a potent vasodilator. Although CGRP plays an important role in central nervous system, its effect on posttraumatic stress is not clear. In the present study, we used social defeat stress model as an animal models for posttraumatic stress disorder. C57BL6J mice were exposed to and suppressed by a single aggressor animal (ICR mice) each day for 10 min for a total of 14 days. After the last defeat, CGRP (0.5 nmol) were administered by intracerebroventricular injection. To investigate the effects of exogenously increased CGRP on depression-like behavior in stressed mice, we used a forced swim test, tail suspension test and sucrose preference test. We found CGRP decreased immobility time in both

forced swim test and tail suspension test. Furthermore, in the sucrose preference test, which measures anhedonic-like deficits, sucrose preferences were significantly lower in stressed mice and increased by CGRP injection as same as control level. These results suggest that CGRP treatment induces anti-depressant-like effects in stressed mice.

**Disclosures:** **N. Hashikawa-Hobara:** None. **S. Mishima:** None. **S. Matsuuchi:** None. **N. Hashikawa:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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SNUH grant 0320140100

BK21-plus education program

**Title:** Dysfunction of microglial STAT3 alleviates depressive behavior via neuron-microglia interactions

**Authors: *S. KWON**[1], J.-K. HAN[2], M. CHOI[1], Y.-J. KWON[1], S. KIM[2], E. YI[1], J.-C. SHIN[3], I.-H. CHO[4], S. KIM[2], S.-K. YE[1]
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**Abstract:** Neuron-microglia interactions play a crucial role in maintaining the neuroimmune system. The balance of neuroimmune system has emerged as an important process in the pathophysiology of depression. However, how neuron-microglia interactions contribute to major depressive disorders has been poorly understood. Herein, we demonstrated that microglia-derived synaptic changes induced antidepressive-like behavior by using microglia-specific signal transducer and activator of transcription 3 (STAT3) knockout ($STAT3^{fl/fl}$;$LysM\text{-}Cre^{+/-}$) mice. We found that microglia-specific STAT3 knockout mice showed antidepressive-like behavior in the forced swim, tail suspension, sucrose preference and open field tests. Surprisingly, the

secretion of macrophage colony-stimulating factor (M-CSF) was increased from neuronal cells in the brains of STAT3$^{fl/fl}$;LysM-Cre$^{+/-}$ mice. Moreover, the phosphorylation of antidepressant-targeting mediators and brain-derived neurotrophic factor (BDNF) expression were increased in the brains of STAT3$^{fl/fl}$;LysM-Cre$^{+/-}$ mice as well as in neuronal cells in response to M-CSF stimulation. Importantly, the miniature excitatory postsynaptic current (mEPSC) frequency in the medial prefrontal cortex was increased in STAT3$^{fl/fl}$;LysM-Cre$^{+/-}$ mice and the M-CSF treatment group. Collectively, microglial STAT3 regulates depression-related behaviors *via* neuronal M-CSF-mediated synaptic activity, suggesting that inhibition of microglial STAT3 might be a new therapeutic strategy for depression.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.16/SS64

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Turkish Academy of Sciences

Hacettepe University, Institute of Neurological Sciences and Psychiatry

**Title:** Electroconvulsive seizures induce neuroinflammatory response: A potential mechanism for BDNF induction

**Authors: Z. SEN**[1], E. EREN-KOCAK[1], B. DONMEZ-DEMIR[1], S. YILMAZ OZCAN[1], M. YILMAZ[1], *T. DALKARA[2,1]
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**Abstract:** Neuroinflammation has been shown to be involved in pathological conditions like cortical spreading depression, seizures and ischemia. Its involvement in physiological processes like neurogenesis, learning and memory has also been reported. Recent research also points out a role for neuroinflammation in intercellular signaling in the central nervous system, which is triggered by homeostatic imbalance. Based on these findings, we hypothesized that neuroinflammation may have a central role in understanding mechanisms of action of electroconvulsive therapy (ECT), one of the oldest and most effective treatments in psychiatry. Limited data point to a microglial activation after ECT, but to our knowledge, the neuroinflammatory response originating from neurons and other glial cells after ECT has not been examined before. In this study, we investigated whether or not a single electroconvulsive

seizure (ECS), a rodent model of ECT, induced neuroinflammatory response. For this, we examined the release of HMGB1, a proinflammatory molecule at 1, 2 , 4 and 6 hours after a single ECS. We observed translocation of HMGB1 from nucleus to cytoplasm or complete loss of HMGB1 signal in some cells in the hippocampus and medial prefrontal cortex by immunohistochemistry starting 1 hour after ECS and, confirmed HMGB1 release by detecting an increased amount of HMGB1 in cerebrospinal fluid compared to sham group. ECS did not induce HMGB protein expression in the hippocampus and prefrontal cortex as detected by Western blotting. Brain derived neurotropic factor (BDNF) levels were increased in hippocampus and prefrontal cortex 1 hour and 6 hours after ECT, respectively. These findings suggest a potential relationship between the HMGB1 release and increased BDNF expression, which is currently being tested by knocking down HMGB1 expression before ECS.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH R01 Grant MH112861

NARSAD Young Investigator

**Title:** Response to antidepressant treatment is mediated by an indirect relationship between dentate gyrus activity and adult hippocampal neurogenesis

**Authors: C. YOHN**, E. DIETHORN, A. GARINO, S. SHIFMAN, *B. A. SAMUELS
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**Abstract:** Depression is a complex psychiatric disorder that remains a burden on society today since only a subset of depressed patients (33%) attain remission upon initial monotherapy with a selective serotonin reuptake inhibitor (SSRI). Chronic SSRI treatment results in increased extracellular serotonin (5-HT) levels, increased adult hippocampal neurogenesis, and increased hippocampal expression of neurotrophic factors such as BDNF. We recently reported that serotonin 1A receptors in the dentate gyrus (DG), a subfield of the hippocampus, are necessary and sufficient for the behavioral, neurogenic, and neuroendocrine response to chronic SSRI treatment. However, further research is necessary to explore the role of the DG in the neural circuitry underlying the antidepressant response and whether the DG mediates the response to other classes of antidepressants. To this end, we assessed possible interactions between

activation of mature DG granule cells during a behavioral experience, adult hippocampal neurogenesis, and behavioral response to chronic antidepressant treatment. Subsequent to chronic corticosterone (CORT) administration to mimic chronic stress, mice were chronically exposed to antidepressant monotherapies with fluoxetine (FLX), bupropion (BUP), or venlafaxine (VEN). Behavioral response to antidepressants was assessed using the Novelty Suppressed Feeding (NSF) task, which permits stratification into responders and non-responders to antidepressant treatment. Following initial exposure to the NSF test, mice were sacrificed and perfused to assess DG immediate early gene expression and adult hippocampal neurogenesis. Preliminary data indicates that behavioral antidepressant response correlates with an indirect relationship between DG immediate early gene expression and adult hippocampal neurogenesis, where responders have more hippocampal neurogenesis and less immediate early gene expression than non-responders to antidepressant treatment.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH R01 Grant MH112861

NARSAD Young Investigator

**Title:** The role of dentate gyrus activin signaling in antidepressant treatment response

**Authors: *M. GERGUES**[1], C. YOHN[2], M. LEVINSTEIN[4], R. HEN[5], B. A. SAMUELS[3]
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**Abstract:** Approximately 32-35 million adults in the US population (16%) experience an episode of major depression in their lifetime, and commonly used treatments, such as selective serotonin reuptake inhibitors (SSRIs), are not ideal since only a subset of patients (~33%) achieves remission with initial treatment. The reasons why some individuals remit to antidepressant treatments while others do not are unknown. Our overall research program addresses this question by assessing antidepressant treatment resistance in mice. Proper assessment of the antidepressant response in mice first requires manipulations that will yield behaviors associated with negative valence constructs that can then be reversed by antidepressant

treatment. Chronic treatment of mice with corticosterone (CORT) effectively induces multiple changes in behavior associated with enhanced responses to potential harm and sustained threats (David et al 2009). Subsequent chronic treatment with antidepressants such as fluoxetine (FLX) reverses these behavioral changes in some, but not all, of the mice, permitting stratification into responders and non-responders to FLX. We looked for changes in gene expression in dentate gyrus, a region that we recently reported is critical for the beneficial behavioral, neurogenic, and neuroendocrine effects of FLX (Samuels et al 2015). We found several significant differences in expression of Activin signaling-related genes between responders and non-responders to FLX in the dentate gyrus. Furthermore, modulating Activin signaling in the dentate gyrus can convert behavioral non-responders to FLX into responders.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Pritzker Neuropsychiatric Disorders Research Consortium

Office of Naval Research (N000141210366 and N000141512224)

Hope for Depression Research Foundation

NIH Grant MH104261

**Title:** Connective tissue growth factor (CTGF) is a novel pro-depressant molecule

**Authors: *C. A. TURNER**[1], V. SHARMA[2], M. H. HAGENAUER[3], C. AYDIN[4], A. M. O'CONNOR[5], R. C. THOMPSON[3], S. J. WATSON, Jr.[3], H. AKIL[3]
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**Abstract:** Little is known about the role of connective tissue growth factor (CTGF) in the brain. We evaluated the expression of CTGF in our selectively bred model of response to a novel environment, where bLRs exhibit increased anxiety-like and depression-like behavior compared to bHRs. We also assessed the expression of CTGF in the human amygdala of individuals with Major Depressive Disorder (MDD). To understand its role in affective behavior, we administered CTGF to rodents acutely and chronically and tested their affective responses. We then assessed

the ability of an anti-CTGF antibody (FG-3019) to alter emotionality and gene expression. Finally, we assessed CTGF expression in various stress paradigms. In three nuclei of the human amygdala, CTGF expression was increased in individuals with MDD compared to controls. In the bHR/bLR model, CTGF expression was significantly increased in the dentate gyrus (DG) of bLRs in adulthood compared to bHRs. When the highly anxious LRs were administered early life fibroblast growth factor-2 (FGF2), a treatment known to decrease anxiety later in life, the expression of CTGF decreased in the DG in adulthood. In outbred animals, acute CTGF (400ng, i.c.v.) increased depression-like behavior. Moreover, both acute and chronic treatment with FG-3019 was antidepressant. Chronic treatment with FG-3019 also altered the expression of molecules that interact with CTGF, as well as decreasing CTGF itself. Finally, four days of social defeat stress in LRs significantly increased CTGF expression in the dentate gyrus. However, two weeks of chronic variable stress in bLRs decreased CTGF expression in the hippocampus. Thus, the region, nature and/or temporal dynamics of the stress are important. In conclusion, molecules that inhibit or decrease CTGF expression may be effective antidepressants.

**Disclosures:** **C.A. Turner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **V. Sharma:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **M.H. Hagenauer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C.. **C. Aydin:** None. **A.M. O'Connor:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **R.C. Thompson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **S.J. Watson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **H. Akil:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C..

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.20/TT2

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH Grants MH045481

NIMH Grants MH093897

the state of Connecticut

**Title:** BDNF release and signaling are required for the antidepressant actions of GLYX-13

**Authors: *T. KATO**[1,2], M. FOGAÇA[1], S. DUMAN[1], X.-Y. LI[1], K. FUKUMOTO[1], R. DUMAN[1]
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**Abstract:** Conventional antidepressant medications, which act on monoaminergic systems, have significant limitations for the treatment of depression, including a time lag of weeks to months and low rates of therapeutic efficacy. GLYX-13 is a novel glutamatergic compound that acts as a modulator of the NMDA receptor with glycine-like partial agonist properties, and like the NMDA receptor antagonist ketamine produces rapid antidepressant actions in depressed patients and in preclinical rodent models. Importantly, GLYX-13 does not produce the dissociative and psychotomimetic side effects observed with ketamine. However, the mechanisms underlying the antidepressant actions of GLYX-13 have not been characterized. Here, we use a combination of neutralizing antibody, mutant mouse, and pharmacological approaches to test the role of BDNF-TrkB signaling in the actions of GLYX-13. The results demonstrate that the antidepressant effects of GLYX-13 in three different models, the forced swim test, novelty suppressed feeding, and female urine sniffing test, are blocked by intra-mPFC infusion of an anti-BDNF neutralizing antibody or in mice with a knock-in of the BDNF Val66Met allele, which blocks the processing and activity dependent release of BDNF. The results also demonstrate that pharmacological inhibitors of BDNF-TrkB signaling or of L-type voltage dependent $Ca^{2+}$ channels (VDCCs) block the antidepressant behavioral actions of GLYX-13. Finally, we examined the role of Rho GTPase proteins, which are required for BDNF mediated stabilization of spines. The results demonstrate that infusion of a selective inhibitor of Rac1 but not RhoA into the mPFC blocks the antidepressant effects of GLYX-13. Together, these findings indicate that GLYX-13 causes activity dependent stimulation of VDCCs, leading to increased release of BDNF, subsequent TrkB-Rac1 signaling, and increased spine synapse number and stability that is required for the rapid and sustained antidepressant effects of GLYX-13.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH Grant MH045481

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State of CT

**Title:** GluN2B subunits on GABAergic interneurons in the medial prefrontal cortex and the antidepressant effects of ketamine

**Authors: *D. M. GERHARD**[1], E. S. WOHLEB[2], R. S. DUMAN[3]
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**Abstract:** Recent studies highlight the rapid antidepressant actions of the NMDA receptor antagonist ketamine. Rodent studies show that ketamine rapidly increases glutamate release, activates mTORC1 signaling and increases translation of synaptic proteins in the medial prefrontal cortex (mPFC) shortly following acute treatment. Furthermore, additional studies show that selective GluN2B receptor antagonists produce similar behavioral and molecular signaling effects. However, the initial cellular trigger underlying the actions of ketamine has not been identified. Collectively, these studies suggest that blockade of GluN2B-containing NMDARs may be a critical mediator for the rapid antidepressant effects of ketamine. We used CaMKII-, GAD67-, parvalbumin (PV)-, and somatostatin (SST)-Cre mice and viral-NR2B shRNA to produce Cre-dependent knockdown of GluN2B receptors in glutamate and GABA neurons, as well as subpopulations of GABA neurons. Mice were then tested in a preswim and open-field test (OFT) to measure baseline effects of cell-specific NR2B knockdown. Viral GluN2B shRNA was infused into the mPFC of the cre recombinase lines and after 3 weeks to allow for recovery and viral expression, the mice were tested before and after ketamine administration in the forced swim test (FST) and novelty suppressed feeding test (NSFT). Viral-mediated knockdown of GluN2B in the mPFC of GAD67-Cre mice produced a significant antidepressant response in the FST, and occluded the antidepressant effects of ketamine in this model; preliminary studies suggest similar effects in the NSFT. Knockdown of GluN2B on

pyramidal neurons in CaMKII-Cre mice did not significantly block the antidepressant response to ketamine in the FST or NSFT. Similar studies were conducted in SST- and PV-Cre mice and preliminary findings suggest that GluN2B on both SST- and PV-Cre neurons mediates the actions of ketamine. These findings indicate that GABA interneurons are the initial cellular trigger for the actions of ketamine. This results in blockade of tonic firing GABA interneurons and disinhibition of glutamate transmission that leads to increased synaptic connectivity and rapid antidepressant behavioral responses. Studies are being conducted to confirm these findings and to extend the results to other behavioral models.

**Disclosures: D.M. Gerhard:** None. **E.S. Wohleb:** None. **R.S. Duman:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.22/TT4

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Title:** Optogenetic stimulation of a specific principle neuron subtype in the prefrontal cortex produces rapid and long-lasting antidepressant effects similar to ketamine

**Authors: *B. D. HARE**, R. J. LIU, S. GHOSAL, K. E. FURMAN, R. J. DILEONE, R. S. DUMAN
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**Abstract:** Activation of pyramidal cells in the prefrontal cortex is implicated in the antidepressant effect of rapid acting antidepressants, including NMDA receptor antagonists such as ketamine. Consistent with this, optogenetic activation of principal neurons in the prefrontal cortex (PFC) has been demonstrated to produce the long term behavioral changes observed after administration of ketamine, as well as many of the proposed mechanistic effects of ketamine treatment. PFC principal cell subtypes have been defined based on morphological complexity, physiological properties, projection targets, and anatomical location within the PFC, and are referred to as Type A and B cells. Recent work demonstrates that Cre-recombinase expression under control of the dopamine D1 (Type B), or D2 (Type A) receptor allows subtype specific targeting with cre-dependent constructs. It is not clear whether the antidepressant response produced by optogenetic stimulation is carried by a particular subtype of principal neuron or whether concurrent activation of both types is necessary. To address this question we expressed cre-dependent channelrhodopsin (Chr2) in type A or type B principal cells within the mouse medial PFC. Behavioral testing occurred 1 day or more after optogenetic stimulation. Stimulation of type B cells produced an antidepressant response in the forced swim test that was evident 24 hours after stimulation and persisted for up to 7 days. Additionally, an anxiolytic response in the elevated plus maze and novelty suppressed feeding test was observed up to 72

hours after stimulation. In contrast, we observed no behavioral changes following stimulation of type A cells. Immediate early gene (IEG) analysis demonstrated that PFC stimulation of type B cells produced activation within the PFC, bed nucleus of the stria terminalis, and basolateral amygdala, but not the dorsal raphe nucleus. Though IEG activation was evident in the PFC after stimulation of type A cells, no changes in IEG expression were observed in downstream regions. Our results indicate that optogenetic stimulation of type B principal neurons in the medial PFC is sufficient to produce a rapid and enduring antidepressant response that is associated with activation of a distributed network implicated in emotional behavior. Experiments are ongoing using inhibitory opsins to test the necessity of type B pyramidal cells to the antidepressant response produced by ketamine administration.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

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**Program#/Poster#:** 613.23/TT5

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH Grant MH045481

NIMH Grant MH093897

**Title:** Characterization of the signaling mechanisms underlying the rapid antidepressant actions of the ketamine metabolite (2R,6R)-Hydroxynorketamine and GLYX-13

**Authors: *M. FOGACA**[1], F. KENICHI[2], R. S. DUMAN[3]
[1]Psychiatry, [2]Yale Sch. of Med., New Haven, CT; [3]Yale Univ. Sch. Med., New Haven, CT

**Abstract:** Low doses of the NMDA receptor antagonist ketamine produce rapid antidepressant actions even in patients considered treatment resistant. Recent studies demonstrate that the ketamine metabolite (2R,6R)-hydroxynorketamine (HNK) produces antidepressant actions in rodent models, without the side effect profile of ketamine. In addition, the NMDA receptor modulator GLYX-13 also produces rapid antidepressant actions in depressed patients and rodent models, without ketamine-like side effects. We are interested in identifying the cellular target(s) and signaling pathways that mediate the actions of these agents, and have reported that ketamine and GLYX-13 cause activity dependent release of BDNF and stimulation of mTORC1/p70S6K signaling in the prefrontal cortex (PFC), as well as in primary cultured neurons. Here we conduct *in vitro* cortical primary culture studies to extend these findings to HNK and to further test the role of BDNF in the regulation of mTORC1 signaling. Preliminary studies demonstrate that

HNK produces a dose and time dependent stimulation of the phosphorylated and activated forms of mTOR and p70S6K, as well as the upstream kinase ERK in primary neuronal cultures. This is consistent with preliminary *in vivo* studies showing that the antidepressant behavioral actions of HNK are blocked by infusion of the selective mTORC1 inhibitor rapamycin, or by infusion of a BDNF neutralizing antibody into the mPFC. Further studies of GLYX-13 in cultured neurons show that incubation with a BDNF neutralizing antibody prevents the stimulation of mTORC1 and ERK signaling, demonstrating a requirement for extracellular BDNF. Incubation with the VDCC blocker verapamil also blocks GLYX-13-induced BDNF release. Additional *in vitro* studies are being conducted to determine if HNK also causes activity and VDCC dependent release of BDNF in primary cultured neurons. Together these results suggest that rapid acting antidepressants cause activity dependent release of BDNF and subsequent activation of mTORC1 signaling. Studies are being conducted to determine if these agents act directly on principle neurons or indirectly on inhibitory neurons to produce activity dependent signaling and synaptic changes that underlie rapid antidepressant responses. *Supported by NIMH Grants MH045481 and MH093897, and the State of CT.*

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIMH MH 045481

NIMH MH 093897

State of CT

**Title:** Sex-dependent persistent alterations in microglial morphology following chronic unpredictable stress

**Authors: *C. Y. XU**, *C. Y. XU, T. C. FRANKLIN, R. S. DUMAN
Yale Univ., New Haven, CT

**Abstract:** Major Depressive Disorder (MDD) is a recurrent mental health illness with more than half of affected patients relapsing after their initial episode. High levels of psychological or environmental stressors are associated with the initial development of MDD and may play a role in recurrent episodes. Interestingly, susceptibility to both stress-induced depressive-like behaviors and immune activation differ between males and females and may play a role in the sexual dimorphic risk associated with psychological disorders such as depression. Our

preliminary results demonstrate that chronic stress promotes long lasting hippocampal microglia morphological changes in male rodents, which coincide with prolonged risk for stress-induced depressive-like behaviors. However, it is unclear if female rodents show similar persistent effects following chronic stress. In this study, we assessed sexual dimorphic effects of long-lasting microglial changes that corresponded with development and recurrence of depressive behavior using a chronic unpredictable stress (CUS) model in rodents. Male and female C57BL6 mice were tested for depressive-like behaviors following CUS (28 days) using a sucrose preference test (SCT) and tail suspension test (TST). Microglial morphological changes were assessed immediately following CUS exposure, and preliminary results demonstrate that female mice show evidence of activated microglia similar to morphological changes to those observed in male mice. To determine if stress-induced microglia alterations persisted in female mice after CUS, additional studies were performed in which males and female mice were exposed to CUS then allowed to recover for 4 weeks. Hippocampal microglia morphological changes were assessed in post stress animals and compared between males and females. Together, these data will provide novel insights into potential sex-dependent differences in stress-induced priming of microglial and subsequent susceptibility to depressive-like behaviors.

**Disclosures: C.Y. Xu:** None. **T.C. Franklin:** None. **R.S. Duman:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** NIH Grant MH093897

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State of Connecticut

**Title:** Characterization of medial prefrontal cortex glutamatergic projections in the antidepressant actions of ketamine

**Authors: *A. M. THOMAS**, B. D. HARE, T. KATO, K. FUKUMOTO, R.-J. LIU, G. K. AGHAJANIAN, R. S. DUMAN
Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

**Abstract:** A single, low-dose infusion of ketamine produces an antidepressant effect in depressed patients within a few hours of administration lasting for up to a week, making it one of

the most promising possibilities in the search for antidepressants that are faster-acting and more reliable than those currently available. The neural circuitry that responds to ketamine has yet to be fully characterized. It has been shown that ketamine induces glutamate release in the medial prefrontal cortex (mPFC), and that blocking glutamate transmission in the mPFC also blocks its antidepressant effect. This laboratory has demonstrated that it is possible to produce long-lasting synaptic and antidepressant-like behavioral responses in rats with a single, one-hour, 10-Hz optogenetic stimulation of excitatory neurons in the mPFC, using an rAAV2-CaMKIIα-ChR2(H134R)-EYFP vector. Here, we have found that a one-hour stimulation of ChR2-expressing axon terminals in the dorsal raphe nuclei (DRN) projecting from cell bodies in the mPFC of rat is also sufficient to produce an antidepressant-like effect in the forced-swim test (FST), but not in other assays of depression and anxiety behavior; this effect is present 24 hours after stimulation, but not 7 days after. This result raises the possibility that the DRN may be an important part of the circuitry that underlies the antidepressant response to ketamine. The effect of ketamine on the mPFC to DRN projection is being directly examined by infusing 8OH-DPAT, which inhibits the release of serotonin, into the DRN to test whether serotonin release is necessary for ketamine's antidepressant effect. To better understand how ketamine affects this circuit, immunohistochemical studies are being conducted to assess how the stimulation of these terminals affects neural activity in the DRN and which cells within the DRN are targeted by the activation of these axons. Finally, we are also examining the effect of optogenetically stimulating mPFC-originating axon terminals in the nucleus accumbens, to understand whether the antidepressant effect of stimulating mPFC-DRN terminals is unique to that pathway.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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VA National Center for PTSD

Connecticut Mental Health Center

**Title:** Transcriptome profiling of post traumatic stress disorder in human subgenual prefrontal cortex

**Authors: *M. J. GIRGENTI**[1], D. A. CRUZ[2], B. CARLYLE[1], D. WILLIAMSON[2], M. FRIEDMAN[3], J. H. KRYSTAL[1], R. S. DUMAN[4]
[1]Yale Univ. Sch. of Med., New Haven, CT; [2]Psychiatry & Behavioral Sci., Duke Univ., Durham, NC; [3]Geisel Sch. of Med. at Dartmouth, Hanover, NH; [4]Yale Univ. Sch. Med., New Haven, CT

**Abstract:** Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder with a life-time prevalence of 7.8% in the general population. Numerous studies suggest that alterations in gene transcription are associated with onset of PTSD. The majority of these studies have focused on peripheral blood transcript changes. While it is likely that many changes observed in the periphery reflect changes in the CNS, there are currently few studies directly examining the transcript changes occurring in brain. However, through recent efforts supported by the VA and the National Center for PTSD, a brain bank is being developed and is providing tissue for studies of the neurobiology of PTSD. We performed high through-put RNA-sequencing on human post mortem subgenual prefronal cortex (PFC) (Brodmann Area 25) from subjects with PTSD, a psychiatric control (major depressive disorder, MDD), and matching controls. Multidimensional scaling revealed significant differences in the gene expression patterns in subjects with PTSD and MDD, as well as some overlap between these groups. Further, we identified gene expression network changes in immediate early genes, inflammatory pathways, and in glucocorticoid signaling. We used classical fear conditioning in rats as an animal model of PTSD and found regulation of several of these transcripts in medial prefrontal cortex (mPFC) after training, consistent with expression changes of these genes in human subgenual PFC. These post mortem and preclinical studies have identified several key factors in PTSD pathophysiology and as a potential new target for therapeutic intervention.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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NIMH MH093897

State of CT

**Title:** Cell-specific ablation of microglial RAGE alters susceptibility to depressive-like behaviors after chronic unpredictable stress

**Authors:** *T. C. FRANKLIN[1], ***T. C. FRANKLIN**[1], C. XU, 06511[1], *Y. ZHANG[2], R. S. DUMAN[1]
[1]Yale Univ. Sch. of Med., New Haven, CT; [2]Yale Univ., New Haven, CT

**Abstract:** Maladaptive alterations that result from severe or chronic stress exposure are associated with increased risk for Major Depressive Disorder (MDD). Chronic stress promotes dysregulation of the innate immune system leading to enhanced inflammatory signaling often associated with depressive symptoms. Growing evidence suggests that innate immune cells such as microglia, promote neuroinflammation in response to stress by releasing danger associated molecular pattern (DAMP) molecules leading to increased inflammatory signaling through binding to pattern recognition receptors such as toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE). Our preliminary studies show that microglial RAGE is upregulated in response to chronic unpredictable stress (CUS) and enhanced microglial RAGE expression coincides with the *onset* and *recurrence* of stress-induced depressive-like behaviors, even weeks after stress exposure. Most importantly, constitutive RAGE KO mice show an attenuation of stress-induced depressive-like behavioral effects. These novel findings indicate that chronic stress exposure leads to long-lasting alterations of microglia, including increased expression of RAGE, that primes microglia to subsequent exposure to stress and susceptibility to depressive behaviors. We therefore hypothesize that deletion of RAGE specifically in microglial will attenuate depressive-like behaviors following stress due to suppressed DAMP signaling. In this presentation, we will present data from ongoing studies that examine the role of RAGE signaling in the development of depressive-like behaviors following chronic stress. To test this hypothesis, we generated $RAGE^{fl/fl}$:$CX3CR1^{CreERT}$ mice and utilized tamoxifen-induced Cre recombinase system for cell-specific deletion of microglial RAGE. Cre negative animals were used as controls. Tamoxifen-induced conditional RAGE knockout (KO) mice will be tested for cognitive, anxiety and depressive-like behaviors at baseline and after CUS exposure using novel object recognition (NOR), open field test (OFT), tail suspension test (TST) and sucrose consumption test (SCT). Microglial morphological changes will be assessed immediately following CUS exposure to determine if microglial RAGE deficient mice display reduced microglial reactivity following chronic stress exposure compared to littermate controls. Together, these data will provide novel insights into the role of microglial RAGE signaling in stress-induced microglial reactivity and the development of depressive-like behaviors.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

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The State of CT

**Title:** Rapid antidepressant actions of ketamine require acute inhibition of GABA interneuron firing

**Authors: *S. GHOSAL**[1], M. FOGACA[1], B. D. HARE[4], D. M. GERHARD[2], M. WU[1], M. ALREJA[1], C. H. DUMAN[3], R. S. DUMAN[5]
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**Abstract:** Imbalance of excitatory and inhibitory neurotransmission within the prefrontal cortex (PFC) circuitry has been implicated in clinical and rodent studies of depression. The rapid antidepressant response to the NMDA receptor antagonist ketamine has been associated with a glutamate burst and activity dependent synapse formation in the PFC. Consistent with these findings we have reported that optogenetic stimulation of principle neurons in the medial PFC produces synaptic and behavioral effects similar to ketamine. Moreover, knockdown of NMDARs on GABA interneurons blocks the antidepressant behavioral actions of ketamine (Gerhard et al., 2017, SfN abstract). These findings suggest that ketamine blockade of tonic firing GABA neurons causes disinhibition of PFC pyramidal neurons, resulting in a glutamate burst and long-lasting synaptic changes that underlie the antidepressant behavioral response. To examine how interactions between distinct types of neurons within the PFC local network contribute to the process of ketamine-mediated antidepressants like effects, we performed a single, one-hour 5-Hz laser stimulation of PFC GAD-positive inhibitory neurons infected with an AAV2-EF1a-DIO-hChR2(H134R)-EYFP construct. We found that optogenetic activation of GABAergic neurons in the PFC attenuated the antidepressant-like effects of ketamine in the forced swim test (FST) and in the novelty suppressed feeding test (NSFT), suggesting that PFC GABAergic interneurons act as the cellular trigger mechanism underlying the rapid antidepressant actions of ketamine. Currently, studies are being conducted to determine if acute, transient suppression of PFC GABAergic interneurons produces antidepressant behavioral effects similar to ketamine. Experiments to test this hypothesis require selective activation and /or inihibition of specific interneuron populations (SST or PV) using a combination of GABA interneuron specific Cre recombinase mice and light or chemically driven manipulation of cell activity. Together these studies will further define the role of GABA interneurons in the actions of ketamine, and determine if ketamine-induced disinhibition underlies a long-term synaptic response that restores the excitatory/inhibitory balances.

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**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

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**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** Navitor Pharmaceuticals

**Title:** Sestrin2 modulator NV-5138, shows ketamine-like rapid antidepressant effects via direct activation of mTORC1 signaling

**Authors: T. KATO**[1,2], R.-J. LIU[1], C. H. DUMAN[1], R. TERWILLIGER[1], G. P. VLASUK[3], E. SAIAH[3], S. HAHM[3], *R. S. DUMAN[1]
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**Abstract:** The NMDA receptor antagonist ketamine produces rapid antidepressant actions, even in patients considered treatment resistant, addressing a major limitation of currently available medications. In preclinical rodent models the antidepressant behavioral actions of ketamine are associated with increased number and function of synapses in the medial prefrontal cortex (PFC) and these effects are dependent on mTORC1 signaling. The mTORC1 pathway is a cellular regulator of protein synthesis and is modulated by neuronal activity, endocrine and metabolic signals, including amino acids, notably leucine, which activates mTORC1 signaling via the binding to sestrin2. Here, we examined the influence of NV-5138, a small molecule modulator of sestrin2 that penetrates the blood brain barrier, on antidepressant behavioral responses and induction of spine synapses in the prefrontal cortex (PFC). The results demonstrate that NV-5138 (160 mg/kg) produced antidepressant effects in the rat forced swim test (FST), female urine sniffing test (FUST), and novelty suppressed feeding test (NSFT), without effecting locomotor activity or home cage feeding. NV-5138 also reversed the anhedonia caused by chronic unpredictable stress exposure. The effects of NV-5138 were long-lasting, observed for up to 72hrs following a single dose when there was no detectable brain or plasma exposure. As expected, NV-5138 activated the mTORC1 pathway in the PFC 1 hr after administration, similar to ketamine, and the antidepressant actions of NV-5138 were blocked by infusion of the selective mTORC1 inhibitor rapamycin into medial PFC. We also tested synaptic responses of layer V pyramidal neurons in the medial PFC and found that NV-5138 administration rapidly increased the number and function of spine synapses in the apical dendrites. NV-5138 also increased levels of synaptic proteins in PFC, including GluA1 and synapsin1. Taken together, the results demonstrate that NV-5138 produces rapid synaptic and antidepressant behavioral responses via the direct activation of the mTORC1 signaling pathway, supporting the possibility that sestrin2 modulation is a novel target for development of rapid acting antidepressants.

**Disclosures:** **T. Kato:** A. Employment/Salary (full or part-time):; Sumitomo Dainippon Pharma. **R. Liu:** None. **C.H. Duman:** None. **R. Terwilliger:** None. **G.P. Vlasuk:** A. Employment/Salary (full or part-time):; Navitor Pharmaceuticals, Inc. **E. Saiah:** A. Employment/Salary (full or part-time):; Navitor Pharmaceuticals, Inc. **S. Hahm:** A. Employment/Salary (full or part-time):; Navitor Pharmaceuticals, Inc.. **R.S. Duman:** None.

**Poster**

**613. Animal Models for Affective Disorders: Therapeutics**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 613.30/TT12

**Topic:** G.04. Mood Disorders: Depression and Bipolar Disorders

**Support:** CONACYT CB-2011-167436Q

**Title:** The bodybuilding supplement ZMA, & Ketamine (1 mg/kg) produce an additive increase in the anti-depressive index, "swimming time", but not in the depressive index "immobility time", in the forced swimming test in BALB/c mouse

**Authors: *V. J. MEDINA ARANDA**[1], A. L. ROSADO-PEREZ[1], M. VILLALOBOS[1], S. VILLALOBOS[1], J. C. PINEDA[2]
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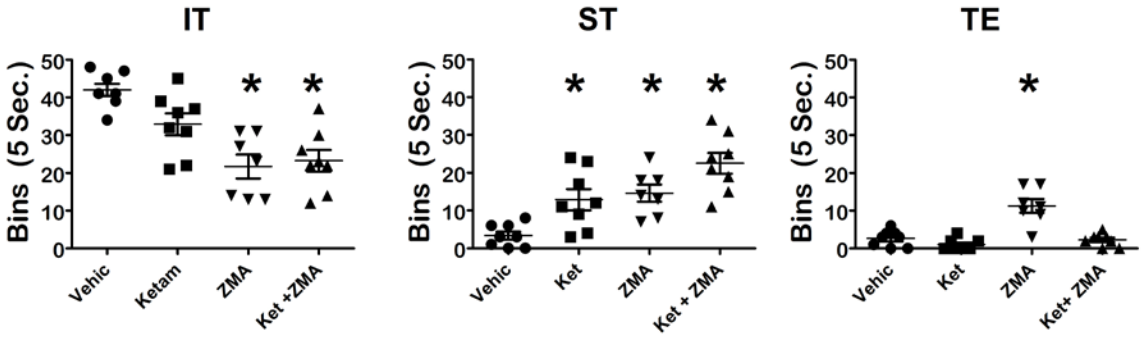
**Abstract:** Depression is a disorder with a high rate of resistance to available treatments. Ketamine, an N-methyl-D-aspartate receptor antagonist, produces a rapid and sustained antidepressant effect in patients with treatment-resistant depression. But in therapeutic doses produces psychotic symptoms that limits its use. The bodybuilding supplement ZMA (11 mg of pyridoxine, 450 mg of magnesium and 30 mg of zinc) may be used as antidepressant drug, given that deficiency of zinc or magnesium produced depressive or anhedonia symptoms. We tested whether ZMA can potentiate the action of ketamine in BALB/c mice. **Methods:** 32 BALB/c mice were divided into 4 groups of 8 mice each, received a dose of: Ketamine (1 mg / kg; a subthreshold dose for antidepressant activity); or Ketamine (1 mg / kg) + ZMA (0.028125mg / kg); or ZMA in the same dose; 24 hrs. before each test. The mice were subjected to the open field test, (6 minutes). One week after the open field test the mice were subjected to the forced swimming test for 6 minutes. **Results.** ANOVA $F_{3, 27} = 12.44$ P = <0.0001. Tukey´s posttest showed that immobility time (IT) was reduced when ZMA or ZMA + Ketamine was applied against vehicle, but not when Ketamine was applied alone. While for the swimming time (ST) in the last 4 min. of the test, ANOVA reveled significant differences: $F_{3, 27} = 11.72$. P = < 0.001. Tukey´s posttest showed that significant differences between Vehicle with ketamine ($p < 0.05$), with ZMA ($p < 0.05$) and with ZMA + Ketamine ($p < 0.001$). No changes were detected in the climbing time. No differences were detected in the distance traveled by the mice in the open field test. **Discussion:** Ketamine (1 mg/kg), and ZMA reduced the depressive index and increased the

antidepressant index during the forced swimming test in BALB/c mice. Since the effect of Ketamine and ZMA differ for the IT, while for the ST the effect was additive, it is possible that the action on these two behaviors is not mediated by the same mechanisms.




**Disclosures:** **V.J. Medina Aranda:** None. **A.L. Rosado-Perez:** None. **M. Villalobos:** None. **S. Villalobos:** None. **J.C. Pineda:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CB 2011- CB167436-Q.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.01/TT13

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Sex differences in the role of ventral hippocampus following retrieval of context fear memory

**Authors: *L. PAN**, A. A. KEISER, N. C. TRONSON
Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

**Abstract:** In males, dorsal hippocampus has been extensively studied, and more recently, ventral hippocampus has also been implicated as a key region in the circuitry underlying context fear memory. In females, however, the precise role of hippocampus and the mechanisms underlying context fear conditioning and retrieval remain less clear. Here we examined the role of, and signaling pathways activated in, ventral hippocampus during retrieval of context fear memory in both males and females. Ventral hippocampus was robustly activated after retrieval of context fear conditioning in both sexes, with females showing greater levels of cFos compared with males. These data suggest a role for ventral hippocampus in retrieval of context fear conditioning in both sexes, but that males and females likely differ in the molecular mechanisms recruited

during memory retrieval. To determine whether ventral hippocampus is required for memory retrieval, male and female mice received infusions of muscimol into ventral hippocampus prior to retrieval of foreground context fear conditioning. We observed decreased freezing, demonstrating that ventral hippocampus is required for context fear memory retrieval in both sexes. Finally, to identify whether males and females recruit different intracellular signaling pathways, we collected ventral hippocampus one hour after retrieval of context fear and conducted western blot analysis to identify the activation of PKA-CREB and mTOR-related signaling pathways. Together, our data demonstrate that ventral hippocampus is required for retrieval of context fear, and that retrieval activates different molecular mechanisms in males and females. These data on contextual fear memory retrieval will advance the development of sex-specific treatments for fear and anxiety related disorders such as PTSD.

**Disclosures: L. Pan:** None. **A.A. Keiser:** None. **N.C. Tronson:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.02/TT14

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NDSEG

**Title:** Retrieval of context fear memory involves sex-specific molecular mechanisms and changes in hippocampal gene expression

**Authors: *A. A. KEISER**[1], L. PAN[2], N. C. TRONSON[2]
[2]Dept. of Psychology, [1]Univ. of Michigan, Ann Arbor, MI

**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 memories. We have previously demonstrated that female mice display greater context fear generalization and show less activation of hippocampus and more activation of amygdala compared with males. Together with findings demonstrating male-specific mechanisms of context fear conditioning, these data suggest that males and females use both different circuit-based mechanisms and molecular mechanisms of memory retrieval. In this study, we examined how sex differences in context fear conditioning affected memory retrieval at remote time points, and identified sex-specific molecular mechanisms of retrieval using RNA-sequencing. In males, context fear memory becomes less hippocampal dependent and more reliant on cortical structures over time via systems consolidation. We show that eight weeks after background context fear conditioning, females but not males exhibit decreased freezing compared to tests one day after conditioning. That is, females show impairments in remote context fear memory.

Immunohistochemistry determined differential recruitment of hippocampus, amygdala, retrosplenial cortex, and anterior cingulate cortex at remote time points in males and females. Such sex differences in remote memory further support the possibility that males and females employ unique mechanisms of context fear conditioning and its retrieval. In order to step away from a strictly male-comparative approach, we employed RNA-sequencing as an unbiased measure to examine regulation of hippocampal gene expression after memory retrieval in females and males. These data will inform molecular pathways activated in females following retrieval of context fear. Understanding the mechanisms mediating retrieval and maintenance of contextual fear memory in males and females will aid in the development of sex-specific treatments for PTSD and potentially lead to methods of cognitive enhancement in healthy individuals.

**Disclosures: A.A. Keiser:** None. **L. Pan:** None. **N.C. Tronson:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.03/TT15

**Topic:** H.01. Animal Cognition and Behavior

**Support:** China 973 Program

**Title:** Isolation induced-forgetting of social memory is regulated by Rac1 activity

**Authors: *Y. LIU**[1], L. LI[2], Y. ZHONG[1]
[1]Sch. of Life Sciences, Tsinghua Univ., Beijing City, China; [2]Tsinghua Univ., Beijing city, China

**Abstract:** During the past decades several independent studies have repeatedly confirmed that long-term social recognition memory is impaired by social isolation (SI) of adult mice and rats. However, the mechanisms mediating this impairment and their whereabouts in the brain are yet to be discovered. Currently, we find that hippocampal Rac1 activity increased in isolated mice whereas Rac1 activity decreased in re-grouped mice. Behavior experiments show that inhibiting Rac1 activity in the hippocampus can block the SI induced-social memory impairment. Furthermore, the LTP deficiency in isolated mice can also be rescued by inhibiting Rac1 activity, providing electrophysiological evidence that Rac1 activity mediates social memory impairment. Thus, based on our previous studies of Rac1 regulated-active forgetting, the current findings here indicate that SI induced-social memory impairment might be a kind of active forgetting caused by hippocampal Rac1 activation.

**Disclosures: Y. Liu:** None. **L. Li:** None. **Y. Zhong:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.04/TT16

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NNSF of China 31471079

**Title:** Paternal methyl donor-rich diet reduces kcnmb2 expression and causes synaptic function and memory deficits in offspring F1 mice

**Authors: *L. GUO**
Qingdao Univ., Shandong, China

**Abstract:** BK channel is a known regulator of neuronal excitability, synaptic plasticity and memory. Our previous study showed that a paternal methyl donor-rich diet reduces the expressions of *kcnmb2* which *encodes BK channel beta subunit 2*, and caused memory deficits in offspring mice. To explore the underlying cellular mechanism, we measured the BK current, the neuronal excitability, synaptic transmissions and plasticity in CA1 pyramidal neurons of the offspring F1 mice which father was fed with either methyl donor-rich diet (MD) or regular control diet (CD). Whole-cell patch-clamp recordings revealed reduced resting membrane potential, a decrease in inhibitory synaptic transmission and unchanged excitatory synaptic transmission in CA1 pyramidal neurons of MD F1 mice. Meanwhile, field recordings revealed impaired LTP in hippocampal slices of MD F1 mice. Over-expressions of *kcnmb2* in CA1 region with a viral vector (AAV1-hSYN1-Kcnmb2-IRES-GFP-WPRE) reversed the changes in neuronal excitability, synaptic function and memory deficits observed in MD F1 mice. Those findings thus suggest that reduced expression of *Kcnmb2* by DNA methylation may alter activity of inhibitory neurons in hippocampal network and cause impairment in synaptic plasticity and memory formation.
This work was supported by NNSF of China (31471079 to Y.Z.)

**Disclosures: L. Guo:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.05/TT17

**Topic:** H.01. Animal Cognition and Behavior

**Support:** China 973 program

**Title:** Role of Rac-specific GAP proteins in the forgetting of fear memory

**Authors: *L. LV**, Y. LIU, Y. ZHONG
Sch. of Life Sci., Tsinghua Univ., Beijing City, China

**Abstract:** Molecular mechanisms underlying active forgetting have only recently begun to reveal. Several important proteins have been identified, and their contributions to the forgetting of memory have been assessed in the mushroom body of *Drosophila* and in the hippocampus of mice such as a small G protein Rac1. However, the upstream mechanisms of Rac1 underlying forgetting have not been fully understood. Here we explored the functions of Rac-specific GAP proteins in the forgetting of contextual fear memory induced by weak training. We found that knockdown of one of the GAPs in dorsal hippocampus induces forgetting of fear memory without affecting remote fear memory. Conversely, overexpression of this GAP attenuated forgetting of fear memory. In addition, knockdown of the GAP gene also accelerated forgetting of 24-hr social memory. Electrophysiological evidences show bi-directional regulation of this GAP in the adult dorsal hippocampus. Our findings suggest that at least one of the Rac-specific GAPs is crucial for forgetting of fear memory and social memory in adult mice.

**Disclosures: L. Lv:** None. **Y. Liu:** None. **Y. Zhong:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.06/TT18

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH MH101477

NIH OD011132

NSF DGE-1444932

**Title:** Adolescent corticosteroid exposure disrupts decision-making, trkB, and cortico-limbic anatomy in adulthood

**Authors: *E. T. BARFIELD**[1,2,3,4], K. J. GERBER[5], S. L. GOURLEY[1,2,3,4]
[1]Yerkes NPRC, [2]Dept. of Pediatrics, [3]Grad. Program in Neurosci., [4]Dept. of Psychiatry & Behavioral Sci., [5]Mol. & Systems Pharmacol., Emory Univ., Atlanta, GA

**Abstract:** Prolonged exposure to elevated glucocorticoids, as occurs with chronic stress, disrupts the structure and function of cortico-limbic brain regions involved in decision-making, planning, and motivation, and impairs neurotrophin signaling that supports dendritic spine growth and plasticity. However, neurodevelopmental factors remain largely uncharacterized, despite evidence that adverse experiences during adolescence may be particularly impactful. Here, we tested the hypothesis that adolescents are vulnerable to the long-term neurobehavioral consequences of prolonged, elevated glucocorticoid levels. We exposed mice to the primary glucocorticoid, corticosterone (CORT), for 11 days and found that CORT exposure in early adolescence, but not adulthood, impaired goal-directed decision-making, biasing behavior towards stimulus-elicited habits, in adulthood. CORT exposure also disrupted the ratio of tyrosine receptor kinase B (trkB) isoforms throughout multiple cortico-limbic brain regions. Further, dendritic spine densities on excitatory pyramidal neurons in the orbital prefrontal cortex (oPFC) were reduced in adult animals with a history of adolescent CORT exposure. Additionally, goal-directed behavior was positively correlated with the density of mature, mushroom-shaped spines in the oPFC. Stimulation of trkB during adolescence blocked decision-making deficits in CORT-exposed mice. Finally, we examined how CORT±trkB stimulation impacted inputs to the oPFC, in particular those from the ventral hippocampus. Our findings indicate that adolescent CORT exposure induces long-term alterations in neuronal structure, function, and decision making. Pharmacological interventions that augment trkB signaling may be particularly efficacious in blocking enduring neurobehavioral deficits in adolescents exposed to chronic stress.

**Disclosures: E.T. Barfield:** None. **K.J. Gerber:** None. **S.L. Gourley:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.07/TT19

**Topic:** H.01. Animal Cognition and Behavior

**Support:** H2020-MSCA-IF-2015 Project ID 703285

BIAL Foundation grant # 64/12

**Title:** Cognitive bias in zebrafish

**Authors: *F. ESPIGARES**, R. OLIVEIRA
Inst. Gulbenkian De Ciência, Oeiras, Portugal

**Abstract:** According to cognitive appraisal theories, organisms use a set of stimulus evaluation checks in this process that allow them to evaluate its properties (intrinsic valence, familiarity,

predictability, prediction error) and the available coping mechanisms (capacity for control). The fact that cognitive appraisal is involved in the evaluation of stimuli creates the potential for cognitive biases that produce subjective evaluations (i.e. some individuals will consistently evaluate ambiguous stimuli as negative - pessimistic, and others as positive - optimistic). In this study, we have developed a cognitive bias behavioral test for zebrafish, a model organism for which genetic tools are available for visualizing and manipulating neural circuits, establishing therefore the occurrence of cognitive bias in this species. The behavioral test consists in training the fish to discriminate between a positive (P; i.e. reward) and a negative (N; i.e. punishment) color cue. Once fish are capable of discriminating between P and N color cues (as indicated by different latencies in reaching each one), their responses to an ambiguous (A) color between the positive and the negative is tested. Each individual (n=70) was, therefore, classified in an optimistic/pessimistic dimension. Our results, based on qPCR and RNAseq analysis, show that optimistic and pessimistic individuals are characterized by different basal levels of stress-related genes, suggesting that cognitive bias towards optimistic/pessimistic judgments are potentially associated with different stress coping styles.

**Disclosures:** **F. Espigares:** None. **R. Oliveira:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.08/TT20

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Vikings Children Fund and Minnesota Medical Foundation to P.V.T.

**Title:** TMEM35 modulates pain and drug seeking behavior

**Authors:** *T. MATVEEVA[1], ***T. MATVEEVA**[1], J. C. GEWIRTZ[3], P. V. TRAN[2]
[1]Psychology, [2]Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, MN; [3]Dept Psychology, Univ. of Minnesota Dept. of Psychology, Minneapolis, MN

**Abstract:** Previously we have shown that TMEM35 (NACHO) KO mice exhibit heightened pain sensitivity (Abstract 2016-S-14895-SfN). Given the comorbidity of pain and drug-seeking behavior, the present study investigated whether TMEM35 KO mice also show increased risk for drug seeking behavior. To test this, we examined conditioned place preference (CPP) for morphine and nicotine in separate experiments. Following habituation, one side of the CPP chamber was paired with morphine (10mg/kg s.c.) or nicotine (0.5g/kg base i.p.) and the other with saline. Mice were given 4 trials with drug and 4 trials with saline. WT and KO male mice were handled for 12 consecutive days. On the day following conditioning, all animals were injected with saline and allowed to explore the apparatus freely for 15 minutes. Preference for

the drug-paired side of the apparatus was measured. TMEM35 KO mice exhibited significantly stronger CPP for both morphine and nicotine than WT controls. For morphine, there was a main effect of genotype and conditioning context, and a significant genotype X context interaction, revealing that the effect of morphine on CPP was greater in the KO animals. Similarly, TMEM35 KO mice showed a significant increase in preference for the nicotine-paired context compared to pre-test, while WT animals showed no change in preference for the drug-paired side. Together, these results provide strong evidence implicating TMEM35 in drug-seeking behavior. Given a recent finding implicating TMEM35 in the expression of functional nicotinic acetylcholine receptors (nAchRs), these behavioral effects in TMEM35KO mice may be associated with the reduced expression of nAchRs. Contrary to a previous report showing a complete loss of a7 expression in TMEM35 KO mice, we report residual a7 expression in KO mouse brain, suggesting alternative mechanisms underpinning these drug-seeking behaviors. We are now conducting a transcriptomic analysis to identify alternative candidate mechanisms.

**Disclosures: T. Matveeva:** None. **J.C. Gewirtz:** None. **P.V. Tran:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.09/TT21

**Topic:** H.01. Animal Cognition and Behavior

**Support:** JSPS Grant-in-Aid for Young Scientists B 16K16559

**Title:** Mild exercise causes dynamic change of hippocampal gene expression in response to acute stress

**Authors: *M. OKAMOTO**[1,2], A. C. PEREIRA[1], J. D. GRAY[1], R. L. DAVIDSON[1], J. F. KOGAN[1], C. S. LARSON[1], B. S. MCEWEN[1], H. SOYA[2]
[1]The Rockefeller Univ., New York, NY; [2]Univ. of Tsukuba, Tsukuba, Japan

**Abstract:** Exercise induces resistance against stress-related brain dysfunction, such as memory deterioration and mood disorder. Previous studies have demonstrated mild exercise activates the hippocampus and enhances neuronal plasticity and function. However, the effects of mild exercise on stress resistance remain unknown. In this study, the effects of mild exercise on working memory and gene expression after an acute stress exposure were examined using RNA-Sequencing (RNA-Seq) to understand the mechanisms underlying the beneficial effects of exercise. Mice were subjected to 6 weeks of mild exercise training using a treadmill (5 times per week for 30 min/day). The running speed was 7 m/min, which is considered of mild intensity. Mice in the sedentary group remained sitting on the treadmill for the same amount of time without running. After the last day of training, one cohort of mice was subjected to 2h of acute

restrain stress (ARS). Subsequent to ARS, mice were returned to their home cage for 30 min before working memory was assessed with the Y-maze. Hippocampal tissue was collected 24h after ARS and behavioral testing. mRNA was sequenced using an Illumina NextSeq550 to collect 150bp 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. The results of the behavior assessment showed significantly decreased spontaneous alternations, a measure of working memory, after exposure to ARS in the sedentary group. Importantly, mild exercise training prevented the ARS-induced decline in working memory. RNA-Seq results showed ARS induced 1,053 changes in gene expression compared to control mice. Exercised mice showed 5,668 differentially expressed genes after ARS. These results provide evidence that regular mild exercise induces resistance to acute stress related memory decline, and the transcriptome under analysis will provide insight into its underlying molecular mechanisms.

**Disclosures: M. Okamoto:** None. **A.C. Pereira:** None. **J.D. Gray:** None. **R.L. Davidson:** None. **J.F. Kogan:** None. **C.S. Larson:** None. **B.S. McEwen:** None. **H. Soya:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.10/TT22

**Topic:** H.01. Animal Cognition and Behavior

**Title:** “Chemobrain”: Cytosine arabinoside (Ara-C) used in acute myeloid leukemia treatment is associated with disruptions to the birth and differentiation of adult-born neurons

**Authors: *Q. WU**[1], K. CHIANG[2], M. Q. GOH[1], E. H. KOO[1,2]
[1]Natl. Univ. of Singapore, Singapore, Singapore; [2]Dept Neurosciences, UCSD, La Jolla, CA

**Abstract:** “Chemobrain” syndrome, the adverse cognitive impact of chemotherapy treatment on cancer patients, has been characterised in a range of rodent models. To date, however, the bulk of our understanding is limited largely to breast cancer drugs and the causal and mechanistic links between chemotherapy and cognitive function have yet to be established. We studied the neurological sequelae in mice treated with Ara-C, the first line treatment for acute myeloid leukemia (AML) and other haematological malignancies. Here, we describe our studies in adult, wild-type C57BL6 mice treated intraperitoneally with Ara-C for five consecutive days then assessed by behavioural, morphological, and molecular measurements. We found that treated animals developed remote, hippocampal-dependent memory impairment, in contextual fear conditioning tests. The deficit occurred when mice were trained immediately after treatment then tested 4 to 8 weeks after training; but short-term memory, as well as learning and memory

assessed 6 months post-treatment, was intact, indicating a specific impairment of remote memory reconsolidation. BrdU labeling and Ki67 staining revealed marked inhibition of cell proliferation in the neurogenic niches of the brain as well as in the corpus callosum immediately post-treatment, indicating mitotic arrests in glial and neural progenitors. Gene expression of cell cycle regulator p21 (cdkn1a) was concomitantly and significantly elevated in the hippocampus, cortex and cerebellum of treated mice 24 hours after cessation of treatment, suggesting possible alterations in cell growth and proliferation pathways. Besides cell birth, cell fate choice was also affected: a lower percentage of adult-born granule cells (abGCs) pre-labelled with BrdU became doublecortin (DCX)- or NeuN-positive neurons. Our results extend current understanding of the "chemobrain" syndrome by highlighting changes in neuronal signalling and other processes that add to the alterations in neurogenesis that have been described previously; processes which in sum could affect memory and cognitive processes in animals, representing potential mechanisms that underlie the human syndrome.

**Disclosures: Q. Wu:** None. **K. Chiang:** None. **M.Q. Goh:** None. **E.H. Koo:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.11/TT23

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Chronic mild stress elevates GRK2 expression in BNST of female rats

**Authors: *A. KULP**, M. RUSS, B. LOWDEN, J. D. JOHNSON
Biomed. Sci., Kent State Univ., Kent, OH

**Abstract:** Prior exposure to environmental stressors enhances contextual fear learning. Greater beta-adrenergic receptor signaling in animals with prior stress exposure mediate the enhanced contextual fear learning as administration of propranolol, a beta-adrenergic receptor antagonist, prior to fear conditioning prevent rats from showing exaggerating freezing 24h later when re-exposed to the context. Sensitization of beta-adrenergic signaling can be mediated by G protein-coupled receptor kinase 2 (GRK2) that decouples the receptor from intracellular signaling cascades and pro-inflammatory cytokines. Here, we investigated whether GRK2 and inflammatory cytokines (IL-1, IL-6) are altered by prior stress exposure in the bed nucleus of the stria terminalis (BNST), a brain region previously demonstrated to be critical for stress induced enhanced learning. Twelve female rats were subjected to a 4-day chronic mild stress protocol or remained in their cages to serve as non-stress controls. Brains were collected 24h after the last stressor exposure and the BNST was laser captured from each rat. Following, GRK2, inflammatory cytokines (IL-1, IL-6), and GAPDH were analyzed through real time PCR to measure gene expression. A significant increase of GRK2 mRNA was detected in stressed

animals compared to controls, while cytokine expression was undetectable. These results suggest repeated stress exposure can cause changes in beta-adrenergic receptor signaling in the BNST. Future studies are aimed to alter GRK2 expression in the BNST to determine whether GRK2 is necessary/sufficient for the stress enhanced contextual fear learning.

**Disclosures:** **A. Kulp:** None. **M. Russ:** None. **B. Lowden:** None. **J.D. Johnson:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.12/TT24

**Topic:** H.01. Animal Cognition and Behavior

**Title:** The effect of HDAC2 inhibition on cognitive measures in C57/BL6 mice: Assessing pharmacological, antisense oligonucleotide, and genetic manipulations

**Authors: *H. M. ARNOLD**, S. GLASS, R. DRISCOLL, P. LEACH
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**Abstract:** A growing body of research suggests that histone deacetylase 2 (HDAC2) is a key negative modulator of memory formation and that inhibition of this enzyme could be a therapeutic target for reversing or slowing cognitive impairment in neurodegenerative disease and perhaps for cognitive enhancement more generally. In the current studies we aimed to confirm and extend findings from the literature suggesting such cognitive benefits of HDAC2 inhibition in normal mice. We began by testing the efficacy of HDAC inhibitors to enhance contextual fear conditioning, as has been reported several times in the literature. We dosed two prototypical HDAC inhibitors, SAHA and CI-994 (both at 0, 10 or 30 mg/kg, IP), once daily for 10 days prior to contextual and cued fear conditioning in C57/BL6 mice, but observed no behavioral effects of these treatments on conditioned context or cue freezing 24 h later. We then tested a more selective HDAC2 inhibitor, FRM-0334, after daily dosing for 1, 3, or 10 days prior to training, but again, no enhancement of contextual fear was observed under any dosing regimen. To take advantage of the long-lasting effect antisense oligonucleotides ASOs have on gene and protein knock-down (>12 weeks) we tested whether an ASO, selective for HDAC2, would enhance contextual fear conditioning in C57/BL6 mice. Again, no improvement in fear conditioning was observed nor was performance enhanced in two other cognitive tasks (two trial Y-maze and pre-pulse inhibition of startle). Selectively knocking down HDAC2 using a genetic approach (CRISPR-based knock-down) also failed to alter performance in these three behavioral tasks. Thus, these observations are not consistent with the premise that HDAC2 inhibition is a viable strategy for cognitive enhancement. As these studies were all conducted in healthy, relatively young mice the possibility remains that HDAC2 inhibition could be effective in

instances and circumstances in which cognitive performance is impaired due to age, disease, or neurodegeneration.

**Disclosures:** **H.M. Arnold:** A. Employment/Salary (full or part-time):; Biogen. **S. Glass:** A. Employment/Salary (full or part-time):; Biogen. **R. Driscoll:** A. Employment/Salary (full or part-time):; Biogen. **P. Leach:** A. Employment/Salary (full or part-time):; Biogen.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.13/TT25

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH grant 3F32DA041778-01A1S1

NIH grant 5R00DA034681-04

**Title:** The role of Gadd45b in DNA demethylation and cocaine action

**Authors: *F. SULTAN**, G.-E. GRAHAM, K. SAVELL, J. J. DAY
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**Abstract:** Introduction
Epigenetic mechanisms are central regulators of the function and information storage capacity of neuronal systems. Methylation of cytosine nucleobases in DNA is a multifunctional epigenetic regulatory modification capable of exerting powerful control gene. In the brain, activity-dependent changes in DNA methylation are critical for synaptic plasticity and memory formation, and have been implicated in a broad range of neuropsychiatric disease states, including drug addiction. However, although activity-related DNA demethylation requires the *Gadd45* (*Growth arrest and DNA-damage-inducible*) protein family, very little is known about how DNA demethylation regulates the function of brain reward circuits or the role that *Gadd45* family members play in behavioral responses to drugs of abuse.
Methods
Here, we combined unbiased genome-wide transcriptional profiling, pharmacological tools, and CRISPR/dCas9 transcriptional activation with traditional knockout and behavioral approaches in rodent model systems (both *in vitro* and *in vivo*) to dissect the role of *Gadd45b* in dopamine-dependent epigenetic regulation.
Results
We show that acute cocaine administration induced upregulation of *Gadd45b* mRNA in rat nucleus accumbens, but did not alter expression of other methylation-related transcripts. Similarly, acute dopamine treatment in striatal neuron cultures increased expression of *Gadd45b*

and *Gadd45g* mRNA. This effect was mimicked by the Drd1 agonist SKF-38393, suggesting upregulation in Drd1-containing neurons. *In vitro*, CRISPR-targeted transcriptional activation of either *Gadd45b* or *Gadd45g* with a dCas9-VP64 fusion construct was capable of unsilencing a methylated reporter gene, suggesting a mechanistic link between *Gadd45* induction and DNA demethylation. Finally, we show that both dopamine treatment (*in vitro*) and cocaine administration (*in vivo*) induce DNA demethylation, and that *Gadd45b*$^{-/-}$ mice exhibited impaired conditioned place preference for cocaine.

Conclusions

These results suggest that striatal *Gadd45b* functions as a dopamine-dependent immediate early gene to coordinate demethylation of DNA at downstream target genes, and that this action is important for cocaine-related behavioral plasticity.

**Disclosures: F. Sultan:** None. **G. Graham:** None. **K. Savell:** None. **J.J. Day:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

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**Program#/Poster#:** 614.14/TT26

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Austrian Science Fund (FWF): P21930-B09

ERC grant agreement no. 311701

**Title:** Experience induces rapid nucleus-scale movements of chromatin in cortical neurons

**Authors: *S. RUMPEL**[1], D. F. ASCHAUER[1], T. R. BURKARD[2], F. GRÖSSL[3], W. HAUBENSAK[3], M. PETER[3]

[1]Johannes Gutenberg Univ. Mainz, Mainz, Germany; [2]IMBA Inst. of Mol. Biotech. of the Austrian Acad. of Sci., Vienna, Austria; [3]Res. Inst. of Mol. Pathology (IMP), Vienna, Austria

**Abstract:** The interphase nucleus is functionally organized in active and repressed territories defining the transcriptional status of the cell. Dynamic chromatin movements at the scale of the nucleus have been observed during interphase in a variety of systems ranging from yeast to mammalian cell lines and are believed to support transcriptional alterations. Despite early evidence for nucleus-scale reorganization of chromatin in neurons, to date, no chronic imaging data of chromatin dynamics in living animals is available. Applying *in vivo* two-photon imaging in a novel transgenic mouse model allowing photolabeling of histones, we show that chromatin organization of neurons can undergo rearrangements within minutes, indicating an actively controlled process. In an *in vitro* brain slice model chromatin remodeling can be observed upon pharmacological manipulation of neuronal activity. Furthermore, we show that auditory cued

fear conditioning, a paradigm prompting changes in gene expression patterns in the auditory cortex, induces chromatin remodeling in neocortical neurons *in vivo*. We propose that global chromatin motility acts together with local gene-specific modifications to promote new nuclear organization patterns permissive for transcriptional plasticity at time scales that could not be realized by passive diffusion. Together, our findings open a framework to chronically study chromatin dynamics at the nucleus-scale in a physiologically relevant context.

**Disclosures: S. Rumpel:** None. **D.F. Aschauer:** None. **T.R. Burkard:** None. **F. Grössl:** None. **W. Haubensak:** None. **M. Peter:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.15/TT27

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant RO1 MH094792 to TJC

**Title:** Conserved modules of insulin-like signaling modulate neural function in *Aplysia*

**Authors: *N. KUKUSHKIN**[1], S. P. WILLIAMS[2], T. J. CAREW[1]
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**Abstract:** Insulin-like signaling is ubiquitous in animals, but its interaction with the nervous system remains largely unexplored. Here we show that the insulin-like system in *Aplysia californica* modulates functional changes in sensory neurons (SNs) induced by human insulin-like growth factor 2. IGF2 has diverse effects: it (i) increases the rate of neurite outgrowth in cultured SNs, it (ii) decreases their excitability, (iii) suppresses a distinct form of activity-dependent growth, and (iv) causes synaptic depression in co-cultures of SNs and motor neurons (MNs). In *ex vivo* preparations of pleural-pedal ganglia, SNs showed similar inhibitory responses to IGF2: reduced excitability in parallel with a decrease in total cellular phosphotyrosine, generally associated with signaling through receptor tyrosine kinases (RTKs). This surprising effect was sensitive to (i) an inhibitor of PTP1B (a tyrosine phosphatase involved in mammalian insulin signaling), (ii) to the mTOR inhibitor rapamycin, and (iii) to GSK1838705A, an inhibitor of mammalian insulin and IGF1 receptors. Blocking synaptic activity reversed both the biochemical and electrophysiological effects of IGF2 in ganglia. Under these conditions, the peptide elicited a stimulatory effect on both excitability and total tyrosine phosphorylation in SNs. In cultured SNs lacking synaptic contacts, inhibitory responses to IGF2 could also be reversed if preceded by a period of nutrient deprivation, suggesting possible modulation by cellular energy status and/or in vivo signaling from synaptic partners. We have identified two insulin-like receptors and five insulin-like ligands expressed in *Aplysia* CNS and confirmed that

a recombinant form of one of these peptides has a glucose-lowering effect. We propose that in response to nutrient-related signals, a subset of endogenous *Aplysia* insulin-like peptides suppresses sensory input by inhibiting RTK signaling in SNs via activation of a PTP1B-like phosphatase, while enabling some long-term stimulatory effects such as elevated rates of neurite growth. This response may partially account for behavioral suppression we observed in *Aplysia* after food intake, possibly representing a "rest-and-digest" response well documented in a wide variety of animals. While the possible combinatorial complexity of this multi-receptor/multi-ligand system in *Aplysia* remains an open question, its responsiveness to a human insulin-like peptide offers an insight into a conserved toolkit of molecular modules integrating neuronal activity, metabolism, and growth.

**Disclosures: N. Kukushkin:** None. **S.P. Williams:** None. **T.J. Carew:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.16/TT28

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant MH 096816

**Title:** Mechanistic Target of Rapamycin complexes in mGluR-LTD and related cognitive behaviors

**Authors: *P. ZHU**, C.-J. CHEN, M. COSTA-MATTIOLI
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**Abstract:** Information storage is thought to have a physical basis in long-lasting modifications of synaptic function in selective brain circuits. At hippocampal synapses, repeated low activity weakens the efficacy of synaptic connections in a processes called long-term depression (LTD). Interestingly, different types of learning processes are associated with LTD. The mechanistic target of rapamycin (mTOR) signaling has been implicated in hippocampal LTD, but most of the evidence supporting its role is based on the use of the mTOR inhibitor and pharmacological agent rapamycin. mTOR forms two complexes: mTOR complex 1 (mTORC1), which is sensitive to rapamycin, regulates protein synthesis and is defined by the adaptor protein *raptor;* and mTORC2 that is largely insensitive to rapamycin, regulates actin dynamics and is defined by the adaptor protein *rictor.* To investigate the role of mTOR complexes in mGluR-LTD, we use genetics to independently silence mTORC1 and mTORC2 functions. Here we report that, mGluR activation triggers the activity of both complexes, mTORC1 and mTORC2. Strikingly, conditional deletion of *rictor* (mTORC2), but not *raptor* (mTORC1), in the postnatal murine forebrain impairs chemical and electrically induced mGluR-LTD. Moreover, rapamycin (1 μM)

blocks mGluR-LTD in both control and mTORC1-deficient hippocampal slices. Consistent with the electrophysiological recordings, hippocampal-dependent object place learning, a behavioral task associated with mGluR-LTD in the hippocampus, was only impaired in mTORC2-deficient, but not mTORC1-deficient, mice. Thus, mTORC2 signaling is required for mGluR-LTD and related behaviors and is a promising novel target for the treatment of mGluR-LTD-linked cognitive dysfunction.

**Disclosures: P. Zhu:** None. **C. Chen:** None. **M. Costa-Mattioli:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.17/TT29

**Topic:** H.01. Animal Cognition and Behavior

**Title:** GSK-3β-Nrf2 signaling pathway as a neuroprotective mechanism in Alzheimer's disease

**Authors: *C. LEE**[1], G. PARK[2], J.-H. JANG[1]
[1]Keimyung Univ., Daegu, Korea, Republic of; [2]Kyungpook Natl. Univ., Daegu, Korea, Republic of

**Abstract:** β-Amyloid peptide (Aβ) is the major component of senile plaques accumulated in the brains of patients with Alzheimer's disease (AD) and has been reported to cause neuronal cell death via oxidative stress. Therefore, attention has been focused on identifying redox-sensitive transcription factors and their target genes protecting against Aβ-induced oxidative cell death. Nrf2 plays a pivotal role in the transcriptional regulation of antioxidant proteins and detoxification enzymes and blocks apoptosis caused by a wide array of death signals. Ectopic expression of Nrf2 rescued cells from Aβ-induced cytotoxicity, apoptosis, intracellular accumulation of reactive oxygen species and oxidative damages. Moreover, Nrf2 overexpression increased the expression of γ-glutamylcysteine ligase (GCL), a rate-limiting enzyme in cellular glutathione biosynthesis and heme oxygenase-1 (HO-1), a key enzyme in heme degradation process. Conversely, knockdown of Nrf2 gene expression with siRNAor dominant negative mutant Nrf2 exacerbated Aβ-induced oxidative cell death. To further elucidate the upstream regulator for Nrf2 activation, we have focused on glycogen synthase kinase-3β (GSK-3β). Inhibition of Aβ-induced GSK-3β activation by pharmacological inhibitors such as LiCl led to nuclear accumulation Nrf2 and transcriptional activation of Nrf2 downstream target genes and protected against Aβ-mediated oxidative cell death. In another experiment, some dietary and medicinal phytochemicals attenuated Aβ-induced oxidativecell death via suppression of GSK-3β and subsequent activation of Nrf2. Taken together, these findings suggest that GSK-3β-Nrf2 signaling pathwaymay act as a survival mediator against AD.

**Disclosures:** **C. Lee:** None. **G. Park:** None. **J. Jang:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

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**Program#/Poster#:** 614.18/TT30

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH grant 5R01MH078064-11

**Title:** Proteomic and epigenetic determinants of state-dependent memory

**Authors: *V. JOVASEVIC**[1], F. SANANBENESI[3], J. WIKTOROWICZ[4], A. FISHER[3], J. RADULOVIC[2]
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**Abstract:** Fear-inducing memories can be state-dependent, meaning that retrieval of a memory is the most efficient when occurring under the same state of consciousness as when the memory was encoded. Restricted access to such memories can present a risk for psychiatric disorders and hamper their treatment. We have previously demonstrated that heightened activity of hippocampal extrasynaptic GABAA receptors enabled state-dependent encoding and retrieval. The susceptibility to state-dependent processing of fear memories was determined by miR-33, a microRNA which regulates several GABA-related proteins. Here we analyzed the global changes at the molecular level to identify individual molecules and cellular pathways contributing to state-dependent processing of fear memories. We performed high throughput analyses on samples from mice treated with gaboxadol: (1) proteomic analysis to identify proteins whose amount or activity is altered by the treatment; (2) epigenetic analysis to identify changes in the epigenome specifically associated with state-dependent memory processing. We also performed proteomic analysis on samples from mice treated with miR-33 inhibitor to identify proteins through which miR-33 confers susceptibility to state-dependent memory processing. Results of the proteomic analyses showed that gaboxadol treatment induced alterations in the amount and activity of a limited number of proteins. These proteins were clustered in a very narrow subset of cellular pathways. Proteins whose levels change as the result of miR-33 inhibitor treatment were also clustered in a narrow subset of pathways overlapping those affected by gaboxadol treatment. These results suggest that changes of cognitive states require targeted alterations of a very small number of cellular pathways.

**Disclosures:** **V. Jovasevic:** None. **F. Sananbenesi:** None. **J. Wiktorowicz:** None. **A. Fisher:** None. **J. Radulovic:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant P40 OD010440 (Caenorhabditis Genetics Center)

**Title:** GCY-28 mediates naïve approach to benzaldehyde and associative learning of benzaldehyde and starvation in different neurons in *C. elegans*

**Authors: *N. LI**, D. VAN DER KOOY
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**Abstract:** Information regarding the innate odor preferences of many animals is encoded in chemosensory neurons. These olfactory responses, however, can be modified by various processes, such as associative learning, whereby an attractive conditioned stimulus is paired with an aversive unconditioned stimulus. To better understand the behavioral changes driven by associative learning, we use the simple model organism *Caenorhabditis elegans*. This nematode worm is naïvely attracted to benzaldehyde, an odorant sensed by the pair of AWC neurons, $AWC^{ON}$ and $AWC^{OFF}$, but learns to avoid it after paired exposure with starvation. Here, we reveal a previously unknown role of GCY-28, a receptor-like guanylate cyclase that has been implicated in mediating attraction to odors sensed by the $AWC^{ON}$ chemosensory neuron as well as sensory integration of conflicting cues in the AIA interneuron. We show that mutants lacking *gcy-28* not only less prefer $AWC^{ON}$-sensed odors but also cannot learn to associate benzaldehyde with starvation, and that GCY-28 modulates these behaviors in distinct neurons: naïve attraction to $AWC^{ON}$-sensed odors in $AWC^{ON}$ versus associative learning of benzaldehyde and starvation in AIA. We further show that the cyclic nucleotide-gated channels CNG-1 and CNG-3 are the downstream effectors of GCY-28 in AIA, and that the observed learning deficit lies in memory retrieval, not acquisition or storage, as memories formed during training are transferrable and can be retrieved within a different context. Using odorants that are differentially sensed by $AWC^{ON}$ or $AWC^{OFF}$, our data indicate that GCY-28 is only required during memory retrieval when both AWC neurons are activated by chemical cues, suggesting that it functions in AIA to mediate the resolution of different signals released from $AWC^{ON}$ and $AWC^{OFF}$ following sensory stimulation. We propose that during signal transduction, GCY-28 functions upstream of the release of INS-1, an insulin-like peptide orthologous to human insulin, from AIA to AWC, and that in the absence of GCY-28 in AIA, INS-1 transmission is diminished, explaining the inability of *gcy-28* mutants to learn. Our results offer new insights about how information is processed in the neural network of *C. elegans* during associative learning. Understanding the components of

associative learning pathways will provide insights into the broader neural mechanisms involved in memory formation and retention.

**Disclosures: N. Li:** None. **D. van der Kooy:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.20/TT32

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Prdx6 knockout mice exhibit increased fear memory to trace fear conditioning

**Authors: *S. PHASUK**[1,3], T. PAIROJANA[1], P. SURESH[1], S. P. HUANG[2], N. PAKAPROT[3], S. CHOMPOOPONG[4], I. Y. C. LIU[1]
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**Abstract:** The peroxyredoxin6 (PRDX6) is a *Prdx* family member and demonstrates both peroxidase and calcium-independent phospholipase A2 (iPLA2) activities. Recent studies have reported that the PRDX6 plays a critical role in various neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. PRDX6 is also highly expressed in the hippocampus, a brain area which is important for memory formation. Prdx6-iPLA2 activity appears to inhibit neurite outgrowth in neural precursor cells. Moreover, postmortem brain sections taken from schizophrenia patients also showed upregulation of PRDX6. Though PRDX6 seems to be involved in various brain functions and neurodegenerative diseases, the physiological role of PRDX6 in the regulation of synaptic plasticity, learning and memory has not been investigated. Thus, in this research, we aimed to investigate the function of PRDX6 in learning and memory. The 14-week-old Prdx6 knockout (KO) mice were used for behavioral phenotyping using various paradigms including open field, three chamber test, elevated plus maze, Morris water maze tests, and fear conditioning. Results showed that the Prdx6 KO mice exhibited normal locomotor activity, sociability, anxiety-like behaviors and spatial learning and memory, but impaired social novelty (control, $p = 0.017$, $n = 7$; Prdx6 KO, $p > 0.05$, $n = 13$) and increased freezing behavior in both contextual and tone test ($p = 0.01$ and 0.019, respectively, $n = 10$) . Enhanced retrieval of fear memory in the Prdx6 KO mice was accompanied by 2-fold upregulation of *Bdnf* mRNA ($p = 0.0178$, $n = 3$) during memory consolidation. These results indicate that PRDX6 may play a critical in suppressing fear memory formation. Further experiments are ongoing to investigate the underlying cellular and molecular mechanism.

**Disclosures: S. Phasuk:** None. **T. Pairojana:** None. **P. Suresh:** None. **S.P. Huang:** None. **N. Pakaprot:** None. **S. Chompoopong:** None. **I.Y.C. Liu:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.21/TT33

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH

NSF

**Title:** Molecular organization of octopus brains reveals first insight into unique memory center signaling

**Authors: *G. C. WINTERS**[1], C. BOSTWICK[1], L. HATFIELD[2], A. B. KOHN[1], L. L. MOROZ[1]
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**Abstract:** Cephalopods (*Octopus,* Squid, Cuttlefish, and *Nautilus*) have evolved a degree of behavioral flexibility that rivals that of many mammals. The Vertical Lobe (VL), a structure unique to cephalopods containing intricate memory circuitry, parallels mammalian analogues like the hippocampus in cell number and function, but has evolved independently within the distant molluscan lineage. We used integrative Next-gen sequencing technology and bioinformatic analyses, followed by anatomical validation using in-situ hybridization, to identify the first molecular maps of signaling molecules implemented in cephalopod memory circuitry. We constructed, sequenced and analyzed *Octopus* neural transcriptomes of various tissues (including VL, CNS, SFL-Superior Frontal lobe, and Arm Cords), and individual cells from subpopulations within the VL and other neuronal tissues, including the Amacrine (Am) Interneurons and Large Efferent (LE) Neurons that make up the VL. We compared these transcriptomes to the available Octopus genome and our gastropod mollusc neural transcriptomes including *Aplysia californica*. We identified 16,194 transcripts in the VL (TPM >/=1) and found 4,139 (25.5%) appear to be cephalopod-specific. Remarkably, indicators for many memory related signal molecules and transmitters like NO (NOS) and GABA (GAD) were not identified in VL transcriptomes, suggesting a distinct cephalopod-specific complement of molecules independently recruited in the organization of learning and memory-forming circuits. We used both targeted and unbiased bioinformatic approaches to identify 104 putative secretory molecules in *Octopus* nervous tissues approximately half of which have not been described before in any species, and most of which were uncharacterized in cephalopods. We have systematically cloned and mapped expression of NPs, and have localized 16 NP to the components of the VL circuit. Three peptides are abundantly expressed in the cell bodies of the MSF, where the afferent tract to the VL originates. An additional four peptides label the small amacrine interneuron population in the VL gyri, three of which appear to be unique to

cephalopods like the VL itself. The remaining 9 VL peptides label distinct sub-populations of the large efferent neurons, providing the first evidence for more than one type of large efferent neuron. This expansion of novel signaling molecules in the VL circuit is likely a key feature of the unique memory systems of cephalopods, further implying extensive parallel evolution of cephalopod brains and memory circuits in particular.

**Disclosures: G.C. Winters:** None. **C. Bostwick:** None. **L. Hatfield:** None. **A.B. Kohn:** None. **L.L. Moroz:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.22/TT34

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Neocortical SHANK1 shRNA knockdown impairs associative learning of whisker-trace-eyeblink conditioning

**Authors: *S. M. COLLINS**, R. GALVEZ
Psychology, Univ. of Illinois at Urbana-Champaign Dept. of Psychology, Urbana, IL

**Abstract:** Learning-induced neocortical synaptic plasticity is a well-established mechanism mediating memory consolidation. In support of this theory, our laboratory has demonstrated that the forebrain-dependent associative learning paradigm whisker-trace-eyeblink conditioning (WTEB) induces an increase in neocortical dendritic spine density that returns to preconditioning levels with over-training. In WTEB, rodents learn to associate a whisker stimulation followed by a short stimulus-free delay with a mild periorbital shock. Our previous dendritic spine analyses demonstrated neocortical synaptic remodeling with WTEB, a now widely accepted mechanism for neocortical dependent learning. Although synaptic remodeling is generally accepted as a mechanism of learning, the underlying molecular processes mediating this plasticity are poorly understood. Interestingly, recent studies have identified SHANK1 as a possible mediator of, or at least an important factor for this process. SHANK1 overexpression has been shown to increase dendritic spine density. In contrast, global genetic knockout of SHANK1 both decreases dendritic spine density and impairs learning of cued fear conditioning. These studies have collectively suggested a role for SHANK1 in dendritic spine plasticity; however, a detailed analysis of SHANK1 in specific memory networks during learning has not been explored. The current study aimed to directly examine the role of SHANK1 in associative learning-induced neocortical networks in the following two experiments. Experiment 1: SHANK1 protein expression in primary somatosensory cortex (S1) was examined during distinct learning phases with WTEB. These analyses demonstrated that SHANK1 expression in S1 increased during specific learning phases that are known to exhibit dendritic spine plasticity, further suggesting a

role for SHANK1 with learning-induced dendritic spine modification. Experiment 2: Using shRNA, the effects of knocking down SHANK1 in S1 was assessed on acquisition of WTEB. Our findings demonstrated that knocking down SHANK1 expression in S1 significantly impaired acquisition of WTEB. These findings are consistent with previous studies implicating SHANK1 as a mechanism for synaptic plasticity necessary for learning and further suggest a role for SHANK1 in learning-induced plasticity of specific memory systems. In addition, these studies provide further evidence for SHANK1 as a potential mediator of learning-induced neocortical plasticity underlying memory consolidation.

**Disclosures: S.M. Collins:** None. **R. Galvez:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.23/TT35

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Multi-colored single-molecule fluorescence in-situ hybridization reveals complexity of neural ensemble representation of experience

**Authors: *B. J. GONZALES**, D. MUKHERJEE, B. IGNATOWSKA-JANKOWSKA, N. BLEISTEIN, A. CITRI
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**Abstract:** Long-term information coding in the nervous system requires temporally defined waves of induced transcription, the earliest of which is comprised of immediate early genes (IEGs). Individual IEGs are widely used to identify neuronal ensembles recruited by distinct salient experiences, with the assumption that different IEGs are co-expressed within ensembles encoding a common experience. However, this assumption has not been tested. We set out to address this notion and to investigate the complexity of neural ensembles recruited by distinct experiences. We have previously observed that different experiences are encoded by unique and robust transcriptional responses, and identified subsets of IEGs sufficient to unambiguously decode the recent salient experience of an individual mouse. To identify the spatial expression within tissue, we performed RNA In-Situ Hybridization (RNA ISH) against robustly induced candidate IEGs following the rewarding experience of acute cocaine. We found that induced expression maps to sub-regions within defined brain nuclei of the reward circuitry. Within the striatum (Str), robust expression was localized the dorsomedial (DM) and ventrolateral (VL) regions. To address whether induced IEGs co-localize in discrete populations with a common functional role, we utilized multi-colored single-molecule fluorescence ISH to compare induction within the two major neuronal populations of the striatum (D1R and D2R expressing neurons), known to have opposing roles in behavior, following acute cocaine. Strikingly,

analysis of overlapping induced transcription of candidate IEGs including *Egr2*, *cFos*, *Arc* and *Nr4a1*, revealed specific enrichment in D1R- but not D2R- expressing neurons of the VL-Str (*Egr2* in 80.7% of D1R-, but only in 35.8% of D2R-expressing neurons; 92.4% of *Fos* expressing neurons were Egr2+/D1R+). Conversely, in the DM-Str similar ratios of D1R and D2R expressing neurons were recruited according to induced IEG expression (*Egr2* in 79.4% of D1R-, and in 65.7% of D2R-expressing neurons; 65.5% of *Fos* expressing neurons were Egr2+/D1R+ and 56.8% were Egr2+/D2R+). These results show that salient experiences induce unique spatially defined transcription signatures, recruiting specific neuronal ensembles. However, these data also imply that neuronal ensembles defined in this manner can be comprised of cells with opposing functionality, revealing unaddressed complexity of neuronal ensembles recruited in salient experiences. Precise manipulation of identified genes and defined engaged neuronal ensembles may determine novel and precise roles in memory formation underlying the development of adaptive behavior.

**Disclosures: B.J. Gonzales:** None. **D. Mukherjee:** None. **B. Ignatowska-Jankowska:** None. **N. Bleistein:** None. **A. Citri:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIMH RO1 MH 041083

NIMH 5T32 MH019524

NIMH T32 MH 963314

**Title:** Members of a novel family of cysteine-rich neurotrophic factor-like proteins show differential gene expression during synaptogenesis in *Aplysia* cultured neurons

**Authors: *A. ALEXANDRESCU**, N. V. KUKUSHKIN, T. J. CAREW
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**Abstract:** Growth factor signaling is a highly conserved molecular mechanism that is critically involved in neuronal developmental plasticity and is re-engaged in the adult during synaptic plasticity underlying long-term memory formation. The marine mollusk *Aplysia californica* is a powerful model system for studying the cellular and molecular mechanisms of long-term synaptic facilitation in identified sensory (SNs) and motor neurons (MNs), which form monosynaptic connections *in vivo* and in an *in vitro* co-culture system. Our laboratory has identified a novel neurotrophic factor, *Aplysia* cysteine-rich neurotrophic factor (ApCRNF),

which shares structural and functional characteristics with mammalian neurotrophins. We previously showed that ApCRNF is released in the CNS in an activity-dependent manner and is required for the induction of long-term facilitation of SN-MN synapses in culture. Here we describe the identification and characterization of a novel family of cysteine-rich neurotrophic factor-like (ApCRNF-like) proteins. Members of the ApCRNF-like protein family and ApCRNF show conservation of critical cysteine residues known to be essential for the formation of the cysteine-knot structural motif characteristic of growth factors. We show here that the ApCRNF-like transcripts are expressed in the *Aplysia* CNS. In addition, using single-cell qPCR, we have performed an analysis of the gene expression profile of the ApCRNF-like gene family in single cultured L7 and L11 MNs as well as in pleural SNs. The *Aplysia* SN-MN co-culture system offers single-cell spatial resolution for studying endogenous growth factor signaling in the context of synapse formation and plasticity. Moreover, *Aplysia* neurons demonstrate synapse-specificity in culture as *in situ*: SNs form functional chemical synapses with target L7 MNs, but not with non-target L11 MNs. Interestingly, we found changes in the gene expression profile of ApCRNF-like transcripts between single and co-cultured SNs and MNs, as well as between co-cultured SN-MNs with or without putative synapses. These results suggest that gene expression of the ApCRNF-like proteins in single neurons can be differentially regulated in the presence of target or non-target synaptic partners, raising important opportunities to examine the dynamic regulation of this gene family during synapse formation.

**Disclosures: A. Alexandrescu:** None. **N.V. Kukushkin:** None. **T.J. Carew:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.25/TT37

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIMH RO1 MH 041083 Grant to TJC

**Title:** Growth factor mediated post-transcriptional regulation of the immediate early gene c/ebp by the RNA-binding protein ELAV is critical for long-term memory formation in *Aplysia*

**Authors: *A. A. MIRISIS**[1,2], T. J. CAREW[1]
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**Abstract:** We have previously shown that signaling through distinct growth factor (GF) pathways is required for long-term memory (LTM) formation in unique temporal and spatial domains in *Aplysia*. Specifically, (i) TrkB signaling is required in the sensory neuron (SN) synaptic neuropil during Trial 1 of a two-trial LTM training paradigm, and (ii) TGFβ signaling is

required at the SN cell body during Trial 2. TrkB signaling is required for gene expression of *c/ebp*, an immediate-early gene required for LTM formation, at 45 min following Trial 1, but prolonged *c/ebp* gene expression at 60 min requires the presence of TGFβ signaling during Trial 2. This prolonged gene expression is also dependent on TrkB signaling during Trial 1, precluding the possibility that Trial 2 independently increases *c/ebp* gene expression (Kopec et al., 2015). Interestingly, *c/ebp* mRNA contains multiple AU-rich elements (AREs) in its 3' UTR, which confers to it unique characteristics including increased susceptibility to degradation and/or stabilization due to its ability to bind to various RNA-binding proteins. *Aplysia* is known to express ApELAV, an RNA-binding protein which promotes stabilization of *c/ebp* mRNA by binding to AREs in its 3' UTR (Yim et al., 2006). Here we show that: (i) Trial 1-dependent *c/ebp* gene expression at 45 min is transcription-dependent, whereas Trial 2-dependent *c/ebp* gene expression at 60 min is transcription-independent, (ii) treatment with TGFβ1 at 45 min is sufficient for increased *c/ebp* gene expression at 60 min (even in the presence of transcription inhibitors), and (iii) when delivered during Trial 2, CMLD-2, a potent inhibitor of HuR - mRNA interaction, significantly reduces Trial 2-dependent *c/ebp* gene expression at 60 min and blocks LTM formation. Our results directly implicate post-transcriptional regulation and, specifically, stabilization of Trial 1-induced *c/ebp* mRNA by ELAV-like proteins during Trial 2, as a prerequisite for LTM formation.

**Disclosures: A.A. Mirisis:** None. **T.J. Carew:** None.

**Poster**

**614. Learning and Memory: Molecules and Mechanisms II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 614.26/TT38

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH

**Title:** Memory allocation is disrupted in conditional Wilm's Tumor 1 $KO^{DG}$ mice

**Authors: *L. MUNARI**[1], C. MARIOTTINI[2], E. GUNZEL[3], R. D. BLITZER[4], R. IYENGAR[3]
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**Abstract:** Memory allocation is the process that determines which neurons in a neural network will store a specific memory. Recent findings reported that shared neuronal populations may link distinct memories close in time, and increasing neuronal excitability affects the probability of a neuron to participate in a memory trace or engram (Cai *et al*., 2016). Contextual memories are encoded in sparse populations of neurons in the hippocampus, and the dentate gyrus (DG) seems

to play a major role in this process (Leutgeb *et al.,* 2007). Recently, we have identified Wilm's Tumor 1 (WT1) as a key protein involved in memory retention. Mice lacking WT1 in the forebrain showed better recall in two hippocampus-dependent tests, but memory impairment when both tests were combined in a sequential learning task, suggesting memory interference. Here, we injected AAV8-Cre virus into the DG of $Wt1^{flox/flox}$ mice in order to generate animals that expressed a non functional WT1 protein specifically in that sub region (WT1 $KO^{DG}$). A separate group of animals was injected with both AAV8-Cre and AAV8-hM4D(Gi) viruses. Mice were challenged in a context discrimination test. They were allowed to explore three different contexts (A, B, C), but only one (context B) was paired with a footshock. Freezing time related to each context was assessed 24 hours after contextual fear conditioning. Mice lacking functional WT1 showed increased freezing time in the context A (never shocked) after being shocked in context B, suggesting impairment in context discrimination. We hypothesized that this effect was mediated by an increase in neuronal excitability due to knock down of WT1 in the DG. Hence, we tried to block this effect using a CNO/hM4D(Gi) receptor strategy, and showed that we could reverse the context discrimination impairment previously observed. In addition, we observed that WT1 $KO^{DG}$ mice show significantly enhanced LTP in DG compared to control littermates. In summary, our findings suggest that blocking WT1 activity in DG cells disrupts memory allocation, leading to formation of false memory. We hypothesize that this effect is mediated by WT1's control over hippocampal neuronal excitability.

**Disclosures: L. Munari:** None. **C. Mariottini:** None. **E. Gunzel:** None. **R.D. Blitzer:** None. **R. Iyengar:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Program#/Poster#:** 615.01/TT39

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant T90DA043219

**Title:** Synchronized neocortical and hippocampal dynamics during NREM sleep

**Authors: *D. LEVENSTEIN**[1], J. M. GORNET[2], B. O. WATSON[3], G. BUZSAKI[4], J. M. RINZEL[5]
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**Abstract:** During non-REM (NREM) sleep, neuronal populations in the forebrain show highly synchronized patterns of spontaneous activity. In the neocortex, this manifests as the NREM

slow oscillation: large 0.5-4Hz deflections in the extracellular field potential concurrent with population-wide periods of inactivity (DOWN states), which alternate with periods of spiking (UP states). These UP/DOWN alternations ("synchronized" dynamics) are reminiscent of those seen in the neocortex during other periods of behavioral quiescence such as quiet wakefulness and under anesthesia. Using an idealized neural population model, we show that synchronized dynamics are characteristic of recurrent populations under conditions of low external drive and are promoted by neural adaptation mechanisms. The balance between adaptation, recurrence, and neuronal excitability can give rise to either noise-induced or adaptation-induced transitions, which allows for a variety of synchronized regimes with distinct statistics of UP/DOWN state durations, or dwell times. Using a nonparametric method to match dwell time distributions, we find that, unlike other in vivo synchronized regimes, NREM sleep in the rodent neocortex is best represented by an "Excitable$_{UP}$" regime: a network state in which perturbation of a stable UP state results in transient DOWN states. This model is able to account for multiple features of NREM sleep thought to be important for mnemonic and homeostatic functions, such as stochastic or perturbation-initiated slow waves and high frequency oscillations at the DOWN->UP transition. Contrary to the neocortex, the hippocampus shows generally low levels of spiking with occasional bursts of synchronized population activity, or sharp wave ripple events (SPW-R). We find that the in vivo distributions of SPW-R/interSPW-R durations are also well-matched by the same model, albeit in an "Excitable$_{DOWN}$" regime: a network state in which periods of low-activity are punctuated by stochastic or externally-induced population bursts. These results provide a general theoretical treatment of slow adaptation-mediated oscillations in the forebrain, and suggest that despite their apparent differences, hippocampal and neocortical dynamics during NREM sleep may result from similar underlying mechanisms that are tuned to promote hippocampal-cortical interaction.

**Disclosures: D. Levenstein:** None. **J.M. Gornet:** None. **B.O. Watson:** None. **G. Buzsaki:** None. **J.M. Rinzel:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.02/TT40

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Sir Henry Wellcome Postdoctoral Fellowship

NIH R01 MH054671

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NIH U01 NS094349-01

**Title:** Medial and lateral entorhinal cortex gamma inputs entrain different hippocampal populations during learning and navigation

**Authors: *A. FERNÁNDEZ RUIZ**, A. OLIVA, G. BUZSAKI
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**Abstract:** The two main extrinsic input regions to the hippocampus are the medial and lateral areas of the entorhinal cortex. Both regions process different types of information. While medial entorhinal cortex (MEC) preferentially encode spatial information and distal visual cues, lateral entorhinal cortex (LEC) encodes local item features. Both inputs converge in the dentate gyrus and CA3 areas where they likely support the pattern completion and separation functions characteristic of these hippocampal regions. There is extensive anatomical evidence of entorhinal axons innervating all excitatory (pyramidal neurons, granular and mossy cells) and inhibitory cell types in these areas. However, functional data on how entorhinal inputs modulate the firing dynamics of these cell populations during behavior is lacking. To address these questions, we employed large-scale silicon probes (up to 512 channels) to record local field potentials (LFPs) and single-unit activity simultaneously in all hippocampal subfields and layers of MEC and LEC in rats during various learning and navigational tasks. As a first step, we employed source-separation techniques and pharmacological manipulations to isolate the contribution of MEC and LEC inputs to hippocampal LFPs. We found that both inputs elicit distinct gamma frequency oscillations at different phases of the theta cycle. Coherent oscillations between the dentate target layers and layer 2 of MEC and LEC, respectively, were identified. Different dentate and CA3 neuronal populations were entrained preferentially by MEC and LEC gamma inputs. This input selectivity correlated with the different degrees of spatial coding and remapping properties found in the different cell classes. During running and REM sleep, theta rhythm coordinated gamma oscillations and synchrony across the entire hippocampus-MEC-LEC circuit. The driving strength of these inputs and interregional network coordination changed according to behavioral and memory demands. Our results show that MEC and LEC coordination with DG and CA3 occur at different gamma frequencies and theta phases and contributes differently to behavior. Entorhinal inputs target preferentially different cellular classes in the hippocampus, and this preference may account for the functional specialization of cell types during behavior.

**Disclosures: A. Fernández Ruiz:** None. **A. Oliva:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** U01NS099705-02

**Title:** Inter-cortical communication over the course of development

**Authors: *J. GELINAS**[1], D. KHODAGHOLY[3], G. POUCHELON[5], C. MAYER[2], G. J. FISHELL[4], G. BUZSAKI[6]
[2]Physiol. & Neurosci., [1]New York Univ., New York, NY; [4]Smilow Neurosci. Program, [3]New York Univ. Langone Med. Ctr., New York, NY; [5]NYU Neurosci., New York, NY; [6]New York University, Sch. of Med., New York, NY

**Abstract:** Neural networks undergo profound changes over the course of development that are critical for establishing cortical functions. These changes are reflected in the spontaneous oscillations generated by developing cortex. Due to the small size and fragility of neonatal brains, determining the characteristic electrophysiological patterns of specific developmental stages in various cortical regions is difficult. Here, we developed and tested a conformable, conducting-polymer based surface electrode array (NeuroGrid) to record from multiple cortical regions simultaneously in awake and sleeping mouse pups (P2 - P14). We detected network oscillations including spindle and gamma, key component of spontaneous neural activity in developing rodents, and determined that features of these oscillations are altered with maturation. Due to the large-scale cortical coverage possible with surface recording, we were able to describe the spatial extent of these oscillations across development. In addition, we observed that precise temporal coordination of spindle oscillations within and between distinct cortical regions was not present at early stages of development, arising only late in the second week of life. Understanding how spontaneous activity emerges and is altered during development will provide insight into the physiological functioning of these early neural networks, and how disturbances of brain oscillations prior to maturation can predispose to neuropsychiatric disease.

**Disclosures: J. Gelinas:** None. **D. Khodagholy:** None. **G. Pouchelon:** None. **C. Mayer:** None. **G.J. Fishell:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Title:** Neuronal circuit mechanisms of pattern separation in the hippocampal dentate gyrus and CA3

**Authors: *Y. SENZAI**[1], G. BUZSAKI[2]
[1]New York Univ., New York, NY; [2]New York University, Sch. of Med., New York, NY

**Abstract:** Theoretical work, clinical imaging, and lesion experiments suggest that an important computational function of the hippocampal dentate gyrus (DG) is pattern separation. Physiological support for such function has been hampered by a lack of well-defined characteristics that can identify granule cells and mossy cells. We developed a neurophysiology-based classification method to conclusively identify dentate granule cells and mossy cells in neural data acquired from freely moving mice. These two excitatory cell types can first be distinguished from dentate gyrus interneurons based on their wide action potential waveform and increased tendency to burst firing. Three main characteristics then separated the excitatory neurons into granule cells and mossy cells: (1) cell body location relative to the reversal of the DS2; (2) relative firing rates in NREM and waking states; and (3) waveform shape. Granule cells had cell bodies located near the depth of DS2 reversal, had increased firing in NREM compared to waking, and had a sharper waveform. In contrast, mossy cells were located well below the depth of the DS2 reversal, had equivalent firing rates in NREM and waking, and had a more symmetric waveform. We validated these physiological characteristics with optogenetic methods, establishing firm criteria for granule cell and mossy cell identification in vivo. Our classification method allowed us to investigate how these cell types interact and encode spatial information. Granule cells were typically low firing and had a single place field, whereas mossy cells were higher firing and had two or more place fields. Although the sparse coding of spatial information by granule cells could be the basis for the pattern separation, granule cells showed weaker remapping across different contexts in the same room compared to their downstream targets, mossy cells and CA3 pyramidal cells. Furthermore, despite the strongly facilitating nature of granule cell-mossy cell synapses, place fields of most mossy cells could not be explained by the ‘‘inheritance’’ of place fields from single granule cells. These findings suggest that the joint action of granule cells, mossy cells, and CA3 pyramidal cells may be critical for pattern separation (Senzai and Buzsáki, 2017). In order to reveal the contribution of the granule cells to the pattern separation in DG and CA3, we will optogenetically inhibit granule cells in DG and analyze its effect on the place information coding and pattern separation by mossy cells and CA3 pyramidal cells. For this purpose, we will employ POMC-Cre::Ai35 mice that express Arch specifically in matured granule cells in DG.

**Disclosures:** **Y. Senzai:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Title:** Gamma oscillations, real spurious and their relationship to spikes

**Authors: M. DING**[1], *B. O. WATSON[2], G. BUZSAKI[3]
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**Abstract:** Gamma oscillations have been observed in many brain regions, and hypothesized to be involved in sensory and cognitive functions. Furthermore, previous work has demonstrated a correlation between broadband gamma spectral power and spike rates at the seconds timescale [1]. It has been proposed that gamma rhythms emerge from excitation-inhibition interactions and enable communication in local cell assemblies [2], but direct demonstration of correlation with inhibitory-excitatory balance has been lacking. Since gamma oscillations are measured as local field potentials (LFP), there is no immediate link between LFP and spiking and here we examine both how the spikes themselves contribute to LFP and secondly how they are coordinated by LFP fluctuations. We used electrophysiological recordings in rodent cortex with high-density silicon probes to answer these questions. First, we found that spikes could directly and prominently contribute to high frequency power in electrodes very localized to the spike itself - this was more true for larger waveforms; therefore, we isolated our analysis of spike-oscillation interactions to distant sites. In our initial analysis, we found that population firing rate changes over time in both pyramidal excitatory and inhibitory cells are positively correlated simultaneous with gamma power changes, especially at higher frequency over about 70 Hz. We then quantified the ongoing E/I ratio over time and found it did not correlate with broadband gamma

oscillation power, but was specifically decreased when power was high in the 70-200 Hz range. It was also found that some neurons were phase-modulated at certain gamma frequency bands detected across several neighboring shanks. Finally, we found instances when gamma power is negatively correlated with spiking. These results imply that gamma oscillations might coordinate activity of individual neurons in cortical circuits in a manner more complex than previously described. 1. Nir, Y. et al. (2008). Interhemispheric correlations of slow spontaneous neuronal fluctuations revealed in human sensory cortex. *Nature neuroscience*, *11*(9), 1100-1108. 2. Buzsáki, G., & Wang, X. J. (2012). Mechanisms of gamma oscillations. *Annual review of neuroscience*, *35*, 203-225.

**Disclosures: M. Ding:** None. **B.O. Watson:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIHMH54671

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NSF PIRE

MH107159

**Title:** Pyramidal cell-interneuron circuit architecture and dynamics in hippocampal networks

**Authors: *D. F. ENGLISH**[1], S. MCKENZIE[1], T. EVANS[1], K. KIM[2], E. YOON[2], G. BUZSAKI[1]
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**Abstract:** Action potential transmission from presynaptic to postsynaptic neurons is the common currency of neural computation. Spike transmission efficacy is thought to critically underlie neural computation and changes in efficacy are critical for information storage. In particular, connectivity from pyramidal cells (PYR) to local interneurons (INT) (feedback inhibition, including lateral inhibition), gates afferent drive and dictates competitive PYR-PYR interactions. Therefore, a mechanistic understanding of circuit operation requires elucidating not only a connectomic map, but also the parameters that affect the dynamic properties of individual

synapses.
Synaptic connectivity and strength are usually assayed through paired intracellular recording of the pre- and postsynaptic cells, which is not practical in behaving animals.. An alternative, indirect, approach is *in vivo* multi-electrode extracellular recordings, which provide spike timing of hundreds of neurons recorded in parallel.. With such data, monosynapses can be deduced from the reliability and precision of spiking in one neuron in the milliseconds after a spike in another. However, detection of such connections requires their isolation from indirect polysynaptic drive and common 'third party' inputs, which have the potential to synchronize neurons that lack direct synaptic coupling. A reliable way to rule out third party coordination is to demonstrate that postsynaptic spikes are causally related to the spiking of a single presynaptic neuron.
To test for monosynaptic connections between neuron pairs, we decoupled pre-synaptic PYR from the ongoing network activity through single cell juxtacellular current injection or optogenetic stimulation. We generated a "ground-truth" dataset to confirm monosynaptic PYR drive of local INT, and validated a model for monosynapse detection. We then identified monosynaptic connections in nearly 30,000 PYR-INT pairs, and examined the functional architecture of the PYR-INT circuit in CA1.
We found that neighboring CA1 neurons were more likely to be connected and to have stronger connections, though superficial PYR projected more to deep INT. Connection probability was skewed, with a minority of highly connected hubs. In general, high frequency pre-synaptic firing led to short-term depression. Synaptic drive was moderately affected by the firing rate of the post-synaptic INT and strongly modulated by its prior spike timing. Finally, we established a short-latency integration window for convergent PYR inputs. These results support a non-homogenous circuit organization and provide a framework for constructing transiently active cell assemblies.

**Disclosures: D.F. English:** None. **S. McKenzie:** None. **T. Evans:** None. **K. Kim:** None. **E. Yoon:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

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Fyssen Foundation

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MH107396

NS 090583

**Title:** Coordinated hippocampus-amygdala reactivations of a place-threat association during sleep

**Authors: *G. GIRARDEAU**[1], I. INEMA[2], G. BUZSAKI[3]
[1]New York Univ. Langone Med. Ctr., New York, NY; [2]McGill Univ., Montreal, QC, Canada; [3]New York University, Sch. of Med., New York, NY

**Abstract:** The association of a context with a specific threat requires both the hippocampus and the basolateral amygdala (BLA). However, how the two structures interact to sustain the consolidation of such an association is still unknown. We designed a novel task where rats have to learn daily the location and direction of an aversive airpuff on a linear track. We recorded large neuronal ensembles simultaneously in the hippocampus and BLA during training and extensive sleep periods preceding and following training. Our results show that the pairwise BLA-hippocampus activity recorded during training is reactivated during NREM sleep following training. These reactivations preferentially involve a subset of BLA cells that are up-modulated during hippocampal ripples. Moreover, the reactivations peak during hippocampal ripples and the joint place-threat representation (airpuff trajectory) is preferentially reactivated compared to the neutral representation (safe trajectory). These results suggest that place-threat associations are consolidated through coordinated BLA-hippocampus activity during NREM hippocampal ripples.

**Disclosures: G. Girardeau:** None. **I. Inema:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** Sir Henry Wellcome Fellowship

U01 NS094349-01

**Title:** Slow gamma frequency dynamics during sharp-wave ripples

**Authors: *A. OLIVA GONZÁLEZ**, A. FERNANDEZ-RUIZ, D. ENGLISH, G. BUZSAKI
Neurosci. Dept., New York Univ., New York, NY

**Abstract:** Sharp-wave ripples (SPW-Rs) are population synchronous events which occur in the hippocampus during consummatory behaviors and non-REM sleep. SWP-Rs have been demonstrated to support memory consolidation of previous experiences (Buzsaki 1989, Girardeau et al., 2009). Although the CA2 region has been shown to play an initiating role during SPW-Rs (Oliva et al., 2016), they are maximally reflected in the local field potentials (LFPs) of the CA1 region. SPW-Rs are composed of a high frequency oscillation (150 - 200 Hz), ripple, in the pyramidal layer and a slow frequency wave (5 - 10 Hz), the 'sharp wave', in the mid-apical dendritic layer. In order to investigate the cellular mechanisms of SPW-Rs, we recorded with high-density large-scale multishank silicon probes from the CA1, CA2 and CA3 hippocampal sub-regions of Long-Evans rats during different memory tasks and sleep. We found that, in addition to the ripple and sharp-wave components, some SPW-Rs events were accompanied by an additional gamma frequency oscillation between (25-30 Hz), as reported earlier (Carr et al., 2012). This gamma oscillation was present in the dendritic LFP signals as well as in the intracellular membrane potential of CA1 pyramidal cells. We classified SPW-Rs into different categories according to sharp wave duration and found that events in these categories also differed in spectral properties and spiking content organization. Longer duration sharp waves often appeared as two overlapping sharp wave events, giving rise to longer duration ripples as well as a 'double-wave'. The presence of the double wave was reflected in the spectrum as slow gamma power and correlated with the synchronous firing of different populations of interneurons in the CA3 region. Interneurons had been shown to synchronize and shape the participation of the principal cells within ripples (Csicsvari et al., 1999, Stark et al., 2015). Therefore, we hypothesized that different subpopulations of pyramidal neurons, putatively processing different pieces of information, coordinate during SPW-Rs to form an integrated representation. These organized representations could sub-serve the replay during sleep of recently acquired memories and could possibly constitute the cellular correlates of trajectories in a spatial memory task (Pfeiffer et al. 2015). We are currently investigating how the slow gamma power during SPW-Rs is affected by a spatial learning task.

**Disclosures:** **A. Oliva González:** None. **A. Fernandez-Ruiz:** None. **D. English:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.09/TT47

**Topic:** H.01. Animal Cognition and Behavior

**Support:** R01MH054671

R01 MH107396-01

U01 NS094349-01

**Title:** Place-action transformation in the hippocampus-lateral septum axis during a run-jump-run sequence

**Authors: *D. TINGLEY**[1], G. BUZSAKI[2]
[1]Neurosci., NYU Neurosci. Inst., New York, NY; [2]New York University, Sch. of Med., New York, NY

**Abstract:** Since the discovery of the hippocampal spatial code, many experiments have extended our knowledge of when and how this code remaps, warps, or maintains representations of the external world. Prior to the demonstration of this spatial code, it was clear that hippocampal activity could also be tuned to other variables, such as a specific object or motor sequence (Ranck 1973).
In the current set of experiments, we attempt to examine how hippocampal activity may integrate these disparate coding regimes into a cohesive signal that is transmitted out of the hippocampus. We designed a behavioral task that includes both the traversal of allocentric space and the completion of a specific motor sequence, while allowing for the dissociation of these two variables. Animals were trained to navigate across an elevated linear track, where a gap could be introduced at any location along the track. Correct traversal required the animal to run, jump across the gap, and continue running fluidly, with the jump location and distance of the gap varying on different blocks of trials.
In agreement with previous studies (Lenck-Santini et al., 2008), neurons were found in the CA1 and CA3 regions of the dorsal hippocampus with firing rates that were reliably tuned to the jumping sequence, independent from allocentric position. These neurons also demonstrated single-trial theta phase precession across different phases of the jump sequence, including the duration where animals were airborne.
To examine how these representations of space and action may be integrated, simultaneous recordings from a major output of the CA1/CA3 regions, the lateral septum(LS), were obtained. Using population firing rate vector analyses, we find that LS neurons carry a less precise spatial code and show stronger ‘remapping’ relative to the jump location. These results suggest the LS firing rates carry a stronger representation (relative to HPC) of specific salient actions, while a degraded allocentric spatial code.
In addition to these population firing rate codes, we will present findings that septo-hippocampal cell pairs participate in cross-regional gamma time scale cell assemblies. Furthermore, these functional assemblies appear to be dependent on whether or not the lateral septal cell demonstrates theta phase precession relative to hippocampal theta and allocentric position.

**Disclosures: D. Tingley:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.10/TT48

**Topic:** H.01. Animal Cognition and Behavior

**Support:** The Danish Council for Independent Research

**Title:** Theta-gamma rhythm perturbation by focal cooling of the medial septum in freely moving rats

**Authors: *P. C. PETERSEN**[1], G. BUZSAKI[2]
[1]NYU Neurosci., [2]NYU Neurosci. Inst., New York Univ., New York, NY

**Abstract:** The theta rhythm coordinates neuronal activity in the hippocampus, entorhinal cortex and other areas involved in spatial memory. Theta oscillations are present during behavior and sleep, and the power and frequency of theta increases with running speed (McFarland, 1974; Ravassard, 2013). Lesions and pharmacological inactivation of the medial septal area has shown that the area is vital for theta rhythms in the hippocampus (Lawson and Bland, 1993; Vertes and Kocsis, 1997) and its damage impairs learning and memory (Winson, 1978; Chrobak et al., 1989; Givens and Olton, 1994; McNaughton et al., 2006).
Yet, it is not known what mechanisms generate the rhythm and what role the frequency and amplitude plays. Towards answering these questions, we have performed local thermal perturbation of the medial septum, to alter the frequency of theta. Temperature perturbation is a powerful tool to study brain rhythms as they affect time-scales of natural occurring processes equally without forcing any external dynamics.
We implanted silicon probes in CA1 in hippocampus and a cooling probe in the medial septum in rats. The cooling effect was either attained using dry ice or a Peltier device, mounted in a cooling device on the head of the animal. The cooling-device was connected to a silver rod implanted in the medial septum, that was thermally isolated along the length to attain a focal effect of the cooling (Aronov and Fee, 2011). The animal performed alternating trials on a figure eight maze, with ongoing cooling sessions, for behavioral quantification and to allow us to study the effects on place cell neuronal activity in the hippocampus. We successfully lowered the theta frequency by 2Hz during behavior. We observed degraded performance during the cooling as the animal made more errors on the maze. We also observed increased running during the cooling, and typically also a temporary decrease in movement as the temperature reversed to normal range. Further, we found a sharp drop in mid-frequency gamma power in the hippocampus as an effect of the cooling, an indicator of reduced entorhinal input (Fernández-Ruiz et al., 2017). Finally, we recorded place cells in the hippocampus to learn how the altered theta rhythm affects phase precession.

In summary, our results, to date, suggest that the theta rhythm can successfully be altered by cooling the medial septum and it leads to memory impairment.

**Disclosures: P.C. Petersen:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** CRCNS NSF Grant 1608077 (HGR)

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CRCNS BSF Grant 2015577 (ES)

**Title:** Inhibition-based theta spiking resonance in a hippocampal network

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**Abstract:** Neuronal oscillations emerge in neuronal systems as the result of the interplay of positive and negative feedback effects with different time scales. These mechanisms involve the synaptic interactions between neurons, especially connections between excitatory and inhibitory neurons, and the neuronal intrinsic ionic currents. It is currently unknown how intrinsic cell properties interact with network dynamics to create neuronal oscillations. In this project we address this issue theoretically in the context of a hippocampal microcircuit composed of excitatory (PYR) and inhibitory (INT) cells. *In vitro,* PYR exhibit a preferred subthreshold frequency response to oscillatory inputs (membrane potential resonance) at theta (4 - 10 Hz) frequencies. Contrary to expectation, *in vivo,* these cells do not exhibit spiking resonance in response to direct oscillatory optogenetic activation, though spiking resonance in PYR can be seen when INT are activated. We explain the underlying mechanisms for these seemingly contradictory observations by combining biophysical modeling, numerical simulations and dynamical systems analysis. In our model, direct PYR activation causes PYR subthreshold resonance that fails to be communicated to the spiking regime, in alignment with *in vivo* observations. This failure is primarily due to the strong oscillatory response that remains above threshold over a broad range of input frequencies. On the other hand, direct INT activation causes PYR theta-band spiking resonance as a result of a combination of rebound spiking and a timing mechanism. A hyperpolarization-activated current (h-) is critical for the generation of

rebound spikes at input frequencies that are low enough for supra-threshold voltage responses in PYR. Alone, rebound spiking creates a low-pass spiking filter that is insufficient to generate spiking resonance. The timing mechanisms are responsible generating the theta band-pass filter by either "erasing" the spikes generated by input frequencies lower than theta (deleting mechanisms) or disrupting the production of spikes for these input frequencies (preventing mechanisms). We identified three possible timing mechanisms: (i) network-mediated inhibition from OLM interneurons, (ii) synaptic depression of INT synapses onto PYR, and (iii) presence of subthreshold gamma resonance in INT. We explain how each of them contributes to the generation of theta spiking resonance. The principles identified in this study are applicable to other systems that exhibit theta resonance such as neocortical networks.

**Disclosures: H.G. Rotstein:** None. **T. Ito:** None. **E. Stark:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.12/TT50

**Topic:** H.01. Animal Cognition and Behavior

**Support:** U01NS099705-02

**Title:** The role of inhibition in hippocampal ictal and interictal activity

**Authors: *S. ROGERS**, J. GELINAS, D. KHODAGHOLY, J. DIMIDSCHSTEIN, G. FISHELL, G. BÜZSÁKI
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**Abstract:** Epileptic seizures reflect a perturbation of the balance between excitation and inhibition, yet the exact role of inhibitory interneurons in the initiation and propagation of epileptic seizures is widely debated. Evidence has emerged suggesting that interneurons play both an anti-epileptic and pro-epileptic role in the maintenance of epileptic brain networks. Enhanced inhibition can suppress spiking of principal cells, but also assists in synchronization of neuronal populations. Specifically, we examined the role of inhibitory interneurons in the dentate gyrus (DG) and CA3 on normal and pathological brain oscillations and spiking activity in a kindling model for temporal lobe seizures in behaving rats. Using a viral construct that restricts the expression of a desired gene to interneurons, we induced expression of an excitatory designer receptor exclusively activated by designer drug (DREADD) into all subtypes of DG and CA3 interneurons of the hippocampus and implanted silicon probes to record LFP and single unit responses. We first tested the effect of tonically increasing inhibitory tone by administering the ligand for the DREADD, clozapine N-oxide (CNO), intraperitoneally in non-epileptic rats to examine the contribution of inhibition from these areas on gamma (40-100 Hz), theta (5-8 Hz),

and ripple (150-200 Hz) oscillations in behaving rats. We next investigated the role of sustained inhibition on afterdischarge and behavioral seizure duration, severity, and development of kindled seizures in animals that were treated with CNO to activate the interneurons every other day. In addition, we compared the rate of interictal epileptiform discharges (IEDs), pathological population spikes that are hypothesized to play a role in the cognitive deficits associated with chronic epilepsy, between days when the animal was given an inert vehicle injection and CNO. Activation of interneurons had a profound effect on the LFP patterns in the intact hippocampus and also reduced the number of IEDs. Unilateral activation of DG and CA3 interneurons produced similar effects but largely confined to the inhibition-activated hemisphere. Surprisingly, tonic enhancement of inhibition exerted a relatively small effect on afterdischarge duration, despite the increased time to develop a fully kindled state. These findings suggest that afterdischarges depend on mechanisms other than disinhibition. The mechanisms underlying physiological and epileptic changes are under investigation.

**Disclosures: S. Rogers:** None. **J. Gelinas:** None. **D. Khodagholy:** None. **J. Dimidschstein:** None. **G. Fishell:** None. **G. Büzsáki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.13/TT51

**Topic:** H.01. Animal Cognition and Behavior

**Support:** U01NS099705-02

**Title:** Learning-enhanced coupling between ripple oscillations in associational cortices and hippocampus

**Authors: *D. KHODAGHOLY**[1], J. GELINAS[2], G. BUZSAKI[3]
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**Abstract:** Consolidation of declarative memories requires hippocampal-neocortical communication. While substantial experimental evidence supports the role of sharp wave-ripples in transferring hippocampal information to the neocortex, the exact cortical destinations and the physiological mechanisms of such transfer are not known. We used an organic-based conformable microelectrode array to record local field potentials and neural spiking across the extent of the dorsal cortical surface of the rat brain, combined with silicon probe recordings in the hippocampus, to identify candidate physiological patterns. Parietal, midline and prefrontal but not primary cortical areas displayed localized ripple (100-150 Hz) oscillations during sleep, concurrent with hippocampal ripples. Coupling between hippocampal and neocortical ripples

was strengthened during sleep following learning. The findings suggest that ripple-ripple coupling supports hippocampal-associational cortical transfer of memory traces.

**Disclosures: D. Khodagholy:** None. **J. Gelinas:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** MSCA-IF-GF 707359

NIH

**Title:** Medial septum and the formation of hippocampal place fields

**Authors: *V. VARGA**, G. BUZSAKI
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**Abstract:** Encoding of locations by hippocampal place cells is a prerequisite of mapping the environment. In the navigating animal information flow in the spatial navigation circuitry is coordinated by theta rhythm paced by the three components , cholinergic, GABAergic, glutamatergic medial septal theta generating network. During theta oscillations, the medial septal input rhythmically disinhibits as well as directly excites pyramidal cells. Depolarization or the induction of dendritic plateau potential may transform hippocampal silent cells to place cells. We asked if the medial septum, by providing all constituents of place cell induction, may be capable and / or necessary for transforming pyramidal cells to place coding units. We recorded the effect of the location-contingent optical stimulation of the medial septal network on the spatial firing of hippocampal neurons. A channelrhodopsin2-carrying, neuron-specific construct was injected into the medial septum and about a month later a multishank, 64-channel silicone probe was implanted into the dorsal CA1 region. Water-restricted animals were tracked at 120 Hz while running for water reward either on a cheeseboard maze or on a modified T-maze. The medial septum was stimulated in a closed-loop fashion by a 450 nm laser diode at 1 Hz or 8 Hz using sine wave stimulation while crossing a predefined area monitored by distance sensors. Stimulation significantly modulated the firing pattern of hippocampal units by inducing either elevation or suppression of activity. Importantly, medial septal activation also affected spatial firing by evoking spikes at the stimulated location but this effect was not carried over to non-stimulated periods. In subsequent experiments we aim to locally manipulate septo-hippocampal fibers and test the effect of stimulation in both familiar and novel environments.

**Disclosures: V. Varga:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIHMH54671

NS 090583

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NIMH F32 MH107159

**Title:** Optogenetic probing for pattern completion across hippocampal subfields

**Authors: *T. EVANS**[1], S. A. MCKENZIE[2], D. F. ENGLISH[3], K. KIM[4], E. YOON[4], G. BUZSAKI[2]
[1]Inst. of Cognitive Neurosci., UCL, London, United Kingdom; [3]Neurosci., [2]New York University, Sch. of Med., New York, NY; [4]Univ. of Michigan, Ann Arbor, MI

**Abstract:** Episodic memory recollection is thought to be supported by unique computations performed within and across the hippocampal subfields. Theoretically, the CA3 to CA3 recurrent connections contribute to rapid learning through the formation of novel associations between excitatory neurons co-active during learning. Under this theoretical framework, recall is supported by a “pattern completion” process in which partial drive to a specific CA3 network can re-instate the full pattern of co-active neurons that became associated during learning. The lack of recurrent connections in CA1 have led theorists to conclude that this region must contribute to memory in some other way. We tested these fundamental theories of hippocampal processing by optogenetically driving CA1, CA3 and dentate gyrus circuits with patterned light stimulation *in vivo*. First, probe trials were given in which a subset of neurons were driven with focal light stimulation. After these baseline trials, we repeatedly co-activated neurons up to 1000 times with synchronous light stimulation. Different pairing protocols were tested, such as: theta burst stimulation, high intensity/low frequency square pulses, and closed-loop Gaussian stimulation triggered when mice occupied a fixed point in space. Following these pairing trials, we cued pattern completion by giving the partial pattern of light stimulation that had been delivered prior to pairing. In CA1, cued pattern completion was never observed. Moreover, there was no increase in the spontaneous correlation between pairs of neurons that were forced to be co-active during light stimulation. Preliminary analysis of CA3 and the dentate gyrus suggests a similar failure to observe any evidence for cued pattern completion. These negative results

suggest that repetitive co-activity of a small number of neurons does not produce sufficient plasticity to redefine which patterns of activity are stable and which are not.

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**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.16/TT54

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Kavli Foundation 423380

**Title:** Ground truth dataset for validating extracellular spike sorting algorithms

**Authors: *M. VOROSLAKOS**[1], D. F. ENGLISH[3], S. A. MCKENZIE[4], E. YOON[2], G. BUZSAKI[5]
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MI; [3]Neurosci., NYU Neurosci. Inst., New York, NY; [4]NYUMC, New York, NY; [5]New York University, Sch. of Med., New York, NY

**Abstract:** Over the last 50 years, the number of *in vivo* simultaneously recorded neurons has been increasing progressively. Advances in microfabrication technology permitted developments of high-channel count and high-density silicon probes (NeuroNexus - 256 sites, IMEC Neuropixel - 966 sites) and allowed electrophysiologists to record the activity of dozens of neurons at the same time *in vivo*. However, it is still challenging to correctly assign spiking signals to their single neuron source. This problem is especially challenging due to overlapping spikes from multiple sources, changes in waveform shape during bursting and electrode drift. Multiple “spike sorting” algorithms have been developed to deal with such large-scale extracellular recordings (KlustaKwik, KiloSort, SpyKING CIRCUS and MountainSort), though there is no community consensus as to which works best, a major impediment being the lack of sufficient ground truth data. Such data necessarily consists of single neurons spikes obtained with a technique which unambiguously assigns spikes to a single cell (such as intra- or juxtacellular recording) and simultaneous extracellular recording of a neural population which includes this same cell.
To produce such a dataset, we performed combined juxtacellular and silicon probe recordings. A 32-channel silicon probe (A1x32-Poly2) was attached to a juxtacellular glass pipette with light cure dental acrylic, and acutely implanted in awake head fixed mice. With this preparation, baseline recordings (at least 20 minutes) were obtained, after which 100 ms pulses of ~1-10 nA were applied to the juxtacellular pipette at 1 Hz until about 1000 spikes were elicited. We began collecting neurons with dual juxta-, extracellular recording methods. These neurons have clusterable size amplitude. Our aim is to collect sufficiently large numbers of simultaneously recorded neurons and test multiple popular spike sorting algorithms to provide the community with a physiological basis for making decisions about which spike sorting algorithms to choose.

**Disclosures: M. Voroslakos:** None. **D.F. English:** None. **S.A. McKenzie:** None. **E. Yoon:** None. **G. Buzsaki:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.17/TT55

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Investigating hippocampal-neocortical dialogue using electrophysiology and wide field calcium imaging *In vivo*

**Authors: *R. A. SWANSON**[1], D. F. ENGLISH[3], J. D. LONG II[2], G. BUZSAKI[4], J. BASU[5]
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New York, NY; [4]New York University, Sch. of Med., New York, NY; [5]Dept. of Neurosci. and Physiol., Neurosci. Institute, New York Univ. Sch., New York, NY

**Abstract:** Systems consolidation (SC) is a proposed process whereby recently learned information is gradually transferred from labile hippocampal (HPC) circuits to distributed networks in the neocortex (CTX). The mechanisms underlying SC are thought to rely on repeated spontaneous replay of task-related patterns of neural activity in HPC and CTX during "offline" periods of quiet wakefulness and sleep. This is facilitated by temporal coordination of neural populations in both structures during HPC sharp-wave ripple oscillations (SPW-Rs). Two findings suggest that SPW-Rs are temporally aligned with the transient reorganization of whole-brain dynamics, in service of SC: (i) On the *micro* (local population) scale, spontaneous replay in HPC is coordinated with similar reactivations in task-relevant lower- and higher-order cortices. (ii) On the *macro* (whole-brain) scale, SPW-Rs have been found to coincide with a decrease in BOLD across subcortical regions and increase in BOLD across nearly all CTX regions. Due to the spatial limitations of extracellular physiology and the temporal (and spatial) limitations of the BOLD signal, however, the temporal and regional dynamics of this whole-CTX response to SPW-Rs are unknown. In this study, we outline the development and application of widefield imaging of a dorsal cortical hemisphere, combined with high-density silicon probe recordings in HPC and CTX. This technique allows us to investigate hippocampo-neocortical interaction in the mouse across learning and during subsequent rest, and makes it possible to observe region-wide variation in CTX response to SPW-Rs events with high temporal precision. We find that cortical widefield activity during active periods is distinct from that seen during behavioral quiescence. Active states are characterized by small, rapid fluctuations in fluorescence, corresponding with theta activity in the HPC. Contrastingly, during quiescent periods we observe synchronous activation of regional cortical motifs, reminiscent of the resting state networks observed in fMRI. This activity is accompanied by large irregular activity in the HPC LFP, with interspersed SPW-Rs. Preliminary results suggest that SPW-Rs are associated with a stereotyped spatiotemporal pattern of regional cortical activation. Together, these findings represent progress towards understanding whole-brain coordination during the consolidation of multi-sensory memories.

**Disclosures: R.A. Swanson:** None. **D.F. English:** None. **J.D. Long II:** None. **G. Buzsaki:** None. **J. Basu:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** Wellcome Trust-DBT Alliance IA/S/16/2/502727

Ministry of Human Resource Development, India

**Title:** An emergent model of hippocampal sharp wave ripple complexes reveals sublayer-specific stratified disparities

**Authors: *M. SINHA**, R. NARAYANAN
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**Abstract:** The hippocampal CA1 region comprises a heterogeneous population of deep and superficial pyramidal neurons whose somata manifest hyperpolarizing and depolarizing transients, respectively, during a sharp wave ripple (SPW-R) event. Here, we present a forward modeling approach that elucidates the emergence of the SPW-R complex and its relationship to the differential properties and responses of deep and superficial neurons. To generate deep and superficial neuronal populations, we accounted for deep-superficial differences in basal dendrites and *h* channels and employed a multi-parametric (12 active and passive properties) multi-objective (12 *in vitro* measurements) stochastic search spanning 5000 models for each population. An additional layer of search and validation, based on *in vivo* measurements, was imposed when valid models from this search (7 deep and 20 superficial) were subjected to high conductance state (HCS). Further, deep neurons received larger inhibition, and the placement of deep and superficial somata translated to iso-distant Schaffer collateral fibers establishing contacts respectively on their distal and proximal dendrites. To match electrophysiological observations during SPW-R epochs, we introduced dendritic plateau potentials (DPPs) and perisomatic ripple frequency inhibition (RFI) in these neurons. We found that HCS lowered the probability of generating an axo-somatic action potential despite the presence of a DPP. Importantly, the distally placed DPP in deep neurons attenuated to a larger extent compared to their proximal counterparts in superficial neurons, which in conjunction with enhanced inhibition in deep neurons resulted in the differential transients observed in deep *vs*. superficial somata during SPW-R epochs. Next, we built a neuropil made of 440 deep and superficial neurons (220 each), with their somata placed within the stratum pyramidale (SP). We computed local field potentials using the line source approximation method at 17 locations when DPPs and RFI impinged on these neurons. With reference to sharp waves in the SPW-R complex, DPPs in the superficial and deep neurons contributed predominantly to proximal and distal sinks, respectively, together yielding a broad spread of the sink. The ripples in the SPW-R complex and the source in the SP were primarily mediated by inhibitory afferents onto deep neurons, with relatively small contributions from superficial neurons. Our results unveil the differential contributions of deep and superficial neurons to SPW-R complexes, and suggest localized DPP as a key cellular mechanism that links the SPW-R complex to memory consolidation through plasticity induction.

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

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NINDS F32 NS090753

ONR MURI N00014-16-1-2832

NIMH R01 MH60013

NIMH R01 MH61492

**Title:** Mechanisms of theta cycle skipping in a detailed microcircuit model of the medial entorhinal cortex

**Authors: *M. J. BEZAIRE**, M. E. HASSELMO
Psychological & Brain Sci., Boston Univ., Boston, MA

**Abstract:** Biological and modeling studies indicate a role for HCN channels in precise rebound spike timing and rebound spike theta phase preferences of medial entorhinal cortical layer II stellate cells. These phase preferences vary as a function of dorsoventral location within the medial entorhinal cortex, and may play a role in the gradient of grid cell properties seen along the dorsoventral axis. Here, we developed biologically realistic stellate cell computer models that reproduce the key gradients in electrophysiological properties observed experimentally along the dorsoventral axis. We subjected these models to various input protocols, finding that their rebound spike phase preferences, and the input phases to which they are sensitive, differ according to cell type, theta frequency, and their reliance on the HCN current. We next incorporated these model cells into a microcircuit model consisting of grouped stellate cells and a small number of interneurons. We examined their ability to code for space in one dimension using a model of theta cycle skipping, and found that the different model cell types are differently able to participate in robust theta cycle skipping, and therefore are differently able to reliably code for space in one dimension by way of theta cycle skipping according to our microcircuit architecture.

**Disclosures: M.J. Bezaire:** None. **M.E. Hasselmo:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH-NIMH R01 MH079511

**Title:** Decoding septohippocampal theta cells during exploration reveals unbiased environmental cues in firing phase

**Authors: *J. MONACO**[1], H. T. BLAIR, IV[3], K. ZHANG[2]
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**Abstract:** The hippocampal representations of space by place, grid, and border cells are embedded in a web of subcortical networks synchronized to the theta rhythm (6-10 Hz) governed by the medial septum. Theta-rhythmic modulation of spatial cell activity has been long characterized in phenomena such as phase precession across grid and place fields. However, how spatial information or environmental cues modulate theta cells has remained unclear. Here, we recorded both hippocampal and extrahippocampal theta-rhythmic cells (mostly interneurons) for multiple hours as rats explored an 80-cm cylindrical arena with an orienting visual cue. These long theta-cell recordings allowed for adequate sampling of spikes given moderate firing rates (n=8 rats, mean 15.5 spikes/s) and spatial biases in exploratory behavior such as wall following. We calculated the mutual information between position and theta phase of spikes and found 233/840 recordings had significant spatial phase information ($p<0.02$) averaging 0.36 bits/spike. We computed adaptive-kernel spatial maps for firing rate and theta phase and found that theta cells with spatial phase information >0.1 bits/spike also demonstrated high correlations, both positive and negative, between rate and phase. We define ’phaser’ cells using criteria for minimum spatial phase information (>0.1 bits/spike), significant rate-phase correlations (>0.2 magnitude), and minimum firing rate (>3.5 spikes/s): 101/233 recordings from 5/8 rats are classified as phasers. These phaser recordings had low peak spatial firing rates (median 7.3 spikes/s; s.d. 4.2), most (65/101) had negative rate-phase relationships (mean r=-0.3; s.d., 0.4), and were recorded primarily from lateral septum (72/101) compared to a minority found in hippocampal, midbrain, or other subcortical areas. Strikingly, the phaser subtypes segregate along the theta cycle: positive/negative phasers prefer inphase/antiphase firing with hippocampal theta. To establish that apparent spatial modulation was not due to behavioral sampling biases of the animal’s trajectory, we trained statistical general linear models (GLMs) to predict spikes based on spatial (quadratic functions of position, wall proximity) and trajectory (speed, direction) variables. Fitting L2-regularized GLMs to a grid partition of the arena produced highly accurate

reconstructions (n=671 unique cells, median $r^2$=0.94) of firing ratemaps. Model analysis revealed overwhelming reliance on space, not trajectory. Thus, spatial theta cells may convert environmental cues into temporal phase codes without behavioral bias.

**Disclosures: J. Monaco:** None. **H.T. Blair:** None. **K. Zhang:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.21/TT59

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Relative representation of elapsed time in hippocampal CA1 neurons during a temporal discrimination task

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**Abstract:** To behave adaptively in complex environments, quantification of intervals is one of the most important abilities. In particular, measuring elapsed time between events in seconds to minutes range, referred to as interval timing, is involved in various behaviors, such as, foraging, decision making, associative learning, and sequential motor learning. Although the importance of interval timing is known to, little study has been done to reveal the neural mechanisms of the interval timing. Recent studies have found that hippocampal pyramidal neurons fire sequentially at particular timing between events (Pastalkova et al., 2009; MacDonald et al., 2011). Because of similarity to place cells, these neurons were termed “time cells”. Sequential activities of time cell between events may represent the temporal information between events. However, the characteristics of time cells in interval timing has not been investigated yet. To clarify the characteristics of temporal information encoded by time cells in interval timing, we changed the length of interval which subjects are required to measure within one session. In this study, rats were required to discriminate two different intervals, running on a treadmill, for making a correct choice and experienced different sets of intervals in task conditions, i.e. they experienced 5 s or 10s interval in condition 1, 10 s or 20 s interval in condition 2, and then 5 s or 10 s interval again in condition 3. We recorded neural activities in dorsal CA1 during interval. Here, we found that a subset of CA1 neurons fired at particular moments and showed sequential activities within interval. Comparison with neural activities between condtion1 and condition 2, these neurons changed firing peak to rightward and enlarged firing filed. In condition 3, these neural activates went back to condition 1. Intriguingly even they changed their firing peak and firing field in condition 2, cross-correlations between two neurons was preserved in three task conditions. These results indicated that hippocampal time cells represent temporal information relatively and the order of activation of each neuron was preserved in three task condition. These results is

similar to previous studies related to place cells. So, same neuronal mechanisms are contributed to encode temporal and spatial information in the hippocampus.

**Disclosures:** **A. Shimbo:** None. **E. Izawa:** None. **S. Fujisawa:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.22/TT60

**Topic:** H.01. Animal Cognition and Behavior

**Support:** JSPS KAKENHI Grant 17K00344

**Title:** Attractor transitions for time cell-like elapsed time dependent activity in a hippocampal CA1-CA3 network model

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**Abstract:** The hippocampus is involved in encoding and retrieval of spatial and temporal memory. Place cells and time cells have been discovered in the hippocampal CA1 and CA3 regions. Place cells fire at a specific location in the given environment. Time cells fire at a specific moment within a temporally structured tasks. Transient firing of time cells express elapsed time from an event by firing at a specific moment. In a simple delayed matching to sample task, rats remember odor stimuli across a delay period. Four different odors were represented by different temporally organized ensembles of time cells in the CA1 region. Several theoretical models of time cells activity have been proposed: the temporal context model, the firing chain model, or a combined model. In the temporal context model, the flow of sequential events of cortical activity drives the sequential events in the hippocampus. In the firing chain model, elapsed time dependent activity is explained by a chain reaction of neural firing between excitable cells. A biophysical model of time cells has not been proposed. On the other hand, an attractor model for the pattern completion task has been proposed. Plenty of recurrent CA3-CA3 synaptic connections contribute to pattern completion. We hypothesize that a small group of neurons having strong internal connections represents a given percept and transition dynamics between the attractors can be represented as time cells. In the present study, we reproduced time cell-like elapsed time dependent firing in a hippocampal CA1-CA3 network model consisting of conductance-based neurons. We first constructed a CA3 network model. The elapsed time dependent activity was generated by successive transition of transient firing between cell assemblies. The successive firing transitions were accomplished if neurons belong to a single cell assembly and a part of those neurons had strong cross connections between cell assemblies. In

the CA1-CA3 network model, two temporally organized firing sequences were recalled. The population correlation between firing sequences induced by different initial stimuli were assessed. Evoked firing ensembles of time cells were determined by the initial stimulus. For the same initial stimuli, the population correlation between trials was high, while the different stimuli induced low population correlations. Our results indicate that the temporal representation of time cells is organized by orthogonal memory patterns in the CA3 region. Such sequential firing independently drives multi series of elapsed time dependent activity in the CA1 region.

**Disclosures: K. Takada:** None. **K. Tateno:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.23/TT61

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Lily's Fund for Epilepsy Research

**Title:** Temporal pattern separation through different multiplexed neural codes in the dentate gyrus, in health and epilepsy

**Authors: *A. MADAR**[1], L. A. EWELL[2], J. A. PFAMMATTER[3], E. WALLACE[5], S. RAVI[6], M. T. COWIE[1], M. V. JONES[4]
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**Abstract:** Understanding what computations neurons perform and how they are altered in diseases is an overarching goal of neuroscience. Yet, rare are the investigations of how neurons transform different input spiketrains following various complex patterns. We focused on granule cells (GCs) of the dentate gyrus (DG), a neural network long hypothesized to support pattern separation, the process of transforming similar input patterns into dissimilar outputs. To rigorously assess whether GCs exhibit pattern separation, we used mouse brain slices to stimulate DG afferents with input spiketrains of varying similarity and simultaneously recorded GCs output spiketrains. Because pattern separation is dependent on how one defines (1) a pattern of neuronal activity and (2) the similarity between a pair of patterns, we systematically explored different similarity metrics, at various timescales. The Pearson's correlation coefficient (R), the normalized dot product (NDP) or the binless Spike Time Tiling Coefficient (STTC)[1] and SPIKE metric[2] all showed that single GCs exhibit pattern separation at short timescales (lower than 100 ms) by rearranging their spike times. Pattern separation is also achieved through variations in the

binwise firing rate, which becomes dominant at longer timescales. It was assessed by a new metric, the scaling factor (SF), which considers spiketrains as vectors and compares their norm. We show it depends on differences in the average firing rate and differences in burstiness. Pattern separation as measured by SF is altered in kainate-injected mice with temporal lobe epilepsy, likely because of a subset of GCs exhibiting an occasional bursting behavior. These mice also show a strong impairment in a mnemonic discrimination task. We are currently investigating how changes in neuronal computations are correlated to behavioral performance and epilepsy severity. References: [1]: Cutts & Eglen (2014) Detecting pairwise correlations in spike trains: an objective comparison of methods and application to the study of retinal waves. *J. Neurosci.* 34, 14288-14303 [2]: Kreuz et al. (2013) Monitoring spike train synchrony. *J. Neurophysiol.* 109, 1457-1472

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**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.24/TT62

**Topic:** H.01. Animal Cognition and Behavior

**Support:** BFU2014-56692-R

**Title:** Role of identified claustral neurons during classical eyeblink conditioning in behaving rabbits

**Authors: *J. DELGADO-GARCIA**[1], M. REUS-GARCIA[1], J. LEDDEROSE[2], M. T. HASAN[3], A. GRUART[4]
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**Abstract:** The claustrum (CL) has been the subject of different theoretical studies with regard to its hypothetical role in integrative neural functions, including consciousness. Anatomical studies have revealed that the CL is a sheet-like neural structure located between the putamen and the insular cortex. It is assumed that claustral neurons are originated from the insula. The CL sends projections and receive afferents from all cortical regions. At present, it is assumed that CL neurons could be involved in cognitive processes and in the integration of different sensory modalities. Nevertheless, CL functions studied in alert behaving animals still remain unknown. We have recorded in behaving rabbits the firing activities of CL neurons during the acquisition of a classical eyeblink conditioning. Recorded cells were identified by their antidromic activation from the medial prefrontal (mPFC) or motor (M1C) cortices of both sides. For conditioning, we

used a delay paradigm: a tone as conditioned stimulus (CS) followed by an air puff as unconditioned stimulus (US) that co-terminated with it. Conditioned responses were determined from the electromyographic activity of the orbicularis oculi muscle. Neurons were recorded with glass and multiple metal electrodes across habituation and conditioning sessions. CL neurons were rarely activated by single stimuli of different modalities (air puffs, tones, light flashes). In contrast, CL neurons were activated during the first sessions of paired CS-US presentations, but their firing was less active in well-conditioned animals. Neurons activated from the M1C were located more superficially in the nucleus and presented different firing profiles than those projecting to the mPFC. Local field potentials recorded in the CL presented a characteristic ≈ 20 Hz oscillation during CS-US presentations. Electrical stimulation of the CL did not evoke any noticeable eyelid motor response even in trained animals. We developed recombinant adeno-associated viruses (rAAVs) equipped with chemically-controlled inducible genetic switches for inducible and reversible silencing of synaptic transmission by expressing a novel destabilized tetanus toxin light chain in the two CLs of rabbits. Silencing of synaptic transmission of CL projecting neurons was effective to delay the acquisition of the classical eyeblink conditioning, but did not evoke any significant effect on the rate of conditioned responses in well trained animals. Therefore, the CL seems to play an important role in the acquisition of associative learning tasks, mostly in relation to the novelty of CS-US association, but not in the expression of conditioned eyelid responses.

**Disclosures:** **J. Delgado-Garcia:** None. **M. Reus-Garcia:** None. **J. Ledderose:** None. **M.T. Hasan:** None. **A. Gruart:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.25/TT63

**Topic:** H.01. Animal Cognition and Behavior

**Support:** MH065561

MH073057

**Title:** Prelimbic cortex infusion of antidepressant nomifensine increases distractibility

**Authors: *A. R. MATTHEWS**[1], M. WILLIAMS[1], M. BUHUSI[2], C. V. BUHUSI[2]
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**Abstract:** Emotional processing is dysregulated in affective disorders such as depression, phobias, schizophrenia, and post-traumatic stress disorder. Among the processes impaired by emotional distracters, and whose dysregulation is documented in affective disorders, is the ability

to time in the seconds-to-minutes range, i.e., interval timing. Presentation of task-irrelevant distracters during a timing task results in a delay in responding, suggesting a failure to maintain subjective time in working memory, possibly due to attentional and working memory resources being diverted away from timing, as proposed by the Relative Time-Sharing (RTS) model (Buhusi & Meck 2009, Phil Trans R Soc B 364: 1875-1885). According to the RTS model, attentional and working memory resources are diverted away from timing when distracters are presented, depending upon the discriminability of the distracter (Buhusi 2012, J Exp Pscyh: Anim Behav Process, 38: 30-39). We have previously identified the prelimbic cortex as a structure that is involved in the RTS of attentional resources that are reallocated during distractions (Matthews et al. 2012, Front Integr Neurosci 6: 111). In Matthews et al. 2012, some rats received noise presentations paired with foot shock, while others received noise presentations without foot shock pairing. Either way, the noise was a familiar distracter. Here, we sought to further evaluate the effect of nomifensine on novel distracters using a similar procedure as Matthews et al. 2012. Because in Matthews et al. 2012 nomifensine decreased the delaying effect of the distracter, we hypothesized that if nomifensine shifts attentional resources back to the primary timing task, it would decrease the delaying effect of novel distracters. Whereas, if nomifensine affects only the emotional components of attention, it would have no effect on the delay after novel distracters. In contrast to these hypotheses, nomifensine dose dependently increased the delayed in trials with novel distraction presentation, $F(2,18) = 5.68$, $p < 0.05$. These results indicate that while nomifensine has a beneficial effect upon working memory under anxiety-inducing distractions, it is detrimental to working memory when the distracters are novel. Results are discussed in relation to the brain circuits involved in RTS of resources, and the pharmacological management of affective disorders.

**Disclosures:** **A.R. Matthews:** None. **M. Williams:** None. **M. Buhusi:** None. **C.V. Buhusi:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.26/TT64

**Topic:** H.01. Animal Cognition and Behavior

**Support:** FAPESP Grant 2016/054732

**Title:** The role of prefrontal cortex-striatum pathway in time tasks

**Authors: *E. F. OLIVEIRA**[1], G. CHIUFFA-TUNES[1], C. LOPES-AGUIAR[2], M. S. CAETANO[1], M. B. REYES[1]
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**Abstract:** The ability to estimate the passage of time is critical for behavioral adaptation and survival. However, the mechanisms underlying this ability still needs to be elucidated. The prefrontal cortex(PFC) and striatum (STR) seem to be key areas involved in time estimation in the seconds-to-minutes range and constitute a pathway looping through the thalamus. Here, we recorded from neurons in PFC and STR during a timing task. We analyzed single-unit activity and local field potentials, evaluated cross-frequency coupling between PFC and STR, described a possible influence of PFC local field potentials in neuronal activity in STR and assessed the functional connectivity within the prefrontal-striatal pathway.

**Disclosures: E.F. Oliveira:** None. **G. Chiuffa-Tunes:** None. **C. Lopes-Aguiar:** None. **M.S. Caetano:** None. **M.B. Reyes:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.27/TT65

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Sloan Scholarship to BJD

R01 supplement to BJD

R01 to NSN

**Title:** D1 v D2 MSN control of timing in the dorsal striatum

**Authors: *B. J. DECORTE**[1], M. S. MATELL[2], N. S. NARAYANAN[3]
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**Abstract:** Time-based decision-making is critical for adaptive behavior and is impaired in a variety of illnesses (e.g., Parkinson's disease, Huntington's disease, etc.). How individuals make decisions based on temporal information can be studied in rats using the peak interval (PI) procedure. In a PI task, the onset of a cue indicates that reward may be earned for responding after a set 'criterion duration' has elapsed. Probe trials are also included in which the stimulus is presented for 3-4 times the length of the criterion duration and reward is omitted. During probe trials, rats abruptly start responding just before and stop responding just after the criterion duration passes. We examined how the dorsal striatum mediates the decision to start and stop responding during probe trials. Rats were trained on a PI task in which a houselight signaled a 6 second delay to reward. During testing, we infused either a D1 antagonist (SCH-23390) or a D2 antagonist (Sulpiride) into the dorsomedial striatum. D2 blockade delayed the decision to both start and stop responding, whereas D1 blockade selectively delayed the decision to stop

responding. This effect was distinct from infusions in the dorsolateral striatum. We next evaluated start/stop-related striatal activity using electrophysiology. Many striatal neurons showed transient activation or suppression around start or stop times. This activity was preceded by linear firing rate changes that spanned several seconds; the rate of which covaried with when a start or stop occurred. Collectively, these results suggest that neuronal activity in the striatum mediates decision thresholds during timing tasks.

**Disclosures: B.J. Decorte:** None. **M.S. Matell:** None. **N.S. Narayanan:** None.

**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.28/TT66

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS)

RIKEN Junior Research Associate Program

**Title:** Blind detection of behavior related population activity by using edit similarity measurement and statistical modeling

**Authors: *K. WATANABE**[1,2], T. HAGA[1], M. TATSUNO[3], D. R. EUSTON[3], T. FUKAI[1,2]
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**Abstract:** Considerable methodological progress suggests that sequential firing of cortical neurons plays an active role in processing behaviorally relevant information, yet methods for analyzing the spatiotemporal structure of population neuronal activity, particularly those for detecting sequential firing patterns, are limited. This research introduces "Edit Similarity" to analyze the temporal structure of spike trains in noisy neuronal population activity. Edit similarity was originally introduced in computer science for analyzing arbitrary strings such as words and genomes. The proposed framework applies this metric to sequences of neuronal population spikes. We show in both artificial and real neural activity data (CA1 hippocampal data taken from CRCNS) and from rat prefrontal cortex that the method enables the detection of spike sequences in the presence of various kinds of noise. The statistical relationship between detected clusters and behavior data are also discussed.This work was supported by "the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from Japan Agency for Medical Research and Development (AMED)" and "RIKEN Junior Research Associate Program".

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**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.29/UU1

**Topic:** H.01. Animal Cognition and Behavior

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CONACYT grant 196

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CONACYT scholarship 164310

**Title:** Time categorization in the primate pre-SMA: single neuron correlates for boundaries, categorical decisions and reward outcomes

**Authors: *G. MENDOZA**[1], J. C. MÉNDEZ[2], O. PÉREZ[1], L. PRADO[1], H. MERCHANT[1]
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**Abstract:** Categorizing intervals as short or long depends on the ability to perceive the passage of time and to assign different stimuli to specific categories, both fundamental primate cognitive skills. To determine the neural mechanism of interval categorization, we recorded the activity of pre-SMA neurons of monkeys executing an interval categorization task in which the short-long limit changed between blocks of trials. A large population of cells encoded the boundary between categories by reaching a peak of activity at a time close to the subjective limit between the short and long intervals. Notably, these cells changed their time of peak activity depending on the categorical boundary of the current block of trials. In addition, pre-SMA cells also represented the category selected by the monkeys and the outcome of the decision. These results suggest that the pre-SMA adaptively encodes the subjective boundary between short and long durations and contains all the crucial neural information to categorize intervals and evaluate the outcome of such perceptual decisions.

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**Poster**

**615. Cortical and Hippocampal Circuits: Timing and Temporal Processing**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 615.30/UU2

**Topic:** F.08. Biological Rhythms and Sleep

**Support:** Springboard Grant by Academy of Medical Sciences and Wellcome Trust, SBF002\1045

Wellcome Trust Grants 095668 and 095669

**Title:** Frequency domain structure of spontaneous infraslow dynamics in local cortical microcircuits

**Authors: *M. OKUN**[1], K. D. HARRIS[2]
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**Abstract:** Cortical activity is organised across multiple spatial and temporal scales. Most neurophysiological research, however, is concerned with activity on timescales of 1ms - 1s, for the simple reason that these timescales suffice for an extensive range of behaviours (e.g. your ability to recognise pictures or sounds, to get up from a chair, or to recall your yesterday's whereabouts). One prominent exception is fMRI: because of technical constraints, it is limited to measurements in the range of 0.01 - 0.5 Hz, thus a substantial body of knowledge on cortical dynamics on timescales slower than a second also exists. However, fMRI is limited not only in its temporal but also in its spatial resolution, thus while we know how entire cortical areas modulate their activity on slow timescales, very little is known about the underlying behaviour of individual neurons. In the present work we addressed this question by analysing multi-hour recordings of neuronal populations in rodent sensory and association cortex, performed using chronically implanted high-density silicon probes.
At the level of single neurons, we found that the spike count Fano factor increases with window size (for windows between 1ms and 500s). To understand this behaviour we developed an algorithm for generating synthetic spike trains with pre-specified inter-spike interval (ISI) distribution and power spectral density (PSD). We found that these synthetic spike trains quantitatively recapitulated the Fano factors in the original data, which was not the case when each constraint (ISI distribution or PSD) was used on its own. For most neurons, the point process PSD of the spike train in frequencies < 1 Hz behaved approximately as $1/f^{\alpha}$ ($\alpha$ was 0.3±0.21), providing a concise summary of slow modulation in neurons' firing rate which is unaccounted for by the fast (intrinsic) spiking dynamics of the ISIs.
In order to understand how the slow firing rate modulations are coordinated among neighbouring cortical neurons, we estimated the coherency between the firing of individual neurons and the

population rate, thus extending the concept of population coupling (Okun et al., Nature 2015) to the frequency domain. On average, in the 0.01 - 1 Hz band coherence fell with increasing frequency, however in ~20% of the neurons this decrease was not monotonic. Most surprisingly, the preferred phase of ~10% of the neurons was strongly frequency dependent, changing on a logarithmic scale by as much as 180 - 360 degrees. These findings suggest that slow modulations in firing rate are controlled by several different mechanisms with distinct effects across the neuronal population.

**Disclosures: M. Okun:** None. **K.D. Harris:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.01/UU3

**Topic:** H.01. Animal Cognition and Behavior

**Support:** NIH Grant R01-MH101297

NSF Grant NSF/CRCNS-1516235

**Title:** Cognitive mapping of a virtual olfactory landscape

**Authors: *B. A. RADVANSKY**, D. A. DOMBECK
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**Abstract:** Olfactory-guided navigation is an animal behavior of crucial ethological significance. But the technical challenge of controlling chemical concentrations in space has posed a barrier to studying the neural basis of this behavior. Here, we overcome this challenge by building an olfactory virtual reality system for mice. This system uses rapid flow controllers and an online predictive algorithm to create and maintain virtual olfactory landscapes. Head-fixed mice on a spherical treadmill in darkness can navigate a linear track comprised of virtual odor gradients while engaging “place cells” in CA1 of the hippocampus. These place cells exhibit similar properties to those previously reported for real and visual virtual environments, demonstrating that olfactory-guided navigation recruits a similar cognitive map to that of multisensory- and visual-guided navigation. In addition to olfactory-guided navigation, this system creates a way forward for studying the neural bases of multisensory integration, innate valence, and low-dimensional sensory-spatial processing.

**Disclosures: B.A. Radvansky:** None. **D.A. Dombeck:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

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**Program#/Poster#:** 616.02/UU4

**Topic:** H.01. Animal Cognition and Behavior

**Support:** CIHR Grant MOP133444

**Title:** The Black Box Effect: Reducing sensory stimulation after spatial learning promotes memory consolidation

**Authors: *D. E. ARKELL**[1], E. ALLISON[1], A. ASIMINAS[1], E. R. WOOD[1], O. M. HARDT[2,1]
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**Abstract:** It is well known that sleeping after learning can enhance memory retention in both humans and non-human animals. Recent studies in humans suggest that this effect may not depend on sleep per se, but can arise from sleep-inherent reduced sensory stimulation, decreasing memory interference (Craig et al, 2016). To address this issue, we tested the role of sensory stimulation after spatial learning on memory retention in rats and explored possible neural correlates of this memory enhancing effect.
We used an object location memory task in our behavioural studies. Rats were habituated for 10 min each day for 4 consecutive days to the empty testing arena, after which they spent 3 h either in a dimly lit resting box or a resting box illuminated with red light, which rats cannot sense. We used gentle handling to prevent rats from falling asleep whenever they were in the resting box. The next day, animals returned to the testing arena that now contained two copies of the same novel object. Rats could freely explore these objects during the 20 min single training trial. Immediately afterwards, rats returned either to their dimly lit or red light resting box. We tested memory retention for the locations of the objects 24 h after learning. Only rats that spent 3 h in the red light expressed significant object location memory.
These results strongly suggest that the lack of visual stimulation alone promotes formation of long-term object location memory, and that sleep is not necessary for this memory enhancement to emerge. To explore the neural mechanisms underpinning this effect, we assessed place field stability as a function of reduced visual stimulation. We recorded place fields in dorsal CA1 during a 10 min session during which rats randomly foraged in a novel testing arena. Rats were placed either in the dimly lit or the red-light testing box for 3 h immediately after, and then returned to their home cage. The same cells were recorded during two further 10 min sessions in the same arena, 6 h and 24 h after the initial exposure. Consistent with our behavioural results, our preliminary data suggest that reducing visual stimulation after learning promoted the stability of place fields in rats.

Overall, we show that long-term memory formation, in terms of behavioural as well as electrophysiological measures, benefits from reduced sensory stimulation after learning: just being exposed to highly familiar visual stimuli suffices to impair long-term memory retention. Our future studies will explore the neurobiological processes by which visual stimulation disrupts memory retention.

**Disclosures: D.E. Arkell:** None. **E. Allison:** None. **A. Asiminas:** None. **E.R. Wood:** None. **O.M. Hardt:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.03/UU5

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Unsupervised learning of neuronal ensemble dynamics reveals representation of space without behavioral measurement

**Authors: *A. RUBIN**, L. SHEINTUCH, O. PINCHASOV, N. BRANDE-EILAT, Y. RECHAVI, N. GEVA, Y. ZIV
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**Abstract:** The traditional experiment in neuroscience relies on measuring neural responses along with an a-priori defined external variable, and then quantifying the extent to which they covary. This approach has many benefits, but also restricts the findings to the pre-selected variables only, potentially overlooking other variables that the researcher did not measure or consider relevant. This is especially unfortunate in in light of the recent technological advances that enable large-scale recording of neuronal activity in behaving animals. To circumvent these shortcomings while taking advantage of the richness of the neural activity data, we developed a novel data analysis approach that allowed us to calculate neuronal tuning curves, regardless of any a-priori external selected variable, but rather with respect to the activity dynamics of other neurons within the same recorded population. We demonstrate the power of our method using several separate datasets with different coding properties: electrophysiological recordings in the thalamus and postsubiculum and $Ca^{2+}$ imaging data from the hippocampus and prefrontal cortex of freely behaving mice. Our analysis uncovered the structure of neuronal activity within the neural code space, exposing key properties of the neural code within these brain regions. Specifically, we were able to reconstruct the internal representation of space and unveil neural tuning curves, but without using any information about the external world. Overall, our approach enables the study of internal representations in neural populations in a generalized and unbiased manner with minimal prior assumptions.

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**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.04/UU6

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Wellcome Grant 099926/Z/12/Z

**Title:** Brain-computer interface relates hippocampal spatial reconfiguration to the encoding of aversion experience

**Authors: *M. TSANOV**[1], O. MAMAD[2]
[1]Trinity Col. Inst. of Neuroscience, TCD, Dublin, Ireland; [2]Trinity Col. Dublin, Institute of Neuroscience, Ireland

**Abstract:** Hippocampus-dependent memories relate rewarding or aversive experiences to environmental context. The hippocampal neurons encode spatial representation but it remains unclear how they encode aversive experiences. Here, we show that the place fields remapped in response to the innately aversive trimethylthiazoline, associated with particular section of the environment. The remapping of the individual place fields followed a specific pattern: the majority of remapped cells showed increased extra-field spiking during the aversive episodes. Optogenetic photostimulation of the basolateral amygdala showed equivalent population and individual remapping pattern. We applied brain computer interface (BCI) to test the hypothesis that the hippocampal remapping is a function of spike-timing proximity between the amygdala activation and hippocampal spiking. We used closed-loop optogenetic photostimulation triggered by selected place cells, during navigation in particular section of the recording track. Immediate, but not delayed BCI photostimulation evoked field remapping of the engaged place cells. Our findings present key evidence that the hippocampal neurons are not merely mapping the static environment but also store aversive episodes, enabling hippocampus-dependent memory for past experience to support future adaptive behavior.

**Disclosures:** **M. Tsanov:** None. **O. Mamad:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.05/UU7

**Topic:** H.01. Animal Cognition and Behavior

**Support:** the Fundamental Research Funds for the Central Universities

**Title:** Optimal orientation of grid cell system

**Authors: *D. CHEN**[1], W.-X. WANG[1], L. WANG[2]
[1]Sch. of Systems Sci., Beijing Normal Univ., Beijing, China; [2]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China

**Abstract:** Grid cells fires when an animal crosses the fields of an hexagonal grid tiling the environment. To provide stable spatial representation, grid cell system must be anchored to external reference frame. Here, we combine winner take all coding and hamming distance coding to analyse population activity of grid cells. We show that the orientation of grid cells offsets from the wall by ~8 degree can minimize the similarity of population coding vector of locations on the segment along the wall within boundary areas. And we propose a reinforcement learning model to achieve the optimal anchoring process. These results explain the experiment data in rodents and point to the underline coding mechanism for grid patterns aligning to the external environment.

**Disclosures: D. Chen:** None. **W. Wang:** None. **L. Wang:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.06/UU8

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Wellcome Trust–DBT India Alliance grant IA/I/11/2500290

University Grants Commission, India

**Title:** Theta oscillations are required to generate spatially periodic receptive fields in the medial entorhinal cortex

**Authors: *A. BALACHANDAR**[1], C. G. ASSISI[2]
[1]Biol., Indian Inst. of Sci. Educ. and Res., Pune, India; [2]Indian Inst. of Sci. Educ. and Res. Pune, Pune, India

**Abstract:** Grid cells in the medial entorhinal cortex (mEC) fire at the vertices of a hexagonal grid that tiles the entire space an animal explores. This pattern serves as an allocentric coordinate system for animals to integrate their movement and determine their current location even in the absence of external cues. The stability and precision of this pattern is remarkable given many experimentally measured variables in the mEC - inputs to stellate cells and variability of local field potential oscillations - vary noisily as the animal navigates its environment. How can a stable spatial representation be built upon such shaky ground? We discover that the answer lies in the interplay between theta oscillations and the intrinsic time scales of the system, namely, the conductances expressed in stellate cells. To illustrate the mechanism we simulate a network of physiologically detailed conductance based model stellate cells coupled via inhibitory interneurons. Competitive interactions between stellate cells cause different groups of neurons to fire at different times. The identity of neurons that form transiently synchronous groups is determined by the topology of inhibition and the history of activation of stellate cells. We show that these spatiotemporal sequences can be easily perturbed by noise to the network. Theta oscillations are required to ensure that the same sequence is stimulated every time the animal traverses a particular trajectory. The reliability of these temporal sequences, in turn, translates into the stability of the grid cell's spatially periodic receptive field. Theta oscillations are themselves fickle, in that, the phase of theta is not pinned to the location of the animal as the spiking activity of grid cells are. Further, changes in movement velocity affect the frequency of theta oscillations. We show that these perturbations to theta do not affect the stability of grid fields. Our simulations concur with experimental data demonstrating that when theta oscillations are selectively and reversibly removed by excising input from the medial septum, grid fields dissipate leaving spatially non-specific and temporally imprecise patterns of activity. Our model shows that the formation of spatially periodic receptive fields is an emergent property of the coupling between theta oscillations and the intrinsic rich temporal repertoire of the mEC network.

**Disclosures: A. Balachandar:** None. **C.G. Assisi:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.07/UU9

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Wellcome Trust–DBT India Alliance Grant IA/I/11/2500290

DST-INSPIRE Fellowship

**Title:** Network mechanisms that represent novelty and familiarity in the Medial Entorhinal Cortex

**Authors: *B. KRISHNAN**, A. BALACHANDAR, C. G. ASSISI
Biol. Sci., Indian Inst. of Sci. Educ. and Res., Pune, India

**Abstract:** Grid cells in the medial entorhinal cortex (mEC) fire whenever the animal is at the vertices of a hexagonal grid. This spatially periodic receptive field is anchored to the external world and can be used to integrate the movement of the animal as it traverses its environment. Specific features of the receptive field can act as markers of the external environment. Changes in the environment can change the orientation of the grid, break its symmetry along specific axes and expand or contract in spatial scale. In this study, we focus on a transformation of the spatial scale of the grid as a function of the novelty of the environment. Here, the environment remains static while the internal state of the animal (its degree of familiarity with the environment) changes over time. When an animal is introduced to a novel environment, the spatial scale of the grid expands and its crystalline symmetry gets perturbed. Over days, with growing familiarity, the scale of the grid contracts and its hexagonal symmetry is restored. The distance between the vertices and the radius of each vertex decreases while the 'gridness', a measure of symmetry, increases. We model this phenomenon using a network of biophysically realistic conductance-based neurons modeled as layer II stellate cells, putative grid cells, that are coupled through inhibitory interneurons. The topology of our model network resembles that of existing continuous attractor network models that have been used to generate the characteristic receptive fields of grid cells. In continuous attractor models, grid formation has been attributed to reciprocal inhibitory interactions between grid cells. We show that stellate cells coupled via an inhibitory intermediary can, under certain conditions, act as reciprocally inhibitory neurons. Inhibitory synapses in the mEC show spike timing dependent plasticity (STDP) and may continually reshape the mEC network as the animal navigates its environment. Further, environmental novelty is also known to affect the frequency of theta oscillations, possibly due to a modulation of acetylcholine levels. We show that a change in theta drive to our model network, coupled with changes in the topology of the inhibitory network effected by STDP can replicate the dynamics observed in the mEC as the animal becomes progressively familiar with its environment.

**Disclosures: B. Krishnan:** None. **A. Balachandar:** None. **C.G. Assisi:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.08/UU10

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Effect of home location on parasubicular grid cells

**Authors: *J. I. SANGUINETTI SCHECK**, M. BRECHT
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**Abstract:** The rat parasubiculum is a long (5.2mm) and narrow (0.3mm) parahippocampal structure engulfing the medial entorhinal cortex (MEC) on its dorsal and medial sides. The parasubiculum outputs predominantly to layer 2 of the MEC, selectively targeting the calbindin positive patches (Tang et al., 2016). This puts the parasubiculum in a prime position to shape both spatial and temporal coding in the MEC. Even though the parasubiculum itself contains most of the same functional cell classes (Grid Cells, Border Cells and Head Direction cells) as the MEC (Boccara et al., 2010, Tang et al., 2016), comparatively little is known about their functional role in navigation and the organization of spatial behavior. Grid cells in the MEC have been described as coding space as a robust hexagonal lattice that tessellates every environment in a similar way. Even though arena shape affects the grid, their stability is only mildly altered by changes in context (Diehl et al., 2017) and may shift with novelty at a very slow pace. However, grid cells in the parasubiculum have not yet been scrutinized for their context dependency or their robustness to changes in the environment. We conducted a preliminary study to test whether parasubicular grid cells are stable to the introduction of biologically relevant landmarks in the environment. The homecage, being the place where the animal spends most of the day, sleeps and receives food, is the most relevant landmark in a laboratory setting. We familiarized Long Evans rats (n=4) to a modified homecage with two lateral doors. After two weeks of familiarization with their home, rats were trained to forage for treats in a squared one meter arena, both in the absence or presence of their homecage. In this environment rats showed home preference, in-home grooming and hoarded food pellets from the surrounding towards the home without specific training. Our preliminary results show that the introduction of the homecage in the familiar environment reorganized foraging behavior, causing the rats to relocate their homebase (preferred location for resting and grooming) towards the home and initiate exploratory trips with the homecage as a starting point. We conducted extracellular single unit recordings of grid cells in the parasubiculum of rats (n=2) foraging the same, familiar, squared environment in the absence or presence of their homecage. We recorded (n=14) single units classified as grid cells, out of which six grid cells shifted their firing fields towards the homecage location. Our preliminary recordings show that home location may alter the grid cell pattern of parasubicular cells.

**Disclosures:** **J.I. Sanguinetti Scheck:** None. **M. Brecht:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.09/DP14/UU11 (Dynamic Poster)

**Topic:** H.01. Animal Cognition and Behavior

**Support:** DFG Forschergruppe FOR2143

**Title:** Differential dynamics of memory representations in the hippocampal dentate gyrus and CA1

**Authors: *T. HAINMUELLER**[1], M. BARTOS[2]
[2]Inst. of Physiol. I, [1]Univ. of Freiburg, Freiburg I. Br., Germany

**Abstract:** The hippocampus is an essential brain center for the acquisition of conscious memories. Each of its major subfields is home to neuronal ensembles which encode memory-specific contents, such as places, sounds, time or individuals (O'Keefe and Dostrovsky, 1971; Quiroga et al., 2005; Aronov et al., 2017). Activation of these ensembles can be used to manipulate existing memories in mice or to craft artificial ones (Ramirez et al., 2013). While there is extensive knowledge on the stability and dynamics of contextual representations by principal cells e.g. in the hippocampal CA1 region, reports of content specific activity in others, such as the dentate gyrus (DG) remain scarce (Leutgeb et al., 2007, Danielson et al. 2016).
The study of neurons in the DG is hindered by the fact that the activity of granule cells (GCs), the principal neurons of the DG, is extremely sparse, with most neurons being silent throughout behavioral recording sessions (Pernía-Andrade & Jonas, 2014). Therefore, a large number of GCs must be observed simultaneously in order to record activity of the few coactive cells that form a functional memory ensemble. This is hard to achieve using standard electrophysiological recording techniques like tetrode-recordings. Only recently, technical advances have enabled the possibility of direct functional imaging of GC activity in awake, behaving mice (Danielson et al, 2016; Pilz et al., 2016).
In our present study, we have employed a novel approach to chronically image the activity of DG neurons in the intact hippocampal formation in awake, head fixed mice behaving in a virtual environment setup (Dombeck et al., 2010). Mice were trained to collect rewards in different virtual environments and to memorize the reward locations. We tracked the development of contextual representation of neurons in the CA1 as well as DG over multiple days. In agreement with previous studies using other recording modalities (Goodsmith et al., 2017, Senzai et al., 2017), we found that many GCs of the DG as well as pyramidal cells in CA1 represent distinct locations in the virtual environment reliably over multiple days. However, principal cells in these two hippocampal subfields show different remapping dynamics when the virtual environment is altered. Furthermore, we found regional differences in the stability of place representations

across days. These findings indicate, that cellular memory engrams in these two hippocampal subfields may be more suitable for certain types of memory tasks and explain earlier findings of specific and distinct memory impairments arising from lesions of either the DG or CA1.

**Disclosures: T. Hainmueller:** None. **M. Bartos:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

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**Topic:** H.01. Animal Cognition and Behavior

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**Title:** Functional breakdown processes of neural circuits in hippocampal CA1 region of Alzheimer′s disease model mice

**Authors: R. TAKAMURA**[1,2,3], *K. MIZUTA[1,2], Y. SEKINE[2], T. ISLAM[2], T. SAITO[2], T. TAKEKAWA[4], M. OHKURA[5], T. FUKAI[2], J. NAKAI[5], T. C. SAIDO[2], Y. HAYASHI[1,2,5,6]
[1]Kyoto Univ., Kyoto, Japan; [2]RIKEN BSI, Saitama, Japan; [3]Waseda Univ., Tokyo, Japan; [4]Kogakuin Univ., Tokyo, Japan; [5]Saitama Univ., Saitama, Japan; [6]South China Normal Univ., Guanzhou, China

**Abstract:** Breakdown of neuronal circuit as a result of deposition of pathogenic proteins leads to the decline of cognitive functions in Alzheimer′s disease (AD). One early symptom in AD patients is the dysfunction of spatial memory, in which the hippocampus plays an essential role. However, the precise process leading to functional breakdown of the hippocampal neural circuits

of AD remains unclear, in part due to the technical difficulty of longitudinally monitoring hippocampal activity during pathogenesis. Moreover, most studies of AD have used transgenic mice highly overexpressing amyloid precursor protein (APP), which caused artificial phenotypes such as memory dysfunctions that do not happen in human patients. To address these issues, we expressed a fluorescent calcium sensor G-CaMP7 in a new AD model mouse with single humanized amyloid precursor protein knock-in carrying Swedish, Beyreuther/Iberian, and Arctic mutations ($App^{NL-G-F}$) (Saito et al., 2014). We could observe the activity of 400-700 hippocampal CA1 pyramidal neurons over months with two-photon microscopy from head-fixed mice behaving under a virtual reality system. When we examined cell activity period, the rate of hyperactive cells (activity rate: ≥20%) during running increased in 7-month-old AD model mice compared with control mice, while it was not different in 4-month-old mice. In addition to activity rate, the number of active cells and percentage of place cells identified in 7-month-old AD model mice declined by 19% and 32% respectively, when compared with 4-month-old AD model mice. These abnormal cell functions may be the important factors resulting in memory dysfunctions, because deterioration of spatial memory starts at 6 months in this AD model mice. Interestingly, in the AD model mice, G-CaMP7-positive aggregations were observed in *stratum oriens* starting around the age of 3 months, and expanded with age. Since these dynamic characteristics were similar to Aβ deposition, and Aβ staining was located close to aggregations, they could be a sign of Aβ in vivo without any staining. These results suggest that the functional circuit including place cells in the hippocampal CA1 of AD model mice breaks down after the increase in Aβ deposition.

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**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.11/UU13

**Topic:** H.01. Animal Cognition and Behavior

**Support:** EU FET GRIDMAP grant

HFSP RGP0057/2016

**Title:** Storage capacity of threshold-linear networks for grid-like continuous attractors

**Authors: D. SPALLA**[1], S. ROSAY[1], *A. TREVES[2]
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**Abstract:** Continuous attractor neural networks have been suggested to be involved in spatial memory, allowing for path integration to be implemented by quasi-frictionless movements of a ‘bump’ of activity representing the current or imagined position within a spatial environment. Like discrete attractor networks, also continuous ones can in principle store multiple environments into as many charts, and in [1] their storage capacity was analytically evaluated in the mean field approximation, for a sparse activity model suited to CA3 place cells. Grid cells, however, appear to map space densely and quasi-periodically, and maybe re-use the same chart for several environments. Can their storage capacity be estimated, too?
We report that it can, for a recurrent network of threshold-linear neurons, adapting what in [1] was a warming-up exercise. As in the sparse model, the calculation reduces to finding the solutions of a single equation, which disappear at a critical value of the storage load; but periodic boundary condition have to be imposed on a two dimensional Voronoi Cell (VC). Imposing a hexagonal VC allows to model grid-like charts and, surprisingly, the storage capacity turns out to be very much higher than in the previous toy model with a square VC, by several orders of magnitude.
Moreover, we show (through both analytical calculation and numerical simulations) that 'stripe' solutions, in which coherence is maintained in only one of the two dimensions of the plane, are favored with a square VC over square grids, allowing for a capacity larger by an order of magnitude. These results match the hierarchy of solutions independently derived in [2] for different 2D tiles, in which hexagonal grids are favored over stripes and both over square grids.
In graded-response networks, mixtures of multiple attractors are known to also be stable, though in limited regions of parameter space [3]. In our network, too, we find, in addition to single bumps, a regime in which external cues trigger retrieval in more than one 2D chart simultaneously, in a similar way as described in [4] for two 1D periodic manifolds.
1. Battaglia F, Treves A**.** Phys Rev E (1998)
2. Kropff E, Treves A. Hippocampus (2008)
3. Roudi Y, Treves A. Phys Rev E (2003)
4. Romani S, Tsodyks M. PLoS Comput Biol (2010)

**Disclosures: D. Spalla:** None. **S. Rosay:** None. **A. Treves:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.12/UU14

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Improved method for measuring the topological dimensions of neuronal firing rate space

**Authors: *S. E. FOX**, J. B. RANCK, Jr
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**Abstract:** The firing of a class of neurons can be represented as a manifold embedded in a larger dimensional space. For N neurons, the space has N dimensions, each point a vector whose components represent the effect of each spike.
The inductive method to measure the topological dimension exploits the property that a boundary of a subspace with unknown topological dimension n, in an N-dimensional space has n-1 dimensionality. A randomly selected point becomes the center of an n-sphere with a radius small enough to fit inside the space. A point on its boundary is then selected as the center of an (n-1)-sphere. The number of times this process must be applied in order to be left with only 2 points is the inductive dimension (d) of the manifold.
This can be appreciated intuitively in a uniformly populated 3-space. The 1st step produces a 2D surface of a sphere, the 2nd, a 1D circle and the 3rd, 2 points: 3 steps equals inductive dimensionality of 3. The rate space of N neurons is a sparsely populated N-space having an embedded manifold with an inductive dimensionality ($d \leq N$, usually $d<<N$) that represents the number of terms necessary to describe the firing in the subspace.
We measured the inductive dimension of an 89D rate space for 89 place cells (courtesy of Dr. Eva Pastalkova) recorded for 1 hr while the rat foraged for food scattered on a 1 m square open field. To deal with sparsity, the inductive method was modified to use a shell for the boundary, rather than a surface. Last year we reported that the result was 3, not the 2 expected for the 2D floor. Because the shell has thickness, the extra dimension is the “experiential curve” itself, the activity along the rat’s path. We have now eliminated the extra dimension by allowing inclusion of only one intercepted point in each pass through the thickness of the shell. The result was 2 for both the real place cells and models of place cells with and without variance, and was not sensitive to exact radius or thickness of the shell. It was unchanged by reducing N to 45, but N=30 was insufficient. Applying the method to the same number of random spikes gave no result.
Whereas the conclusion was predictable for place cells because the firing correlates are well known, there are many neuronal classes and conditions for which there are an unknown number of terms necessary to account for the firing, e.g. firing with no change in behavior, as in sleep. The topological dimension of manifolds is useful because: 1) it allows more extensive mathematical analyses to be used, e.g. maps between manifolds, combining manifolds, and 2) it allows us to define circumstances in which the experiential curve intersects itself, i.e. in which something is the “same”, as is required in learning and recognition.

**Disclosures: S.E. Fox:** None. **J.B. Ranck:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

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**Program#/Poster#:** 616.13/UU15

**Topic:** H.01. Animal Cognition and Behavior

**Support:** ERC

AMIDEX

**Title:** Influence of proximal 3D objects on hippocampal spatial representation in mice navigating virtual linear mazes

**Authors: *R. BOURBOULOU**[1,2], G. MARTI[3,2], M. NOUGUIER[3], F.-X. MICHON[3,2], L. PETIT[3,2], M. PASQUET[3], D. ROBBE[3,2], J. KOENIG[3,2], J. EPSZTEIN[3,2]
[1]INMED UMR901, Marseille Cedex 09, France; [2]Aix-Marseille Univ., Marseille, France; [3]INMED INSERM U901, Marseille, France

**Abstract:** Hippocampal place cells fire at specific locations as a subject moves through space, providing a neural code for the current position during locomotion. This spatial representation can be more or less accurate depending on the quantity and quality of sensory cues available to the animal, and on the level of spatial attention required by the task. The specific contribution of visual cues in the place cell code remains unclear because of their constant mixture with other sensory cues in real environments. In this study, we took advantage of virtual reality to selectively manipulate visual information as mice performed a behavioral task that did not require explicit spatial attention. Mice were trained to go back and forth between liquid rewards at the ends of virtual linear tracks, which differed in the richness of wall patterns, and the presence of virtual 3D objects inside the maze. Once behavioral performance was stable, we performed acute silicon probe recordings (32-64 channels) in area CA1 of the hippocampus. We found that the presence of proximal virtual 3D objects greatly enhanced the quantity of spatially modulated cells (place cells) among active cells and their quality in terms of peak firing rate, spatial information content, stability and in/out field firing. Spatial locations near the objects were over-represented. Virtual 3D objects also favored position over distance coding. Temporal coding of place cells (e.g. theta phase precession) was increased in the presence of the objects. Place cell rate and temporal coding were modified instantaneously upon removal/addition of 3D objects. Furthermore, such online change of 3D objects' availability in a familiar environment (same wall pattern, maze geometry) instantaneously induced an important modification of the place cells' representation (e.g. remapping). We conclude that proximal visual cues, such as visual 3D objects, have a strong impact on hippocampal place cell coding in virtual environments.

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**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

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**Program#/Poster#:** 616.14/UU16

**Topic:** H.01. Animal Cognition and Behavior

**Title:** Dopamine dependence of hippocampal coding and spatial learning

**Authors: *A. RETAILLEAU**[1,2], G. MORRIS[1]
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**Abstract:** The hippocampus (HPC) is often referred to as a cognitive map. However, it is still not known whether and how this map is learnt and what parameters it encodes. Several lines of evidence support tuning of representation in the hippocampus by outcome-related information. We investigate whether local dopamine (DA) input biases hippocampal representation to a subset of available dimensions in a behaviorally relevant manner. We hypothesize that DA input to the hippocampus serves to mold the cognitive map to represent adaptive dimensions of the sensory input. We recorded from multiple single units and local field potentials in area CA1 of dorsal HPC of behaving rats engaged in a navigation task in which two possible sets of cues are relevant to reward collection. The two sets of cues were manipulated in an independent manner so as to dissociate between neuronal encoding of each dimension. Once rats had learnt to follow one set of cues, the paradigm was shifted and animals had to use the second set of stimuli to get reward. Our results show that CA1 neurons can rearrange to represent the reward-relevant representation and neuronal activity can follow the behavioral set-shift rapidly after the switch to encode the most relevant parameters. To study the dependence of this organization on DA, we locally infused D1/D5 dopamine antagonists to block receptors in the HPC at the time of the set shift. Local injection of DA antagonist slows the set shift significantly. Neuronal activity in infused rats seems to persist in encoding the previously relevant parameters for the first 2-3 days after the switch of paradigm. Then, after more training days, blocking of D1 receptors seems to lead to deficits in place-cell properties. From a network point of view, DA receptors blocking leads to a slight shift in the theta frequency towards slower rhythms. Moreover, beta activity found in sham rats during the first days of learning of the new paradigm is suppressed in the infused rats.

**Disclosures:** **A. Retailleau:** None. **G. Morris:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.15/UU17

**Topic:** H.01. Animal Cognition and Behavior

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**Title:** Cellular mechanisms for the formation and plasticity of hippocampal cognitive maps

**Authors: *M. SATO**[1,2,3,4], K. MIZUTA[1,5], T. ISLAM[1], M. KAWANO[1], T. TAKEKAWA[6], D. GOMEZ-DOMINGUEZ[7], H. YAMAKAWA[1,8], M. OHKURA[3,4], T. FUKAI[1], J. NAKAI[3,4], Y. HAYASHI[1,4,5,9]
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**Abstract:** Pyramidal neurons in the hippocampus participate in dynamic cellular ensemble codes for space and memory. The hippocampus receives spatial and non-spatial information via distinct pathways and combines these two streams of information to create a “cognitive map”, which supports not only spatial navigation but also episodic memory. In these maps, prominent features of the environment can be represented by increased densities of relevant place cells. However, cellular mechanisms for the establishment and reorganization of these maps remain unclear. We thus longitudinally imaged functional cellular maps in the CA1 deep sublayer of novel Thy1-G-

CaMP7 transgenic mice during training on a virtual linear track. In this task, a visual landmark and reward delivery were associated with two distinct locations in a linear track created in a visual virtual environment. Fractions of running time and place cells increased as training progressed. Initially, maps were highly variable but, with repeated training, they increasingly stabilized to an extent correlated with behavioral performance. Representations of environmental salience (i.e., landmark) and motivational salience (i.e., reward) emerged with experience in different rapid versus delayed time courses. Initial establishment of over-represented maps was dominated by selective stabilization of cells that encode each salient location but not by direct formation from non-place cells or lateral recruitment of cells that encode non-salient locations. By contrast, robust reorganization of pre-established maps by rearrangement of these salient features occurred via cooperation between de novo formation, lateral shifts, and selective stabilization of affected representations. These findings reveal the distinct engagement of multiple forms of cellular dynamics in the establishment and reorganization of hippocampal cognitive maps and provide a mechanism through which experience of salient environmental features form lasting and adaptable memory traces.

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**Poster**

**616. Navigating Through Space: Grid and Place Cells**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 616.16/UU18

**Topic:** H.01. Animal Cognition and Behavior

**Support:** Howard Hughes Medical Institute

**Title:** Context-dependent encoding of sensory cues in the hippocampus during virtual navigation

**Authors: *X. ZHAO**[1], J. C. MAGEE[2]
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**Abstract:** Hippocampal place cells play important roles in spatial navigation by encoding specific locations in the environment. It is thus critical to understand what input features give rise to the spatial selective response in place cells. To this goal, we examined CA1 place fields in head-fixed mice with a virtual reality system, in which sensory stimuli can be strictly controlled

and flexibly manipulated. Whole-cell recordings were made from CA1 pyramidal cells during the animal's virtual navigation in a 1-dimensional circular track. Consistent with our previous findings (Bittner et al. 2015), place fields could form after plateau potentials were induced at specific locations. The visual cue closest to the place field was then duplicated at another location on the track. This manipulation did not significantly alter the original field, and generated a smaller but reliable depolarization at the new location. The reduction in depolarization amplitude may indicate contributions from non-sensory inputs, or integration of multiple sensory cues since the animal was able to see more cues than the closest one. To test these hypotheses, partitions were added between each cue to restrict the animal's vision to only one cue at a certain location. With this configuration, duplication of the place field-associated cue generated a second field with similar amplitude compared to the original one. Together, these observations suggest that place fields are strongly driven by sensory inputs during virtual navigation, and multiple cues could be integrated within a single field. Lastly, we recorded place cells when mice were navigating in two dramatically different mazes with one shared cue. Interestingly, the same cue that triggers a robust field in one maze did not produce significant depolarization in the other, which reveals a strong context dependency of sensory-driven responses in CA1.

**Disclosures: X. Zhao:** None. **J.C. Magee:** None.

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**Topic:** H.01. Animal Cognition and Behavior

**Support:** Kaken-hi (15H05569)

Kaken-hi(15H01417)

**Title:** Aversive learning reorganizes ensemble representations of current and prospective locations by hippocampal neurons

**Authors: *S. OKADA**, H. IGATA, T. SASAKI, Y. IKEGAYA
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**Abstract:** Hippocampal place cells specifically discharge when an animal enters a specific zone, so-called a place field. In addition, firing patterns of hippocampal cells are subject to non-spatial information. For example, place fields have been shown to shift to a newly rewarded location, suggesting that the distribution of space represented by place cells is not homogeneous in a space but specific locations related to survival are more strongly represented. On the other hand, little

is known about how aversive environments are encoded by hippocampal place cells. To address this question, we analyzed spiking patterns of neuronal ensembles in the hippocampal CA1 region of rats performing a T-maze alternation task with an aversive airpuff stimulation incorporated onto a specific zone. To obtain reward, the rats needed to actively accept the aversive stimulation. Addition of aversive stimulation altered firing patterns of individual place cells, including changes in firing rates within their place fields, remapping of the place fields, and appearance of new place fields. Especially, spatial representation of place cell ensembles was strongly biased to the aversive zone, same as the preferred representation of reward locations reported in previous studies. Finally, Bayesian decoding analysis revealed that firing patterns during brief pauses at a choice point over-represented the airpuff zone to be visited in near future. These results show that aversive learning reorganizes spatial maps of hippocampal neuronal ensembles for representing both current and future places.

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**Poster**

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**Title:** A single-cell spiking model for the origin of grid-cell patterns

**Authors: T. D'ALBIS**[1], *R. KEMPTER[2]
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**Abstract:** Grid cells are neurons of the medial entorhinal cortex that have multiple spatial firing fields arranged in a strikingly-regular hexagonal pattern (Hafting et al., 2005). Since their discovery, grid cells have been studied extensively, as they are believed to support high-level cognitive tasks such as self-location, memory, and navigation (Rowland et al., 2016). Nevertheless, to date, the origin of grid-cell activity remains unclear.
Treves and coworkers (2008 and 2012) proposed that grid patterns could emerge from a competition between persistent excitation by spatially-selective inputs and the reluctance of a

neuron to fire due to spike-rate adaptation. Their model provided an important proof-of-principle but was formulated at a rather abstract level. In particular, the model included network-level interactions and was limited to rate-based neurons, which made a link to experimental data difficult.
To overcome these issues, we propose here a single-cell spiking model based on similar principles as the work by Kropff and Treves (2008). Our model is, on the one hand, more biologically realistic and, on the other hand, better suited for mathematical treatment. Importantly, we show that grid-like patterns emerge from a single-cell mechanism needless of any network-level interaction. To increase biological plausibility, we consider stochastic-spiking neurons, and we constrain the synaptic weights to positive values. Through rigorous mathematical analysis, we quantitatively predict the requirements for grid-pattern formation, and we establish a direct link to classical pattern-forming systems of the Turing type. Our study lays the groundwork for more biophysically-realistic models of the grid-cell activity.
Hafting, T., Fyhn, M., Molden, S., Moser, M. B., and Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature, 436:801-806.
Rowland, D. C., Roudi, Y., Moser, M. B., and Moser, E. I. (2016). Ten years of grid cells. Annu. Rev. Neurosci., 39:19-40.
Kropff, E. and Treves, A. (2008). The emergence of grid cells: Intelligent design or just adaptation?. Hippocampus, 18:1256-1269.
Si, B., Kropff, E., and Treves, A. (2012). Grid alignment in entorhinal cortex. Biol. Cybern., 106:483-506.

**Disclosures:** **T. D'Albis:** None. **R. Kempter:** None.

**Poster**

**616. Navigating Through Space: Grid and Place Cells**

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NSF Grant SBE-0541957

**Title:** Environmental deformations dynamically shift the cognitive map

**Authors:** ***A. T. KEINATH**[1], R. A. EPSTEIN[1], V. BALASUBRAMANIAN[2]
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**Abstract:** Last year we presented a model that showed how a novel mechanism—boundary-tethered grid shift—could account for diverse effects of environmental deformations on grid and place cells (Keinath et al., SFN 2016). In this model, input from border cells resets the spatial phase of grid cells at environmental boundaries, thus maintaining the learned relationship between grid phase and these boundaries, and transmission of grid input to place cells resets place field locations accordingly. If this model is correct, then distortions observed in the time-averaged activity of grid and place cells during environmental deformations do not arise from rescaling of the grid metric, but rather from discrete `shifts' in grid field locations following boundary contact. Here, we tested this idea empirically. First, we reanalyzed two grid cell deformation datasets (Barry, et al. 2007; Stensola, et al. 2012) and found clear evidence for shifts at boundaries. Indeed, the grid field locations and firing rates in the neurophysiological data closely matched the predictions of our model. Next, motivated by a hypothesized link between grid and place field distortions and human spatial memory during environmental deformations (Hartley, et al. 2004, Chen, et al. 2015), we asked whether we could observe evidence for boundary-tethered shifts in human behavior. We taught participants the locations of objects within a large square virtual reality environment, and then asked them to replace these objects in stretched or compressed versions of this environment. During deformation trials we observed shifts in the replaced locations of objects dependent on the most recently contacted boundary, in agreement with our model predictions. Together, these results provide convergent evidence that environmental deformations do not distort - but rather dynamically shift - the cognitive map, and imply that the internal metric of this map persists unaffected when the environment is reshaped.

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**Topic:** H.01. Animal Cognition and Behavior

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RJS: AI-HS

ELZ: NSERC

ELZ: AI-HS

**Title:** Type of environmental enrichment elicits differential responses in male and female rats

**Authors:** ***E. L. ZELINSKI**[1], M. FIDA[2], B. YOUNG, T1J2J2[2], S. LACOURSIERE[3], R. J. SUTHERLAND[4]

[1]Neurosci., Canadian Ctr. For Behavioural Neurosci., Lethbridge, AB, Canada; [3]Neurosci., [2]CCBN, Lethbridge, AB, Canada; [4]Univ. Lethbridge, Lethbridge Alberta, AB, Canada

**Abstract:** Adult hippocampal neurogenesis is an important physiological process that is not particularly well understood. A reduction in neurogenesis has been linked to deficits in learning and memory performance as well as changes in mood and affect. Likewise, increased neurogenesis has been tied to improvements in mood, learning, and memory processes. Rates of adult hippocampal neurogenesis can be increased by the administration of drugs (e.g., fluoxetine) or through behavioural enrichment including environmental enrichment. Environmental enrichment can be social, spatial, or object based and most paradigms use a combination of all of these. Here, we tested the hypothesis that the type of environmental enrichment would elicit differential effects in male and female rats given each sexes propensity to attend to different types of environmental cues. Expressly, males attend more readily to geometric information whereas females attend to landmarks. Each day for one month, same sex cage mates were placed into an enrichment enclosure for ~1 hour and allowed to freely explore. Pairs were assigned to control (i.e., stable enclosure geometry and landmarks), geometric enrichment (i.e., the environmental geometry/shape was changed daily), or landmark enrichment (i.e., objects within the enclosure were manipulated daily). Animals were injected with BRD-U during the final week of training. c-Fos was used to measure hippocampal neurogenesis. Both enriched groups exhibited increased rates of neurogenesis relative to controls, but a double dissociation between response to geometric or landmark enrichment was observed for the sexes in the predicted direction. These findings imply that we should incorporate innate tendencies in spatial orientation strategies in industrial and environmental design.

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**Poster**

**617. Human Perceptual and Spatial Learning**

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**Title:** Neural mechanisms of vibrotactile category learning

**Authors: *P. S. MALONE**[1], S. P. EBERHARDT[2], C. SPROUSE[1], K. WIMMER[3], R. KLEIN[1], K. GLOMB[3], C. SCHOLL[1], E. T. AUER[2], L. BOKERIA[1], J. RONKIN[1], G. DECO[3], X. JIANG[1], L. E. BERNSTEIN[2], M. RIESENHUBER[1]
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**Abstract:** Recent research has provided insight into how the brain assigns meaning to sensory stimuli in the visual and auditory domains. How vibrotactile (VT) stimuli are categorized, however, remains poorly understood. To examine how the brain assigns meaning to VT patterns, we trained 9 participants to categorize VT stimuli. Pulse trains were presented to the right volar forearm. Analogous to our previous work in the visual and auditory systems, we used a morphing algorithm to create a quasi-continuous stimulus space, divided into two categories. The morphing algorithm was applied to 2 VT category prototypes. The Category A prototype comprised a high-pulse-rate stimulus (100pps) near the elbow and a low-pulse-rate stimulus (25 pps) near the wrist, and the Category B prototype was the opposite. Subjects successfully learned to categorize the stimuli, reaching 92.5% accuracy over ~6.5 hours. Following training, subjects completed an fMRI scan in which short blocks of VT stimulus repetitions were presented. At the end of each block, subjects categorized the stimulus. Multivariate pattern analysis (MVPA) was used to decode the neural representations of the stimuli. In a category-selective MVPA, a classifier was trained to discriminate Category A vs B stimuli. In a separate VT feature-selective MVPA, a classifier discriminated between stimuli within the same category. Our analyses revealed a transition from feature selectivity posteriorly in bilateral superior parietal regions to category selectivity anteriorly in left S2, premotor cortex (PMC), and other frontal regions, with overlap of feature and category-selectivity in the left supramarginal gyrus (SMG). The most significant feature-selective regions were in bilateral superior parietal lobule and the left SMG, and the most significant category-selectivity was in the left SMG, S2, and PMC. MVPA classification accuracy in the left PMC significantly correlated with participants' in-scanner performance. Additionally, whole-brain effective connectivity analyses revealed that the PMC exerted top-down influence over the stimulus-selective regions. Our data are broadly consistent with a two-stage model of perceptual categorization from the visual domain, in which feature-selective representations in higher-level sensory cortices interface with category-selective representations in prefrontal cortex. In contrast to the visual domain, however, our preliminary analyses suggest that in the vibrotactile domain, category-level information appears to gradually emerge as stimuli are processed along a posterior-to-anterior hierarchy from parietal to frontal cortices.

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**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.02/UU24

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF 1420600

**Title:** Spatially-based high school course improves spatial abilities and alters brain network functional connectivity

**Authors: *A. WEINBERGER**[1], E. G. PETERSON[1], C. LYNCH[1], R. KOLVOORD[2], D. UTTAL[3], A. GREEN[1]
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**Abstract:** Spatial ability reliably predicts success in STEM fields. While spatial thinking has been examined – and improved – in laboratory-based interventions (Uttal et al., 2013), real-world school curricula do not typically include an explicit focus on spatial thinking (NRC, 2006). The presented study examined the behavioral and neural effects of teaching spatial thinking embedded within a year-long high school course, the GeoSpatial Semester (GSS). Participants were 63 high school students (39 GSS, 24 comparison) who completed three spatially-based tasks (e.g., mental rotation) and a resting-state scan in an MRI scanner before and after the end of the school year. Students in the GSS course showed greater improvement than comparison students on all spatial tasks following the course. To better understand the neural networks underlying these changes in spatial thinking, we applied graph-theoretic analyses to evaluate changes in resting-state and task-based functional connectivity associated with the GSS course. We calculated individual modularity statistics across a range of resolution parameters, and used these scores to predict academic achievement. Preliminary results indicated that, following the GSS course, students showed significantly greater modularity than at Timepoint 1, particularly at smaller resolution parameters (larger communities). Additional analyses were performed to examine whether brain regions that showed high functional connectivity during rest demonstrated a similar pattern of activity during the spatial tasks, as well as whether the efficiency of the neural networks correlated with task performance. Lastly, we examined connectivity strength and flexibility of key regions of the brain that previous research has implicated in spatial thinking. Our findings suggest that improvements in spatial thinking following classroom instruction may be explained in part by changes at the neural level observed both at rest and during spatial tasks.

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**Poster**

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**Topic:** H.02. Human Cognition and Behavior

**Title:** Shifts in effective connectivity across the head direction network reflect changes in imagined facing direction

**Authors: *M. B. PEREZ-LOPEZ**[1], S. AVRAAM[2], M. AVRAAMIDES[3], T. WOLBERS[1]
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**Abstract:** Imagining changes in facing direction is a crucial ability for everyday life, as it allows us to plan an itinerary, orient ourselves in a map or give navigation instructions to somebody else. This sense of direction is supported by head direction cells, which have been extensively studied in rodents and whose signal has been detected non-invasively in humans over key structures for navigation such as retrosplenial cortex (Marchette et al., 2014), thalamus and precuneus (Shine et al.,2016). However, when imagining a facing direction that differs from one's actual facing direction, one needs to overcome a conflict between both signals. At present, it is unknown if and how the head direction system may contribute to this process.
To address this important question, we systematically manipulated head direction disparity (HDD), the angular difference between an actual and an imagined facing orientation, using a perspective-taking paradigm. Specifically, participants (N=23) were asked to imagine a set of facing directions in a virtual environment (VE) while undergoing 3T fMRI scanning. The VE was memorized prior to testing and consisted of an octagonal room with distinctive objects serving as orientation cues. When instructed to assume a facing direction, participants were also asked to point to a second object for behavioral data collection. FMRI data was analyzed with standard general linear models and region of interest (ROI) analyses, using individually defined masks of the precuneus, RSC and the thalamus. In addition, we tested for changes in effective connectivity between regions by conducting generalized psycho-physiological interactions (gPPI) analyses.
Behavioral results revealed that it is easier to imagine facing directions that are cardinally aligned with the actual facing direction, as opposed to orientations that fall in between these cardinal axes. This effect may relate to the intrinsic axes of the body, which would facilitate access to those orientations aligned with it. Beta estimates extracted from ROI analyses confirmed that orientations of greater behavioral difficulty elicit stronger BOLD responses in precuneus, RSC and thalamus. Furthermore, gPPI analyses results, most notably between precuneus and RSC as well as between thalamus and RSC, show a significant increase in

connectivity for these cardinally-aligned facing directions compared to non-aligned ones. Together, these data suggest a novel mechanism by which the human head direction system may support the imagination of facing directions, even in the presence of conflicting visual and body based cues.

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**Topic:** H.02. Human Cognition and Behavior

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PSC CUNY Research Award

**Title:** Knowledge of ordinal position across lists following transitive inference training

**Authors: *T. KAO**[1,2], B. JURNEY[3], C. E. MICHAELCHECK[2], V. P. FERRERA[4], H. TERRACE[3], G. JENSEN[3]
[1]New York City Col. of Technology/CUNY, Brooklyn, NY; [2]Neuroscience, Columbia Univ., [3]Psychology, Columbia Univ., [4]Neurosci. (in Psychiatry), Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY

**Abstract:** Transitive inference (TI) is the ability to infer that, if A>B and B>C, then it follows that A>C. Most animals that have been tested have demonstrated an ability to perform TI. Specifically, they are able to infer the relative positions of items in an ordered list, ABCDE, based only on trial-and-error training of the adjacent pairs (such that A>B, B>C, C>D, and D>E). To demonstrate inference, tests of TI also measure performance for non-adjacent pairs (B vs D) that were not initially trained. However, training on a single list is not sufficient to discover if participants integrate information about absolute position across lists. In our study, 35 human participants (14 males; 21 females) learned five different 5-item lists within a single 1-hour session (520 trials). During training, only adjacent pairs were presented and the correct response was to choose the earlier list item from each pair. Immediately following training, participants were tested with five "derived" lists constructed by taking one item from each of the five training lists. During the testing phase, participants were presented with all ten possible pairs (A>B, A>C, A>D, etc.) for each of these five derived lists. Not only did participants reliably show evidence of transitive inference during the testing phase, but they also responded accurately to each pair on the derived lists, even though all of those pairings were novel. That is

evidence that participants acquired knowledge of each item's ordinal position during training on the original lists. These results point collectively to a representational framework that not only allows transitive inferences with respect to item rank within a list, but comparison of item ranks across multiple lists. Implications for related topics in serial learning are discussed.
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**Topic:** H.02. Human Cognition and Behavior

**Title:** Effects of estradiol levels on hippocampal-dependent spatial memory

**Authors: *S. ASSUDANI PATEL**[1], A. ARNISTA[1], O. OKIFO[1], C. MITZKOVITZ[1], F. KUHNEY[1], K. M. FRICK[2], P. A. NEWHOUSE[3], R. S. ASTUR[1]
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**Abstract:** Estradiol, the primary estrogen hormone, has been found to regulate hippocampal functions including spatial memory. The relationship between estradiol and the hippocampus is sensitive to varying levels of estradiol, either naturally with fluctuations within a menstrual cycle or artificially with the use of oral contraceptives (OCs). Prior research has indicated that lowered levels of estradiol have been associated with an increased risk of dementia and cognitive decline (Shumaker et al., 2003; Yaffe et al., 2007) and an increased risk of anxiety and depression (Newhouse & Albert, 2015). Additionally, studies have shown that women with high estradiol levels outperform women who have low levels on various memory tasks (Graham & Milad, 2013; Hampson & Morley, 2013). Furthermore, OCs deliver a synthetic form of estradiol at low levels across the menstrual cycle (Beltz et al., 2015). It is unclear how this artificial lowering of estradiol affects memory relative to naturally cycling (NC) women. In order to examine the impact of estradiol levels on spatial memory, women at different points of a natural cycle or on an OC were tested on a hippocampal-dependent task. Participants that were on OCs at the time of testing were grouped as an OC group. NC women were broken down into high estradiol (HE) and low estradiol (LE) groups based on estimation of their menstrual cycle. A total of 126 women completed a virtual Morris water task over two consecutive days. This task is known to be sensitive to hippocampal functioning. On day 1, participants are to learn the location of a fixed hidden platform across 12 trials based on cues in the virtual room. The last trial on day 1 is a probe trial in which the platform is removed, without the knowledge of the participant. On day

2, participants start with a probe trial, complete eight hidden platform trials, and then end with one more probe trial. Results indicated no group differences on the overall distance to find the platform across trials on both day 1 and day 2. Furthermore, there were no group differences in the total percent of distance spent in the correct quadrant in probe trial analyses. There was a negative correlation between the length of time taking an OC and the percent of distance in the correct quadrant for the probe trial at the end of day 1. However, this correlation was not significant ($p > .1$). Overall, the findings of this study indicate no obvious differences amongst groups of women with varying levels of estradiol.

**Disclosures: S. Assudani Patel:** None. **A. Arnista:** None. **O. Okifo:** None. **C. Mitzkovitz:** None. **F. Kuhney:** None. **K.M. Frick:** None. **P.A. Newhouse:** None. **R.S. Astur:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.06/UU28

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF Grant BCS-1630296

NIH Grant R01NS076856

**Title:** Frontal midline theta oscillations increase with ambulation in virtual environments, while posterior alpha oscillations increase with rest

**Authors: *M. LIANG**, M. STARRETT, A. D. EKSTROM
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**Abstract:** Hippocampal theta oscillations increase during movement in spatial environments in both humans and rats. Cortical theta oscillations covary with hippocampal movement-related theta in humans, and thus theta activity recorded by scalp electroencephalography (EEG) might also be expected to show modulations as a function of movement. However, to date, the relationship between movement and frontal midline theta remains unclear. Another issue regards whether movement-related theta is distinct from posterior cortical alpha oscillations, which tend to be robust during “off” periods, although their relationship to still periods remains unclear. One issue in past studies is that most wired scalp EEG recording systems do not permit free ambulation and most human navigation studies have been limited to small-scale environments with limited vestibular input. Here, we address these issues by combining a wireless EEG recording system and an immersive virtual reality allowing free ambulation on an omnidirectional treadmill. To address how frontal midline cortical theta oscillations varied as a function of movement, we asked 10 participants to alternate between two behavioral states,

ambulating in VR and standing still. To detect alpha oscillations, a signature of “off” states, we employed two additional behavioral conditions, eyes open and eyes closed. In block 1, subjects alternated between moving and standing still for 30 second intervals, while keeping their eyes open. In block 2, subjects performed the same task as in block 1 but with their eyes closed. In block 3, subjects alternated between eyes open and eyes closed, while ambulating. In block 4, participants alternated between eyes open and closed while remaining still on the treadmill. We predicted that we would see theta power increases in the frontal midline region specifically during movement, regardless of whether their eyes were open or closed. In contrast, we predicted increases in alpha power at posterior sites during eyes closed compared to eyes open, regardless of movement. Preliminary analyses revealed significant delta and theta power increases (log power differences = 1.373, $p = 0.0236$) in frontal-midline region when subjects were moving compared to still with eyes open. In contrast, we found alpha power increases in posterior regions when subjects were still compared to moving (log power differences = 1.058, trend level $p = 0.1948$). Our findings indicate that frontal-midline theta is associated with movement while posterior alpha is associated with standing still. Together, our results provide a novel means to study human navigation related oscillations non-invasively.

**Disclosures:** **M. Liang:** None. **M. Starrett:** None. **A.D. Ekstrom:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

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**Program#/Poster#:** 617.07/UU29

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF: BCS-1630296

NIH: R01NS076856

**Title:** The effect of body-based cues on human neural representations for space during active navigation

**Authors: *D. J. HUFFMAN**, A. D. EKSTROM
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**Abstract:** A central and unanswered question in human spatial navigation regards the impact of vestibular and somatosensory input, termed body-based cues, on our neural codes underlying spatial navigation. Recent advances in virtual reality (VR) technology now permit the construction of visually realistic, immersive virtual environments, thus allowing laboratory experiments to better approximate real-world experiences. However, even VR rendered with head-mounted displays (HMD) lacks the vestibular and somatosensory input that we typically

experience when we ambulate in real-world spaces. Here, we employed a novel omnidirectional treadmill coupled with an HMD to investigate human spatial memory within large-scale, highly immersive virtual environments under varying levels of body-based cues. A recent paper from the lab (Kyle et al. 2015 eLife) demonstrated environment-specific codes within the hippocampus, reminiscent of “place-specific” maps in the rodent hippocampus. We hypothesized that if body-based cues are important for spatial memory in humans, then we should observe behavioral and neural differences in such representations based on the presence (vs. the absence) of these cues during encoding.
In the present experiment, participants completed 2 behavioral sessions. During the first session, participants freely ambulated an immersive virtual city by finding five target locations (stores). Then, participants performed a judgment of relative direction task, with each trial structured as follows: “Imagine you’re standing at Store X, facing Store Y. Please point to Store Z.” Participants performed a total of 4 rounds of navigation interspersed with the direction task. During the second session, participants navigated 3 virtual cities under 3 different levels of body-based cues: 1) “enriched”: translations with movements on the treadmill and head and body rotations with the HMD, 2) “limited”: translations controlled by a joystick and head and body rotations with the HMD, 3) “impoverished”: translations and rotations controlled by a joystick. Preliminary data revealed that participants were able to learn all 3 cities to criterion (subject-specific mean pointing error determined by performance in the first session). Moreover, during a final retrieval phase, the mean pointing error did not significantly differ based on the city or the order in which the cities were learned, suggesting that our training paradigm minimized between-city interference. Subsequent analysis of fMRI data will determine whether environment-specific representations, both within and outside of the hippocampus, vary as a function of body-based input.

**Disclosures: D.J. Huffman:** None. **A.D. Ekstrom:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.08/UU30

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF: BCS-1630296

NIH: R01NS076856

**Title:** Modulatory influences of bottom-up vs. top-down cues on human spatial representations during navigation

**Authors: *C. E. PEACOCK**, A. D. EKSTROM
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**Abstract:** Models of spatial navigation conceptualize it as a multisensory integration process. Consistent with this focus, past studies have suggested a critical role for bottom-up cues like the environmental boundaries ("spatial geometry") and landmarks ("features") in shaping such multisensory spatial representations. However, humans possess a sophisticated ability to employ verbal cues to navigate (Taylor and Tversky, 1992), but how these top-down cues interact with geometry vs. feature cues remains unknown. We developed three experiments to address the roles of verbal cues and sub-vocal verbalization in spatial navigation, each containing two conditions. In the feature condition, participants were instructed to use a colored wall (Exp. 1 and 2) or a mountain range (Exp. 3) to aid their memory of store locations. In the geometry condition, participants were instructed to use the square boundary to learn store locations. Following the cue, participants encoded store locations during navigation of a virtual environment. They then were tested on store locations with a judgment of relative direction (JRD) task, where questions were designed to test spatial memory with respect to the verbal cues and features/geometry from each condition. Participants also completed the Self-Verbalization Questionnaire (SVQ) as an inventory of inner monologue. All experiments showed no significant interaction between verbal cue, alignment, and imagined field of view, suggesting that the verbal cue played no obvious modulatory role in forming spatial representations. A significant interaction between alignment and field of view showed decreased pointing error when the feature was in the imagined heading on misaligned trials. In the absence of featural information, alignment to the environmental axes significantly reduced pointing error and response times. Further, there was a significant correlation between SVQ ratings and pointing error, where lower ratings were associated with increased pointing error. These results suggest that salient bottom-up sensory input provides a dominant influence on spatial representations compared to top-down verbal cues, with a role of inner-verbal monologue.

**Disclosures: C.E. Peacock:** None. **A.D. Ekstrom:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.09/UU31

**Topic:** H.02. Human Cognition and Behavior

**Support:** BMBF-Project 01GQ1511

DFG 2627/8-1

**Title:** Neural dynamics underlying updating of heading changes as revealed by mobile brain-body imaging (MoBI)

**Authors: F. U. HOHLEFELD**[1], M. KLUG[1], L. GEHRKE[3], *K. GRAMANN[2]
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**Abstract:** Spatial orienting utilizes idiothetic information, including the vestibular and proprioceptive senses, to efficiently update spatial representations during physical body rotations. This kind of movement-related information can be integrated with allocentric representations of landmarks to provide an allocentric heading representation as a central component of spatial updating. However, neural dynamics subserving physical body rotations are poorly understood, due to restrictions of existing brain imaging methods that avoid movement-related artifacts in brain activity recordings by requesting the participants to sit or lie, while spatial updating was investigated using 2D visual stimuli.To investigate brain dynamics accompanying physical body rotations in dynamic 3D virtual environments, the present study uses a Mobile brain/Body Imaging (MoBI) approach allowing for simultaneous assessment of high-density wireless EEG and motion capture of various body parts. Twenty participants performed a spatial orientation task, which required physical body rotations following a moving sphere in 3D virtual reality presented through a head mounted display. The same task was also performed without body rotations by using a joystick control, while visual flow was presented in 2D on a standard display. EEG data were decomposed by independent component analysis in order to separate brain activity from movement and other non-brain sources. The data revealed neural activity in diverse cortical areas in the 4-30 Hz frequency band, which co-varied with different movement parameters.The present study demonstrates the unique possibility to extract neural correlates of spatial updating in fully mobile participants, overcoming limitations of previous brain imaging studies. In addition, the MoBI approach contributes significant new insights into the brain dynamics accompanying embodied cognitive processes utilizing idiothetic sensory information, here in the context of spatial orientation. Furthermore, the unique MoBI setup is of indispensable relevance for contributing to our general understanding of neural dynamics realizing full-range body movements and active interaction with the environment in healthy and possibly clinical populations.

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**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.10/UU32

**Topic:** H.02. Human Cognition and Behavior

**Support:** German Research Foundation SFB 874/B1

**Title:** Cortical and subcortical participation in neural processing of passive perception of visuospatial changes

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**Abstract:** In rodents, passive perception of novel configurations of visuospatial stimuli results in robust synaptic plasticity in the hippocampus, in the form of long-term depression (Kemp & Manahan-Vaughan, 2012, Cerebral Cortex, doi: 10.1093/cercor/bhr233). Hippocampal processing of spatial information may be supported by the cerebellum (Rochefort et al., 2011, Science, doi: 10.1126/science.1207403). Here, we explored in humans, hippocampal and cerebellar participation in the processing of passively perceived visuospatial information. First we assessed event-related potentials (ERPs) recorded during passive perception of novel, repeatedly presented, as well as configurationally, or perspectively, changed three-dimensional objects to verify that passive perception occured. We identified parieto-occipital ERP-components that differentiated between spatially reconfigured, familiar, and novel objects. Using single trial estimation and multivariate approaches, based on rapid event-related fMRI, we then conducted searchlight analysis across the brain, to correlate representational dissimilarity matrices (RDMs) derived from selected regions of interest (ROIs). We observed that the representational profile in the hippocampus significantly correlates with that of the cerebellum across novel, familiar, and spatially changed objects. Looking at the RDMs of our ROIs separately, both the hippocampus and the cerebellar vermis lobules I-V exhibited more unique representations for spatially changed objects. By contrast, vermis lobules VI-X displayed this pattern for all items that displayed some form of novelty. These findings suggest that, during passive visuospatial perception, an evaluative process takes place in the cerebellum that dissociates observer-independent configurational, and observer-related novelty. This process can be expected to support pattern separation in the hippocampus that enables processing of visuospatial change.

**Disclosures: D. Manahan-Vaughan:** None. **M.F. Hauser:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

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**Topic:** H.02. Human Cognition and Behavior

**Support:** The I-CORE program of the Planning and Budgeting Committee and The ISF (grant 51/11).

**Title:** Reactivation of a motor memory modulates perceptual learning

**Authors: *S. KLORFELD**, N. CENSOR
Sch. of Psychological Sciences, Sagol Sch. of Neurosci., Tel Aviv Univ., Tel Aviv-Yafo, Israel

**Abstract:** Motor and perceptual memories are acquired and consolidated with repeated practice, often resulting in between-session performance improvements referred to as offline learning gains. In parallel, extensive studies in animal models and recently in humans, have suggested that brief reactivation of consolidated memories may modulate learning. In order to test whether this mechanism operate across domains, the goal of the current study was to examine whether reactivation of a consolidated motor memory prior to a visual learning task, will generate higher offline gains than expected by visual practice per se. We investigated this notion in the context of the link between the oculomotor saccade system and efficient visual perception. An experimental design combining a saccade motor learning task with a typical visual texture discrimination task (TDT, Karni and Sagi, 1991) was used, targeting the same retinotopic location in each domain. Participants first learned to execute a saccade towards a location that was 45 degrees clockwise, at a distance of 5 degrees of visual angle from a fixation point which appeared on random locations on the screen. The movement execution was cued by the disappearance of the fixation, abolishing any visual information that could influence performance. Gaze position was recorded and online feedback was provided for movement accuracy, which was the end-point measure for the motor task. On the subsequent day, the motor memory was reactivated with 10 trials of the saccade task. Afterwards, subjects learned the visual TDT. In this task, subjects reported whether a target array of 3 diagonal bars was set horizontally or vertically, with the center of the target array appearing at the same location of the saccade task, relative to a central fixation. The target-to-mask asynchrony (SOA) was randomly changed within the session, to obtain a psychometric curve, from which the SOA discrimination threshold was derived. On the following day, subjects performed the TDT session followed by the saccade task. Preliminary results show that subjects improved in both tasks. Strikingly, the between-day offline gains in perceptual thresholds were enhanced, compared to learning in previous reports of visual practice per se. Furthermore, a positive correlation was found between the motor and perceptual improvements. Taken together, these results may indicate an interaction between different learning domains due to synchronized reactivation and consolidation processes.

**Disclosures:** **S. Klorfeld:** None. **N. Censor:** None.

**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

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**Topic:** H.02. Human Cognition and Behavior

**Support:** R01MH069456

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Robert J. Glushko and Pamela Samuelson Foundation

**Title:** Consequences of visual production training on object representations

**Authors: *J. E. FAN**[1,2,3], D. YAMINS[1], K. NORMAN[2,3], N. B. TURK-BROWNE[2,3,4]
[1]Psychology, Stanford Univ., Stanford, CA; [2]Psychology, [3]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [4]Psychology, Yale Univ., New Haven, CT

**Abstract:** In the study of language, production has long been viewed as essential for understanding comprehension. By contrast, studies of vision have centered almost exclusively on comprehension (recognition) and have largely neglected production. Here we investigate visual production in its most basic form — drawing. We test the hypothesis that alternating between drawing two objects differentiates their representations, thereby making them more perceptually discriminable. This hypothesis is motivated by our previous findings that: (1) a deep neural network model of the ventral visual stream trained purely on photographs also recognized drawings, reflecting a common feature representation of objects across production and recognition; (2) training people to draw improved the model's ability to recognize their drawings, resulting from reduced feature overlap in the representations of different objects; and (3) this training led to greater categorical perception for trained objects, as measured by a separate perceptual discrimination task. The current study directly evaluates how practice with drawing objects affects their underlying representation in ventral temporal cortex using fMRI. All three phases (pre, training, post) of the study were scanned. During training, participants alternately drew two objects (e.g., table, bed) on an MR-safe tablet. Before and after training, they viewed these and two other control objects (e.g., chair, bench), so that we could obtain estimates of the neural representation of each object. We defined neural representations as the patterns of parameter estimates over voxels from a GLM that averages activity across all trials for each object. Neural patterns were extracted from an anatomical mask of inferior temporal (IT) cortex in the pre and post phases. Additional exploratory analyses will use a searchlight approach (e.g., to discover effects in dorsal stream, motor, and medial temporal lobe regions). We predicted that IT pattern similarity between objects in the trained pair would decrease from the first to third phases, relative to control objects. Our preliminary data are consistent with this

prediction. We also plan to analyze the relationship between the recognizability of drawings produced during training, as measured by the neural network model and separate human observers, and the hypothesized neural changes. Taken together, this work seeks to bridge computational modeling, functional neuroimaging, and psychophysical approaches to understand how perception and action are coordinated in the brain to support complex natural behaviors, including learning and visual communication.

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**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.13/UU35

**Topic:** H.02. Human Cognition and Behavior

**Support:** ERC 310809

**Title:** Navigating through the air - extending spatial memory and neural representation from two to three dimentions

**Authors: *S. MAIDENBAUM**, M. RABINOVITS, A. AMEDI
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**Abstract:** We live in a three dimensional world, yet research into the navigation and spatial memory and representation has focused to date mainly on two dimensional motion. The few human results exploring full 3D are conflicted, with several previous behavioral results suggesting that humans are affected by a series of biases when encoding vertical vs. horizontal location while others suggest that humans might use a 3D isometric representation of space. This is further confounded by a common focus on the vertical relation between 2d platforms (such as floors in a building), comparisons of still images and passive movies instead of full dynamic 3D motion, and on tasks far from the standardized norm in 2D.
Here, we extend into 3D the classical water maze spatial memory paradigm via a flight simulator approach, allowing participants full dynamic 3D motion through the environment. We explore these tasks both behaviorally (n=30) and while subjects undergo fMRI (n=7). Both groups performed the tasks both in 2D and in 3D versions enabling the extraction of the effect of the 3rd dimension.
We find that while subjects subjectively report increased difficulty with the vertical axis compared to the others, their actual behavioral scores demonstrate that performance on all three axis are equivalent though all worse than in 2D. Furthermore, we find that the basic nodes of the brain's navigation and scene-selective network are preserved with the addition of the vertical

dimension, but that there are clear effects within these nodes (with special emphasis on OPA). We further explore the effect on more complex neural spatial signals, finding their preservation as well.
Taken together, these results suggest that on the subconscious level the neural representation of all 3 dimensions are isometric, despite the additional conscious difficulty of the task.

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**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 617.14/UU36

**Topic:** H.02. Human Cognition and Behavior

**Title:** Female superiority for egocentric spatial navigation; male superiority for allocentric navigation

**Authors: *M. FIDA**[1], S. H. DEIBEL[1], E. L. ZELINSKI[2], R. J. SUTHERLAND, t1k1l8[1]
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**Abstract:** The Morris water task (MWT) is a test of spatial learning and memory commonly used in research on spatial cognition in nonhuman animals. From these studies it is clear that the hippocampus is necessary for accurate navigation and that males and females sometimes perform differently. Most often, a virtual MWT is used with humans. In our lab, we therefore designed a real-world version of the MWT and found that males, by default, use an allocentric strategy, but females by default use an egocentric strategy to navigate. Based on these findings, we designed allocentric and egocentric tabletop versions of the MWT to study use of these strategies. In the first experiment, 60 subjects (30 women) ages 18-25, were asked to perform an allocentric spatial task in which the hidden goal had a fixed location relative to stable room cues. Male performance was significantly better than females. In the second experiment, 60 subjects (28 women) ages 18-25 were tested in a egocentric spatial task in which the hidden goal was in a fixed location relative to body position at the start of a trial. Females outperformed males. A third experiment allowe either strategy and resulted in no sex differences. Together, these results suggest that sex differences in spatial navigation result from different prepotent spatial frameworks guiding performance.

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**Poster**

**617. Human Perceptual and Spatial Learning**

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**Program#/Poster#:** 617.15/UU37

**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF Grant 1420600

**Title:** Structural plasticity in parietal cortex associated with real-world classroom education

**Authors: *E. G. PETERSON**[1,2], P. MENDEZ[1], B. SWEETSER[1], N. DINH[1], R. KOLVOORD[2], D. UTTAL[3], A. GREEN[1]
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**Abstract:** Spatial thinking—the visualization and mental manipulation of visuospatial information—has been identified as a key component of success in STEM fields. There is increasing evidence that spatial thinking is malleable and laboratory-based spatial training has identified structural changes in grey and white matter in regions commonly associated with spatial thinking (e.g., posterior parietal cortex). However, training has been limited to laboratory-based tasks, and the extent to which real-world educational experiences support neural and cognitive changes in spatial thinking has been unexamined. Therefore, the present study investigated whether learning-based structural plasticity observed in laboratory training studies extends to classroom instruction in high school sciences classes. Participants were high school students (N=35) enrolled in a science course designed to teach spatial thinking (Geospatial Semester, N=17) or alternative science electives (N=16). Participants completed measures of spatial thinking and a structural MRI (MPRAGE) before and after a year-long science course. Gray matter changes in thickness, volume, and curvature were calculated using Freesurfer for 36 cortical and 10 subcortical ROIs per hemisphere. Relative to the comparison group, students enrolled in the GSS course had greater increases in cortical thickness (left inferior parietal cortex, left postcentral gyrus, right precuneus) and maintained cortical thickness (right inferior parietal cortext, right superior parietal cortex) in regions commonly associated with spatial thinking. These patterns were accompanied by improvements in multiple measures of spatial thinking (e.g., mental rotation, embedded figures). Findings suggest that training spatial thinking within real-world classrooms promotes structural plasticity in adolescents.

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**Poster**

**617. Human Perceptual and Spatial Learning**

**Location:** Halls A-C

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF EEC-1028725

NINDS R-01-NS065186

NIMH K-01-MH086118

**Title:** Persistent changes in resting state connectivity following skill learning

**Authors: *K. CASIMO**, J. WU, J. G. OJEMANN, K. E. WEAVER
Univ. of Washington, Seattle, WA

**Abstract:** Prior electroencephalography (EEG) or functional MRI studies have investigated changes in brain functional connectivity following skill learning in humans. These studies highlight that connectivity is not a static process, but rather changes cross diverse brain regions after a person acquires a new skill. Further changes in connectivity have been observed in resting state periods following the task, which are not identical to those linked to the execution of the task itself. These prior studies have established that changes in connectivity are spread across a large area of cortex, but are limited by either indirect measurements of brain activity via blood flow in fMRI, or by spatial and frequency resolution in noninvasive electrophysiology such as EEG.

We used electrocorticography (ECoG) collected from epileptic individuals undergoing seizure monitoring. We collected six-minute resting state sessions before and after training on a task, which required participants to navigate a three-dimensional virtual environment with the controller orientation rotated 45, 90, or 180 degrees relative to their position. We evaluated change in neural connectivity from before to after learning of the task, and compared these changes to spontaneous levels of variation that naturally occur in the resting state. Pre- to post-task connectivity changes were assessed in resting state sessions with a range of computational interaction measures, including linear regression, phase locking value, and coherence. We performed these evaluations across all brain regions with sufficient data, as identified and grouped by Brodmann area with a standard atlas.

We found connectivity changes over a wide range of cortex, including but not limited to motor regions. Changes in connectivity linked to learning occurred both within single brain regions and between spatially distant regions. These changes appeared across multiple canonical frequency bands from delta (1-4Hz) through high gamma (70-200Hz). The observed changes were similar but not identical across connectivity measures.

Our findings extend prior work evaluating changes in brain connectivity following skill acquisition into ECoG. We estimated changes in connectivity associated with skill learning go beyond natural levels of variation. We provide a unique combination of fine resolution of the high frequency end of the spectrum and spatial clarity relative to previous approaches. We specifically identify changes in higher frequencies not easily accessible in scalp measurements. Future efforts will examine performance differences in the degree of change following task training relative to skill level attained.

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**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** H.02. Human Cognition and Behavior

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NIH Grant T32 EY007136

**Title:** A dynamic normalization model of temporal attention

**Authors: *R. N. DENISON**, M. CARRASCO, D. J. HEEGER
Dept. of Psychology and Ctr. for Neural Sci., New York Univ., New York, NY

**Abstract: Purpose:** Vision is a dynamic sense that handles a continuously changing stream of input. Although attention is critical for prioritizing the most relevant visual input, most models of visual attention are static. Here, we develop and test a dynamic model of visual attention. **Methods:** We generalized an existing static model of visual attention[1] to the time domain. The model consisted of (1) orientation-tuned channels representing the sensory information, (2) two attention layers controlling the gain of voluntary and involuntary attention, which modulated the gain of sensory responses via feedback, and (3) a decision layer that received input from the sensory layer and represented the model's choice in an orientation discrimination task. Voluntary attention responses were task-related and depended only on the attentional cue. Involuntary attention responses were stimulus-driven and depended only on input from the sensory layer. Sensory neural populations could vary in temporal dynamics, having more sustained or more transient responses. Responses in each layer updated at every time step according to differential equations implementing attentional modulation and normalization. We fit the model to psychophysical data from experiments with human observers in which we manipulated temporal attention - the prioritization of visual information at specific points in time - to a sequence of two

visual grating stimuli with independent orientations. Observers were precued to attend to the first, the second, or both stimuli and postcued to report the orientation of one of the stimuli. The stimuli were separated by a time interval that was fixed, and hence predictable, within a session, but varied across sessions (10 intervals, 100-800 ms). We sought to explain task performance as a function of attention condition, inter-stimulus interval, and stimulus orientation.
**Results and conclusions:** A combination of voluntary attentional gain enhancement and involuntary attentional gain suppression explained the psychophysical data. Voluntary gain enhancement took the form of a limited resource over short time intervals, which was renewed as time passed. This generalizes the idea of limited attentional resources across space at a single moment in time to across time at a single location in space. Including separate neural populations with more transient and more sustained responses reproduced patterns of behavior that depended on the sequence of stimulus orientations. The model makes testable predictions about attentional gain dynamics.
1. Reynolds, J. H., & Heeger, D. J. (2009). The normalization model of attention. *Neuron, 61*(2), 168-185.

**Disclosures: R.N. Denison:** None. **M. Carrasco:** None. **D.J. Heeger:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.02/UU40

**Topic:** H.02. Human Cognition and Behavior

**Title:** Is attention enhanced following performance errors? A test of adaptive control

**Authors: *R. COMPTON**, E. C. HEATON, A. GAINES
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**Abstract:** This study tested whether people adaptively sharpen their attentional focus following performance mistakes, as predicted by current theories of cognitive control. Participants ($n$ = 30) completed a Stroop task in which target stimuli were preceded by an informative spatial cue. Adaptive control theory predicts that following errors, participants should more effectively utilize the spatial cue. Cue validity effects on performance were robust, as were Stroop interference effects, but neither effect was altered by commission of an error on the prior trial ($F$s < 1), counter to predictions of an adaptive control model. Likewise, while predictive spatial cues led to left-right asymmetries in EEG alpha power during the cue-target interval, as expected, and cues also produced validity effects on ERP responses to targets, as expected, neither of these effects was modulated by error commission on the previous trial. Instead, errors were followed by generalized arousal, as measured by decreased EEG alpha power (following errors versus correct responses) during both the response-cue interval and the cue-target interval on the next

trial. Errors were also followed by poorer overall performance in both accuracy and reaction time on the next trial. Results support an alternative theory that post-error changes in neural activity and performance reflect arousal, orienting, or cognitive bottlenecking rather than adaptive control of attention.

**Disclosures: R. Compton:** None. **E.C. Heaton:** None. **A. Gaines:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.03/UU41

**Topic:** H.02. Human Cognition and Behavior

**Title:** Event-related brain potentials to focused and divided attention task

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**Abstract: Introduction**: Focused attention allow to allocate a greater amount of resources for the relevant stimuli, facilitating their processing. On the other hand, dividing attention has a cost. There is evidence that in divided attention tasks reaction time is higher and the percentage of correct responses is lower than in focused attention tasks. It has been proposed that when visual stimuli compete for attention with stimuli of other sensorial modalities, more processing resources are allocated to visual stimuli, however, it remains to be determined whether this preference is associated with perceptual, attentional, or response selection processes. In this work we set out to analyze changes in reaction time and ERPs associated with focused and divided selective attention.
**Method**: ERPs were recorded in five derivations (Fz, Cz, Pz, O1 and O2) under three conditions: a focused visual attention task, a focused auditory attention task and a divided attention task to both modalities. The stimuli were presented for 100 msec with a variable interstimulus interval of 400-600 msec. A two-way repeated-measures ANOVA (stimuli x task) were used to analyze the amplitude of the P1, N1 and P2 components associated with visual stimuli, and the N1 and P2 components produced by the auditory stimuli.
**Results:** In the visual modality, a significant main effect in the reaction time was found ($t(14)=4.11$; $p<0.002$. The reaction time was greater in the divided attention task than in the focused attention task. On the other hand, the reaction time recorded in the auditory attention task showed no differences between conditions. The N1 and P2 components of the visual ERPs showed greater amplitude by the attended stimuli than the no attended ones, but there were no differences between focused and divided attention tasks. In the auditory detection task the P2 component showed a greater amplitude in the divided attention task but there were no differences between attended and ignored stimuli.

**Conclusions**: Visual ERPs showed an effect of attention. This effect was similar between the conditions of focused and divided attention. This effect may be due to the fact that the visual stimuli have a competitive advantage over the stimuli of other sensorial modalities, so that the visual processing is maintained in a similar way when the attention is divided between the visual and the auditory modality.

**Disclosures: S. Meneses-Ortega:** None. **S. Meneses-Ortega:** None. **J. Pérez-Bouquet:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.04/UU42

**Topic:** H.02. Human Cognition and Behavior

**Title:** Attentional capture by contextual violation is modulated by reliability

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**Abstract:** We perceive regularities about how objects are arranged in space. This spatial regularity and the inter-object relationship forms the context. Chun (1998) reported facilitation in finding a target when the spatial configuration of distractor and target was learned. This improvement in search times for a target in familiar configuration is called contextual cueing. Evidence for attentional mechanisms that underlie contextual cueing is inconclusive. To investigate the role of familiar configuration in visual processing, we made the participants learn a given spatial configuration and introduced violations to the learned configuration. This contextual violation is achieved by relocating an item from the learned configuration to a new random location on one-fourth of the trials. We further manipulated the reliability of the relocation by changing probability of the relocated item being a target. Experiment 1 (A & B) tested if there is any change in performance when there is a relocation from the familiar configuration. The result revealed separable effects of reliability on the relocated item for the two levels of reliability on performance. Trials were chronologically divided into epochs. The epoch-analysis showed that when the reliability was high the processing of the relocation item improved over the epochs and the performance was best at the final epoch. In the low reliability condition, the relocation was prioritized in the initial epochs but the performance decreased over the epochs and was worst at the final epoch. To further investigate if there is an attentional prioritization for the relocation, in experiment 2 (A & B), we manipulated the display size and looked for slope effects. The slope values were modulated by the reliability of the relocation and the result confirmed the pattern found in reaction times. The slope values for the relocated item increased over epochs when reliability was low and decreased over epochs when reliability was

high. This result shows how attention is modulated by reliability. The present study shows that when there is a violation of familiar configuration, attention is captured by the relocation and this capture is transient and is modulated by the reliability of relocation.

**Disclosures:** **N. George:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Gandhinagar. **M.M. Sunny:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Gandhinagar.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.05/UU43

**Topic:** H.02. Human Cognition and Behavior

**Support:** FONDECYT postdoctorado 3160403

**Title:** Cold blooded attention: Finger temperature predicts performance in attentional tasks

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**Abstract:** Thermal stress has been shown to increase the chances of unsafe behavior during industrial and driving performance due to a reduction in attentional resources. Nonetheless, a major problem has been to define appropriate safety standards regarding environmental temperature, as modulations in performance would also be affected by task kind, complexity, workload, duration, and previous experience with the task. To bypass this attentional and thermoregulatory problem, we focused on the body rather than environmental temperature. Specifically, we measured tympanic, forehead, finger and environmental temperature accompanied by a battery of attentional tasks and EEG recordings under constant environmental temperature for each participant (environment ranging between 19-25°C). Particularly, we give a 10 minutes' baseline were subjects were instructed to sit and relax, followed by three attentional tasks; a continuous performance task, a flanker task, and a counting task. Using multiple linear regression models we checked which variable(s) were the best predictors of performance. We used EEG spectral tonic activity to compare body temperature measurements with known attentional arousal markers (Alpha and Beta spectral bands). Results showed a drop in finger temperature due to instruction and task engagement which was absent when the subject was instructed to relax. No change was observed in tympanic nor forehead temperatures, while the environmental temperature was kept almost constant for each participant. Particularly, the magnitude of the change in finger temperature was the best predictor of performance in all three attentional tasks. The drop in Finger temperature is concordant with Alpha band power reduction

and Beta band power increment, suggesting an increase in arousal. Our results suggest that finger temperature can be used as a predictor of alertness, predicting better than the environmental temperature the performance in attentional tasks. These findings support strongly that peripheral temperature change can be used to prevent unsafe behaviors and accidents.

**Disclosures: R.C. Vergara:** None. **C. Moenne:** None. **P. Maldonado:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.06/UU44

**Topic:** H.02. Human Cognition and Behavior

**Title:** Discussions of brain activity and eye movement during driving

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**Abstract:** [Background and Objective]90% of traffic accidents are caused by human errors.Human errors are existed by errors in recognition, judgment, and operation in driving.Therefore, it is necessary to develop a driving support system that assists driver recognition, judgment, and operation.In the future driving support system, the quality of driving is improved by using the biological information of the driver.In recent research, acquisition of brain function information using fNIRS, EEG and the like has been carried out.In this study, brain activity and eye movements by fNIRS during driving are measured, and their correlation is examined.
[Methods]In the experiment, driving movie was presented to four subjects.The state of driving by a third person was recorded with driver's viewpoint, and the movie was presented to the examinees.During Rest, plus mark was presented for 30 seconds.During Task, a total of four types of moving images (turn left, two types of straight, turn right) were presented for 30 seconds.Brain activity was measured with the fNIRS device (LABNIRS).Measurement points are the forehead 22 ch and the back head 22 ch.Eye movements were measured with an eye tracker (Tobii X2 - 60).Also, to confirm the driving history, a questionnaire on the latest driving day, driving frequency, and licensing acquisition years questionnaire was conducted.The rest of the cerebral blood flow change model and the integrated value at the time of the task were obtained.Also, gaze point, gaze time and saccade time were calculated.
[Results and Discussion]Subjects were divided into two groups in two-dimensional space of gaze time and saccade time.As a result, it was classified into a group with a lot of gazes and a group with many saccades.The gaze distribution during the left turn of a group with a lot of gazes was concentrated in one central point of the screen. Furthermore, there was a tendency that gaze was

more frequent in groups with more gaze than groups with many saccades. It is said that gaze will gather on one point while driving idly.There is no significant difference from the brain function data, and future study is necessary.As a tendency, in the group in which attention was frequently observed, there were many channels with small integrated values at the time of left turn.Therefore, it is described that the subjects in the group with a high degree of gaze are in a state of seeing the front but in a state in which the brain is not active. In other words, it is conceivable that the subject can't concentrate on the driving.
[Conclusions]Subjects who had less Saccade while driving and focused on one point were observed.In that case, there is a possibility that the concentration status is lacking.

**Disclosures:** **S. Nakamura:** None. **S. Hiwa:** None. **T. Hiroyasu:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.07/UU45

**Topic:** H.02. Human Cognition and Behavior

**Support:** Sidney R. Baer Jr. Research Foundation

**Title:** Correlating state-based functional connectivity with behavioral performance

**Authors: *S. LAGANIERE**[1], W. CHEONG[2], M. ESTERMAN[3], M. A. HALKO[4]
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**Abstract: Background** Several studies have used resting state functional connectivity to predict specific behavior during task, eg. reaction time variability. In these studies, the correlation between resting-state fMRI connectivity and behavior relies on the assumption that rest is a relatively homogeneous state and that task-induced changes in network dynamics are largely similar across individuals. However, resting-state acquisitions may not represent a true *task-free state*. Group-level studies have suggested that task-based functional connectivity may capture relevant changes in network dynamics thereby providing additional insight into the link between network connectivity and behavior. We hypothesized that individuals would exhibit different network connectivity -both within and between networks- at rest vs. task, that task-based connectivity measures between these networks would better predict individual performance, and that individual changes in network connectivity - during the transition from rest to task- would predict performance. **Methods** FMRI was acquired on normal healthy subjects in two different states: a 6-min rest-state and a 10-min task-state during which subjects performed a continuous performance task (gradCPT) for the entire sequence. Commission errors, omission errors,

reaction times, reaction time variability and D' were assessed during the task. Subject-level mean functional connectivity within and between 7 canonical networks -at rest and during task- as well as differences in network connectivity between the two states were computed. Connectivity measures were correlated to behavioral metrics. **Results** Preliminary data in 12 subjects suggests that attentional performance - as measured by D' on the gradCPT- was weakly correlated with connectivity *within* networks both during task and at rest. However, performance was more strongly inversely correlated with connectivity *between* the dorsal attention network (DAN) and the default network (DN). Interestingly, the correlation of the between-network connectivity and performance was stronger in the task-state (r=-0.72) than the rest-state (r=-0.49). The change in connectivity between DN/DAN when transitioning from rest to task at the single subject level also inversely correlated with performance (r=-0.61). **Conclusion** Performance on a sustained attention task is significantly correlated to specific *between* network measures of connectivity. This correlation strengthens during task performance. In addition, increased anti-correlation between DMN and DAN- when transitioning from rest-state to task-state- also predicts improved performance on this task.

**Disclosures: S. Laganiere:** None. **W. Cheong:** None. **M. Esterman:** None. **M.A. Halko:** None.

**Poster**

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**Program#/Poster#:** 618.08/UU46

**Topic:** H.02. Human Cognition and Behavior

**Support:** National Medical Research Council Singapore (NMRC/STaR/0015/2013)

Far East Organization

**Title:** Reward motivation reduces implicit temporal bias in attentional readiness through compensatory control: Behavioral and pupillometric findings

**Authors: *K. SASMITA**, S. A. A. MASSAR, J. LIM, M. W. L. CHEE
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**Abstract: Objective** Maintaining attentional readiness is costly and cannot be sustained over longer periods of time. The implicit temporal structure of an environment can help to bias attention towards moments of greatest target probability. Under a uniform distribution of interstimulus intervals (foreperiod; FP) timing expectations can be formed based on the passage of time within the current trial, and stimulus intervals in preceding trials. Responses tend to be faster after long FPs compared to short FPs (foreperiod effect). The foreperiod effect is

modulated by expectations from preceding trials, being more pronounced when following a trial with long FPn-1 and reduced following a short FPn-1 (sequential effect). Both these effects exert temporal biases on response times.
Reward motivation is well known to improve performance (e.g. faster responses). However, it is unknown how motivation influences the foreperiod and/or sequential effects. In this study we analyzed data from a large sample of participants performing a sustained attention task under different reward conditions, with simultaneous pupillometric recordings.
**Methods & Results** Seventy-three participants performed a sustained attention task in which targets were temporally unpredictable (randomly drawn from a uniform distribution; 2-10sec), once to establish baseline (unrewarded), and once in a rewarded run (10c/fast response). Trials were binned according to current foreperiod (FPn; short: 2-6sec; long: 6-10sec), and preceding foreperiod (FPn-1; short: 2-6sec; long: 6-10sec). Results showed that overall performance (in all FP bins) was better in rewarded runs compared to baseline runs. Moreover, reward reduced temporal bias as evident in smaller foreperiod and sequential effects. Pupillometry revealed that reward increased target-locked pupil dilation, particularly at time bins that are normally characterized by low attentional readiness (short FPn), suggesting a compensatory control mechanism.
**Conclusion** The current findings suggest that reward motivation can improve overall attentional performance, and reduce implicit temporal bias, particularly by deploying compensatory control at moments of incomplete attentional readiness.

**Disclosures:** **K. Sasmita:** None. **S.A.A. Massar:** None. **J. Lim:** None. **M.W.L. Chee:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

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**Program#/Poster#:** 618.09/UU47

**Topic:** H.02. Human Cognition and Behavior

**Support:** Touhara ERATO Chemosensory Signal Project from JST, Japan

**Title:** Attention to a smell and its neural signatures

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**Abstract:** Aims: The capacity to pay attention is a prerequisite for the cognitive ability, for example, evaluating an object for its qualities. However, this role is not clearly established for olfaction. The aim of this study was to examine olfactory attention using electro-encephalography recording (EEG). Specifically, we were interested in exploring how the neural

responses after inhaling the same odor differ between tasks with different attentional requirements and in which areas of the brain.
Methods: Nineteen subjects (10F, mean age=24) participated in the study. The odor of 2-phenylethyl-alcohol (a rose-like smell) was presented to the nose using an olfactometer while the subjects breathed nasally. We estimated changes in the cortical processing of odor in attend and not-attend task using olfactory event related potential (ERP) analysis. We focused on P3 component, which is known to reflect late cognitive processing, and compared its amplitude and latency between attend and not-attend odor trials. We localized the sources of P3 data using standard low resolution brain electromagnetic tomography (sLORETA), and identified the brain regions that showed significantly enhanced neural activity for attend than not-attend trials.
Results: The ERP analysis for attend odor trials revealed significant larger amplitude and smaller latency during in P3 component for attend than not-attend odor trials. This effect was observed in the post-stimulus time interval, 850-1050 ms. The source localization of ERP data for this time interval revealed significantly enhanced neural activation for attend than not-attend trials in inferior orbital frontal region, insula, precuneus, and temporal lobes. The odorless stimuli did not replicate these findings.
Conclusion: The findings from our study aligns with the general idea that attention to a stimulus enhances its neural processing. The sLORETA analysis suggests that the enhanced neural processing during olfactory attention is subserved by a network of brain areas that are associated with specific olfactory and general cognitive functions.

**Disclosures:** **A.K. Singh:** 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.; ERATO Touhara Chemosensory Signal Project from JST, Japan. **M. Okamoto:** A. Employment/Salary (full or part-time):; Touhara ERATO Chemosensory Signal Project from JST, Japan. **K. Touhara:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.10/UU48

**Topic:** H.02. Human Cognition and Behavior

**Support:** National Medical Research Council Singapore (NMRC/STaR/0015/2013)

Far East Organization

**Title:** Increased costs of attentional effort after sleep loss: Evidence from performance, preference and pupillometry

**Authors: *S. A. MASSAR**, J. LIM, K. SASMITA, M. W. L. CHEE
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**Abstract: Objective** Sleep deprivation (SD) has widespread negative effects on cognitive performance in different domains including attention and decision-making. While sustained attention is among the most badly affected domains by sleep loss, most studies have interpreted this as an indication of a reduced capacity to perform. Alternatively, theories of effort-based decision-making propose that performance is subject to a continuous cost-benefit analysis. In the context of sustained attention, the effort associated with task performance can be considered a cost that is weighed against the benefits of performance. We previously found that sleep deprivation alters this effort-based decision process. Here, we examined how performance decrement in sustained attention after sleep deprivation may be explained as an increase in subjective costs.
**Methods & Results** To test the effort-based decision-making model during performance, participants (N=26) performed a sustained attention task (Psychomotor Vigilance Task; PVT) under different incentive conditions (1, 5 or 15 cent for fast responses). Overall, performance improved in higher reward runs, as evident in faster responses, fewer lapses (responses > 500 ms), and less performance decrement with time-on-task. Although performance was poorer during SD for all runs, this SD-effect was more pronounced with lower reward. This suggests a shift in the cost-benefit balance with SD.
To more formally test for this shift, participants performed a discounting task in which they indicated their preference for rewards that were available upon performance of different durations of PVT (1, 5, 10, 20 or 30 min). Results showed that participants discounted reward value based on the proposed duration of the attention task. Importantly, discounting was steeper after SD.
Lastly, pupillometry during the attention task revealed that pupil dynamics were modulated by SD and by reward. After SD, pupil size was smaller and more variable than in the well-rested condition. Incentive motivation increased pupil size and decreased variability, mostly in SD.
**Conclusion** Together, these data demonstrate that attentional performance can be interpreted within a cost-benefit framework. Moreover, findings from both tasks confirm that the allocation of attentional effort in vigilance performance is considered more costly after a night of sleep deprivation, and that reduced capacity does not fully explain the attentional deficits that occur following short-term sleep deprivation.

**Disclosures: S.A. Massar:** None. **J. Lim:** None. **K. Sasmita:** None. **M.W.L. Chee:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.11/UU49

**Topic:** H.02. Human Cognition and Behavior

**Title:** Mindful driving:Brain functional state of mind wandering in driving and PVT task

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**Abstract:** [Introduction] Mind wandering is a state in which attention deviates from the external environment to the inner thought, and many related studies were performed. It is a state opposite to mindfulness and observed in a daily life. Accidents are caused by temporary carelessness when falling into mind wandering at the driving. To prevent accidents, a driving support system that evaluates the driver's attention state is necessary. When driving operations are performed, cognition, processing, and judgment are carried out in the brain. brain activity is widely used as an index for estimating human condition. when elucidating the brain activity during driving, the performance of the driving support system improves. The goal of this study is to examine which part of the brain is related to attention status during driving with information obtained from an electroencephalograph (EEG). For this purpose, judgment index from the brain wave information is expected in the attention state during driving.
[Method] There were four subjects (age:21) in this experiment, and the dual-task was performed. The main task is an automobile driving task of driving highway course of driving simulator for 10 minutes, and the sub task is Psychomotor Vigilance Task (PVT). brain waves were measured using g.USBamp of EEG (g.tec). A caution interval and an inattention interval were defined from reaction time obtained from PVT, and a power spectrum was calculated from brain wave information. These were regarded as feature amounts, and a comparison was operated between attention state and inattention state.
[Result & Discussion] The result of the reaction time shows that when the reaction time is fast, the attention is not paid not to a driving task but PVT directly. the results in the case of a slow reaction time explain that attention is directed to driving tasks. Comparison of the power spectra of the β wave band (14 to 20 Hz) was performed in two states of care and inattention. The results showed a high tendency in the channel of occipital area when attention was given to driving. Previous studies have reported that an increase in the power of the beta wave in the occipital region is associated with visual attention. This is because the visual cortex in the occipital region is responsible for the visual input adjustment function and the beta wave rhythm is synchronized to enhance responsiveness to the visual input. These studies lead to an increase in the power of the β wave in the occipital region during driving requiring visual attention.
[Conclusion] The results of this study suggest that the power spectrum of the β wave in the occipital region is essential as an index for evaluating the attention state during driving.

**Disclosures: Y. Fujiwara:** None. **S. Hiwa:** None. **T. Hiroyasu:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.12/UU50

**Topic:** H.02. Human Cognition and Behavior

**Title:** Reactive shifts of cognitive control under load during continuous performance: An fMRI study

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**Abstract:** During the execution of a complex task, context information that is actively kept in working memory guides behavior in order to proactively achieve desired goals. Since keeping relevant information in working memory can require attentional resources, a more reactive behavior that relies on external stimuli can be alternatively engaged when processing demands are high. Previous work has shown that proactive and reactive mechanisms of control can be flexibly selected according to task demands. Individual factors may contribute to the priority of one mechanism over the other (for instance, age) but the neural mechanism underlying this preference remains obscure. One possible way of studying why some people tend to behave proactively and some reactively in a given task is to induce a reactive shift in healthy, generally proactive populations. The AX-Continuous Performance Task (AX-CPT) has previously been widely used to study proactive vs. reactive cognitive control. The task consists on the identification of a specific cue-probe pair of letters (so called valid or target pair). During the delay period the cue information (context) should be maintained, and previous studies using physiological and neurophysiological measures suggest different patterns of neural processing for the different cue types (valid cue vs. non-valid cue). In the present work, we developed a novel variant of the task by increasing the load in the cue period. The load manipulation successfully induced a more reactive pattern of behavior in healthy subjects (n = 31). While subjects performed the task, we measured brain activity with functional magnetic resonance imaging (fMRI) and found distinct patterns of activity in the right dorsolateral prefrontal cortex (DLPFC) related to the proactive and reactive modes. The results are consistent with and extend previous fMRI studies. We propose that when task demands are high (global context), the use of context within the task (local context) may be hindered due to excessive processing demands. Thus, reactive processing in cognitive control may be driven by limitations in processing capacity or attentional effort, as indexed by reduced context-processing and hypoactivation of the right DLPFC.

**Disclosures: V. Exposito:** None. **T. Hagen:** None. **T. Espeseth:** None.

**Poster**

**618. Functional Mechanisms of Attention**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.13/UU51

**Topic:** H.02. Human Cognition and Behavior

**Title:** Neural correlates of spatial attention deployment

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**Abstract:** Visual attention operates by rapidly selecting information in series of discrete episodes (Wyble & Bowman, 2009). Previous ERP investigations of visual attention have identified the N2pc component as reflecting processes related to the selection of a lateralized target (Eimer, 1996). Recent work has found support for the hypothesis that the component is reflective of localization of target information in visual space (Kiss et al., 2008; Tan & Wyble, 2015). This hypothesis specifically proposes that processes responsible for the selection of space, rather than of target identity, elicits the N2pc. The current work seeks to further explore this hypothesis by using cue stimuli designed to initiate localization processes in absence of processes linked directly to target identity information. Two experiments employed the use of spatially informative cues prior to target onset, and examined the impact of these cues on the elicited N2pc. Experiment 1 demonstrated that laterally presented cues elicited an N2pc and targets following cues did not. Experiment 2 used a central cue to investigate top-down shifting mechanisms and their relation to localization processes. Preliminary Data reveal that while a peripherally presented target designed to trigger localization elicits an N2pc, a centrally presented cue designed to signal a shift in attention from fixation does not. These results suggest a dissociation of the attentional processes involved in automatic localization of visual target information, and top-down shifting of attention to target information.

**Disclosures: A. Drew:** None. **E. Heiny:** None. **A.T. Karst:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.14/UU52

**Topic:** H.02. Human Cognition and Behavior

**Support:** ISF Center of Excellence #51/11

BSF grant 2015385

CIG grant 631265

**Title:** Depth of language processing for unattended speech

**Authors: P. HAR SHAI**, *E. M. ZION GOLUMBIC
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**Abstract:** Speech processing requires analysis of auditory input at multiple acoustic and linguistic levels, and a long-standing research question is which stages of language processing require attention. As suggested by the infamous "Cocktail Party" effect (Cherry 1953), as well as the "Irrelevant Sound Effect" (Beaman et al. 2007), at least some unattended content seems to be fully analyzed and can affect behavior. Nonetheless, individuals are typically unable to recall the content of unattended speech.

Here we studied the depth of processing applied to unattended speech, using a novel experimental approach, exploiting the time-scales within speech to systematically probe different levels of linguistic processing (Ding et.al, 2015). Speech materials consisting of isochronously presented syllables (at 4Hz) were structured such that every two syllables created a Hebrew word, two words constructed a phrase and two phrases constituted a sentence. These stimuli (referred to as "hierarchical isochronous speech"; HIS) allow differentiating neural responses to distinct linguistic levels - words, sentences and phrases – as they are uniquely associated with specific frequencies.

We present data from two "Cocktail Party" style experiments in which HIS was presented concurrently with natural speech (dichotically), and was to be either attended or ignored. Using EEG measurements of neural activity, we tested which linguistic levels are represented in the neural response to HIS speech as a function of attention, while varying the attended task. We found that when individuals engaged in a speech-comprehension task, unattended HIS stimuli could nonetheless be analyzed at multiple linguistic levels, without impairing performance on the attended task. In contrast, when the attended task consisted of more demanding target-detection, unattended HIS was only analyzed at an acoustic level and no evidence could be found for higher linguistic processing. Taken together, these results invite nuance when studying the processing applied to unattended speech. Specifically, they suggest that the depth of processing for unattended speech can vary as a function of task-demands applied to attended speech. While in some cases analysis of unattended speech is limited only to the sensory level, in other cases it can undergo full linguistic processing emphasizing the brains' capability, in principle, to process two streams of speech simultaneously. This study paves the way for future research aimed at characterizing the factors affecting the depth of processing of unattended speech and exploring the capacity and limitations of the brain for parallel processing of speech.

**Disclosures: P. Har Shai:** None. **E.M. Zion Golumbic:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.15/UU53

**Topic:** H.02. Human Cognition and Behavior

**Title:** Anxiety toward fear-relevant stimuli modulates perceptual switch in continuous flash suppression

**Authors: *T. CHIBA**[1,2], K. IDE[3,2], H. MORIYA[4], H. TODA[5], T. YAMAMOTO[1], T. YAMAMOTO[1], M. KAWATO[4]
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**Abstract:** Among mammalian, the ability to rapidly detect life-threatening stimuli is crucial for the transport of genes between generations. The attentional priority of phylogenetic fear-relevant stimuli is mediated by an evolutionary shaped fear module. The fear module preferentially associates fear-relevant stimuli with negative affective acts including unconditioned stimuli and anxiety. Thus, the fear module endows the fear-relevant stimuli with enhanced fear acquisition and resistant to extinction, which result in the high prevalence of phobia for phylogenetic fear-relevant such as snakes. However, to the best of our knowledge, no study demonstrated the attentional priority of fear-relevant stimuli among individuals without fear for such stimuli. Previous study analyzed the participants who might had been already fear conditioned to such stimuli. In this study, healthy individuals with varying degrees of anxieties but without fear for snakes and/or spiders were presented with pictures of snakes, spiders and flowers suppressed from view by continuous flash suppression. The suppression times for snakes relative to flowers have been found shorter than those for spiders relative to flowers most probably because of higher trait anxiety scores. This study indicates that phylogenetic fear-relevant stimulus is preferentially associated with anxiety and anxiety enhances the attentional bias toward the stimuli regardless of subjective fear.

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**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.16/UU54

**Topic:** H.02. Human Cognition and Behavior

**Support:** Grant-in-Aid for Research Activity Start-up (16H06986)

Impulsing Paradigm Change through Disruptive Technologies Program

**Title:** Two-different resting states - mind blanking and mind wandering

**Authors: *T. KAWAGOE**[1], K. ONODA[1], S. YAMAGUCHI[2]
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**Abstract:** Study for resting-state brain networks (RSNs) has become an important area of neuroimaging. Usually, neuroscientists assess RSNs using an instruction like “think about nothing in particular” or “let your mind wander.” Such instructions are probably appropriate and at present we know of nothing better than these instructions. However, the difference between the two types of instructions might cause some RSN differences. In the field of cognitive psychology, mind wandering (MW) has become a hot topic over the past 15 years. MW is defined as thinking unrelated to an ongoing task or activity, which is similar to spontaneous and stimulus-independent thought. Recently, another mental state in humans called mind blanking (MB) has been suggested. MB is defined as a lack of conscious awareness. The MW and MB would relate to the two different instructions mentioned above. For our research, we manipulated the participants’ mental states with instructions designed to uncover RSN differences between the two-different mental states, MB and MW. Self-reports confirmed that our manipulations were valid. RSNs data indicated that the default mode network (DMN; a core network during MW) and salience network (a network for detecting subjective “salience”) had a larger anticorrelation during MB conditions than during MW conditions in addition to a change within DMN connectivity. We concluded that two instructions for MB and MW can differentially affect the relationship between and within RSNs and that MB is neurophysiologically distinct from MW.

**Disclosures: T. Kawagoe:** None. **K. Onoda:** None. **S. Yamaguchi:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.17/UU55

**Topic:** H.02. Human Cognition and Behavior

**Title:** Let's talk about secs: Effects of distraction on speeding and stopping distances in young drivers

**Authors: *A. C. PLOWS**[1], H. N. RIZEQ[1], I. N. NGUYEN[1], K. D. F. RUELOS[1], D. J. GOBLE[1], H. S. BAWEJA[2]
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**Abstract:** Distracted driving is driving while doing another activity that takes attention away from driving itself. The types of distractions involve, but are not limited to: a) visual: taking one's eyes off the road; b) cognitive: taking one's mind off of driving; and, c) manual: taking one's hands off the steering wheel. However, the combined effects of speed and distraction while driving remain unclear. Therefore, the purpose of this study was to examine the effects of speed and distraction on driving performance in young adults using a realistic driving simulator. Twenty-six healthy young adults (Average age: 22.8 years, 12 females) volunteered to participate in the study. The driving simulation task involved lane keeping on a 2-lane road with a leading vehicle stimulus braking and accelerating at quasi-random intervals. All subjects performed a total of 6 trials under two experimental conditions: 1) lane keeping with no distractions, and 2) lane keeping with a secondary task (dual tasking with serial-7s). Both conditions were performed at three levels of difficulties: 60, 75 and 90 mph. Lane keeping accuracy was quantified as the root mean squared error between the intended lane position and steering movement sinusoid created by the subject's tracking. Steering variability was quantified as the standard deviation of the detrended steering movement trajectory. The distance covered during the time between the stimulus and paddle depression was also quantified. Lane keeping accuracy and smoothness decreased with increase in speed. Furthermore, distances covered from stimulus to reaction were inversely proportional to vehicle speed irrespective of acceleration and braking stimuli. We find that distraction degraded driving performance, reaction times, accelerating and stopping distances. These findings suggest individuals may not be able to adequately use information and experience previously acquired while they are distracted. The average driving experience in this cohort of subjects was at least 5 years post-licensing. Yet, the failure to benefit from prior driving experience under distraction as seen in the degraded driving performance with distraction is an important finding. The detrimental effect of distraction on driving is ubiquitous in young adults irrespective of gender and driving experience. Previous work has shown that graduated driver's licensing programs in which participants receive more

on the road training results in reduced fatal crashes. Our work is supportive of this notion and extends previous findings by providing objective measures of driving performance.

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**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.18/UU56

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH R01-EY025648 (JG)

Alfred P. Sloan (JG)

H. Dean and Susan Regis Gibson Research Award

**Title:** Independent and overlapping neural representations of saccades, attention shifts and reference frames

**Authors: *X. ZHANG**, J. D. GOLOMB
Dept of Psychology, Ohio State Univ., Columbus, OH

**Abstract:** When changing our focus from one location to another, we can either move our eyes or shift our attention covertly. Previous research indicates that saccade execution and attention shifts might share underlying brain networks, including intraparietal sulcus (IPS) and frontal eye field (FEF) (Corbetta and Shulman, 2002). To further explore how neural representations of saccades and covert attention shifts interact, we acquired fMRI data during a combined saccade and covert attention task. Participants began each trial by fixating at one of two fixation points while covertly attending to one of three rapid serial visual presentation (RSVP) streams (left, center, right of screen). There were four critical conditions. On eyes-fixed trials, participants either held attention at the same initial location (hold eyes, hold attention) or shifted attention to another stream midway through the trial (hold eyes, shift attention). On eyes-move trials, participants made a saccade midway through the trial, while maintaining attention in one of two reference frames: (shift eyes, retinotopic attention) and (shift eyes, spatiotopic attention). The retinotopic condition involved holding attention at a fixation-relative location but shifting relative to the screen, whereas the spatiotopic condition involved holding attention on the screen-centered location but shifting relative to the eyes. We used multivariate pattern analysis (MVPA) to decode information about saccades (eyes-fixed vs. eyes-move), attention shifts (hold vs. shift attention), and reference frames (retinotopic vs. spatiotopic attention). Regions where saccade information could be decoded partially overlapped with those where attention shifts could be

decoded, mainly in parietal areas, but we found less overlap in visual and frontal areas. Reference frame (task) information could only be decoded in a few areas (e.g., part of left superior parietal lobe, SPL). Further analyses suggest that this left SPL area may contain representations of both spatiotopic and retinotopic attention allocation. Moreover, we compared shift-eyes with hold-eyes conditions to examine how saccade and attention shift processes might be involved in reference frame representation. The activity pattern of "shift-eyes, retinotopic attention" seemed more similar to the "hold eyes, hold attention" condition in earlier visual areas than in later parietal and frontal areas, consistent with the notion that earlier areas are largely retinotopically organized, while other processes such as saccades might more strongly influence representations in later areas.

**Disclosures: X. Zhang:** None. **J.D. Golomb:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.19/UU57

**Topic:** H.02. Human Cognition and Behavior

**Support:** European Research Council, number StG_2011283314

**Title:** The impact of transcutaneous vagal nerve stimulation on the amplitude of the P3 event-related potential implicates the involvement of noradrenergic activity

**Authors: *C. M. WARREN**[1], L. OUWERKERK[2], S. NIEUWENHUIS[3]
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**Abstract:** We used transcutaneous vagal nerve stimulation (tVNS) in concert with electroencephalogram recordings to test for an impact of tVNS on the amplitude of the P3 event-related potential (ERP) component. Nieuwenhuis, Aston-Jones, and Cohen (2005) proposed that the P3 is a manifestation of phasic norepinephrine release. TVNS is a non-invasive treatment of epilepsy that is thought to exert its putative therapeutic effect through increased norepinephrine release. Previously, invasive vagal nerve stimulation has been linked to the P3 in that patients with epilepsy who responded to VNS treatment also exhibited an increase in P3 amplitude, whereas patients who did not show improvement in seizure frequency also did not show an increased P3 (De Taeye et al., 2014). We applied real and sham tVNS to a group of healthy subjects (n = 24) in a subject-blind cross-over design, while they performed a standard set of "oddball" tasks known to elicit a P3. TVNS did not affect reaction time nor accuracy in the oddball tasks. We used principal component analysis (PCA) to decompose the ERP data into a set of linearly uncorrelated waveforms (Spencer, Dien, & Donchin, 2001). PCA identified two

distinct positive components consistent with the classic P3, and the novelty P3. We observed a reduction in amplitude of both the classic and novelty P3 in the real condition relative to the sham condition. We argue that the inconsistency between our results and those of De Taeye and colleagues (2014) must be due to physiological differences between the patients with epilepsy studied by De Taeye et al. (2014) and the healthy participants who took part in our study, as well as the difference between invasive VNS and tVNS. Regardless, our results provide additional support for the role of the norepinephrine system in production of the P3.

**Disclosures: C.M. Warren:** None. **L. Ouwerkerk:** None. **S. Nieuwenhuis:** None.

**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.20/UU58

**Topic:** H.02. Human Cognition and Behavior

**Support:** Open Project Grant of the State Key Laboratory of Cognitive Neuroscience and Learning

National Key Basic Research Program of China

National Natural Science Foundation of China

**Title:** Anticipatory lateralization of HbO and alpha oscillations cooperatively predicts N2pc: A concurrent fNIRS-ERP study

**Authors: *Y. SONG**[1], C. ZHAO[1], Y. TAO[1], J. GUO[1], H. LIU[2], L. SUN[3]
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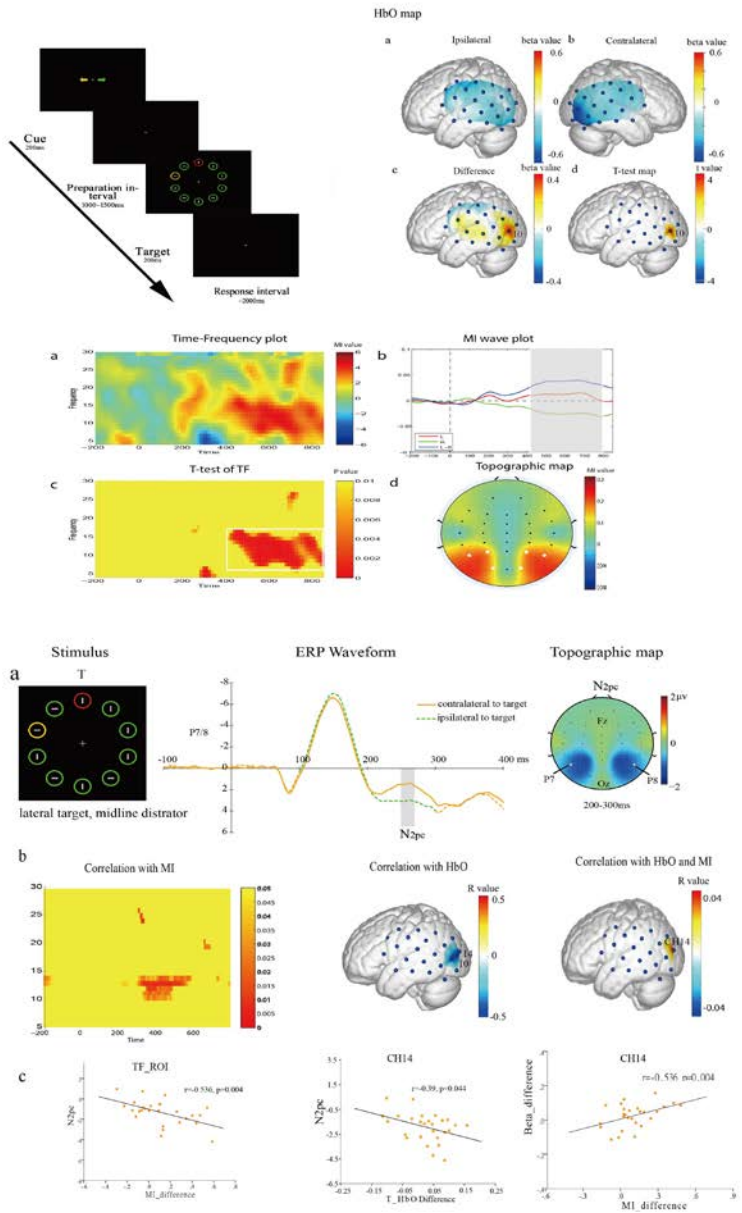
**Abstract:** Understanding the properties of attentional control, along with the neural mechanisms subserving them, has long invited intense scrutiny in research groups. However, it has not been demonstrated how the top-down anticipatory oxygenated hemoglobin and electrical activity activation cooperatively influence the subsequent attentional processing of targets selection. Here, with a concurrent event-related fNIRS-ERP recording, we explored the potential contribution of anticipatory oxygenated hemoglobin (HbO) based activity and low-frequency oscillations to attentional control by examining how HbO and alpha-band oscillations influence the subsequent attentional selection ERP marker. We found that expecting a target led to both a larger increase of preparatory HbO response and a larger decrease of posterior alpha-band (8-12 Hz) oscillations over the visual cortex contralateral to the upcoming target, indicating an increase in cortical excitability in task-relevant sensory neurons to facilitate the processing of subsequent stimulus inputs. Importantly, the magnitude of cue-induced alpha lateralization was positively

correlated with the HbO lateralization in V3 cortex, and such a cue-induced alpha and HbO lateralization were both positively correlated with the subsequent target-evoked ERP N2pc amplitudes assumed to reflect attentional selection. Our results suggest that each individual's attentional target selection ability is predictable in advance via the functional coupling between anticipation-induced changes of HbO and low-frequency EEG signals in the visual cortex.

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**Poster**

**618. Functional Mechanisms of Attention**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 618.21/UU59

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH NCCIH K08AT009385

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**Title:** Decoding the focus of attention during breath meditation

**Authors: *H. WENG**[1,2], J. LEWIS-PEACOCK[3], F. HECHT[1], D. ZIEGLER[2], M. UNCAPHER[1], L. DUNCAN[4], N. FARB[5], V. GOLDMAN[1], M. CHAO[1], S. SKINNER[2], M. ESTEFANOS[2], S. LEE[1], R. LOPILATO[2], A. GAZZALEY[2]
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**Abstract:** Meditation skills learned from mindfulness-based interventions are shown to decrease stress and improve mental and physical health symptoms. However, the mechanisms of action for these benefits are currently unclear due to challenges in measuring internally-oriented attention to the body during meditation practice. The EMBODY task provides a framework to address these measurement issues by applying machine learning methods to fMRI data to identify varied and fluctuating mental states during meditation. The task measures the focus of attention during a core meditation skill, focused attention to the breath, where attention is sustained to sensations of the breath, and brought back to the breath if the mind wanders. The task was piloted and validated in 12 adults, including 7 meditation practitioners ($\geq$ 5 years of weekly practice) and 5 age- and gender-matched novices. In Step 1 of the EMBODY Task, participants were instructed via brief audio instructions with eyes closed to pay attention to 1) sensations of the breath, 2) sensations of the feet, 3) sounds from the scanner, 4) directed thinking about life events, and instructed to 5) stop paying attention and let whatever come to mind (a proxy for mind wandering). Using multi-voxel pattern analysis (MVPA) applied to wholebrain individual-level data, all five neural patterns were recognized above chance (all classification accuracy percents > 41% [chance: 20%], all one sample $t_{11}$'s > 4.52, all $p$'s < 0.001), demonstrating that neural patterns associated with each internal mental state were

distinct. In addition, neural patterns were better recognized in meditators vs. novices specifically for attention to the breath (independent $t_{10} = 2.65$, $p < 0.05$) and not the other conditions ($p$'s > 0.18). In Step 2 of the EMBODY Task, a 10-min period of breath meditation was decoded using the trained neural patterns from Step 1, which produced a second-by-second readout of mental states. These data produced novel metrics of internal attention during meditation, including percentage time spent paying attention to the breath (mean = 22.10%, SD = 7.16) and number of mind wandering events (mean = 27.42, SD = 6.98). The EMBODY task provides a novel framework to measure internally-oriented attention to the body that is trained by meditation, and holds promise to elucidate mechanisms of action from mindfulness-based interventions.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.01/UU60

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Title:** What do you think before you fall? Brain activation during a rock climbing specific hang until failure

**Authors: *K. C. PHILLIPS**, B. NOH, H. MAAT, T. YOON
Michigan Technological Univ., Houghton, MI

**Abstract:** The prefrontal cortex (PFC) plays an important role in cognitive control, motor planning, execution of movement, and allocation of attentional resources. Functional near-infrared spectroscopy (fNIRS) allows for a convenient way of investigating PFC activation through measuring the hemodynamic response to cognitive and motor tasks. Rock climbing is a task that involves both muscular and cognitive demands (i.e. movement planning, fatigue, fear of falling, etc.). The purpose of this study is to examine PFC activation during a climbing specific sustained hang until failure. Four experienced male rock climbers (26 ± 3.1 years) took part in this study. After an upper extremity warm up, participants performed three maximal voluntary contractions (MVCs), with their dominant hand, on a climbing specific finger flexor assessment device. Next, participants were equipped with a 16 channel fNIRS sensor pad over their PFC and a two minute baseline measurement was performed. After the baseline, participants were asked to perform a body weight hang from two large climbing holds until they could no longer maintain themselves on the holds. Within 30 seconds after failure, participants were secured back into the finger flexor assessment device and performed one MVC. Lastly, participants

reported their rating of perceived exertion (RPE) at the beginning and failure of the hanging task. The optodes were averaged in three regions of interest for each participant; left PFC (optodes 1-6), frontopolar PFC (optodes 7-10), right PFC (optodes 11-16). The results of this study showed MVC force significantly declined (490 N vs. 413 N, $P<0.05$) from pre to post task. Additionally, participants RPE significantly increased ($P<0.001$) from the start to failure of the hanging task. The PFC showed significant increases in oxygenation in all 3 areas of interest ($P<0.05$), however, there was no difference in increases between areas. Lastly, optode 13 (right dorsolateral PFC) showed a significant correlation with RPE ($r=0.97$, $P<0.05$). These results suggest that the right dorsolateral PFC may play a role in the determination of task termination during a climbing specific hang until failure.

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**Poster**

**619. Cognitive Control and Performance**

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**Program#/Poster#:** 619.02/UU61

**Topic:** H.02. Human Cognition and Behavior

**Support:** MEXT Special Funds 1111501004

Grant-in-Aid for Young Scientists (B) 16K20930

**Title:** High-intensity intermittent exercise improves executive performance by enhancing Stroop-effect-related prefrontal activation: A fNIRS neuroimaging study

**Authors:** *K. BYUN[1], ***K. BYUN**[1], S. KUJACH[2], K. HYODO[1], K. SUWABE[1], T. FUKUIE[1], R. LASKOWSKI[2], H. SOYA[1]
[1]Fac. of Hlth. and Sport Sci., Univ. of Tsukuba, Tsukuba-Shi, Japan; [2]Gdansk Univ. of Physical Educ. and Sport, Gdansk, Poland

**Abstract:** High-intensity interval training (HIT), also called high-intensity intermittent exercise (HIE), is well known as a time-efficient exercise regimen that enhances endurance capacity. Current studies suggest that HIE not only has an impact on cardiovascular fitness, but also on cognitive brain functions. However, how acute HIE improves cognitive functions, and its neural substrates, is still unknown. To address this issue, we examined the effects of acute HIE on executive function using the color-word matching Stroop task (CWST), which produces a cognitive conflict in the decision-making process and its neural substrate, with functional near infrared spectroscopy (fNIRS) (Hitachi, Japan). Twenty-five young adults (mean age: 21.6 ± 1.4 years; 9 females) participated in two counter-balanced sessions: HIE and resting control. The HIE session consisted of two minutes of warm-up exercise (50 W load at 60 rpm) and eight sets

of 30 s of cycling exercise (mean: 127 W ± 29.5 load at 100 rpm) followed by 30 s of rest on a recumbent-ergometer. Participants performed a CWST before and after the 10-minute exercise session, during both of which cortical hemodynamic changes in the prefrontal cortex were monitored using fNIRS. Acute HIE led to improved Stroop performance reflected by a shortening of the response time related to Stroop interference. It also evoked cortical activations related to Stroop interference on the left-dorsal-lateral prefrontal cortex (DLPFC), which corresponded significantly with improved executive performance. These results provide the first empirical evidence, to our knowledge, that acute HIE improves executive function, probably mediated by increased activation of the task-related area of the prefrontal cortex, the left-DLPFC.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.03/UU62

**Topic:** H.02. Human Cognition and Behavior

**Support:** Internal support though NYU

**Title:** The effects of a semester of aerobic exercise on fitness, cognition, mood, and GPA in college students

**Authors: *J. C. BASSO**, C. CROSTA, M. RASKIN, A. WANG, D. KADAKIA, J. CHOI, E. MILBURN, R. TRIVEDI, W. A. SUZUKI
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**Abstract:** Long-term aerobic exercise enhances mood state and improves a range of cognitive functions including attention, information processing speed and both short- and long-term memory. Though a recent study found that first-year medical school students who regularly exercised attained higher grades than those who remained sedentary, little has been done to assess whether long-term exercise in undergraduate students positively influences cognitive function and academic performance. Therefore, the present longitudinal study examined the effects of an aerobic exercise intervention on mood, cognitive function and academic performance in first-year college students. Preliminary data on thirteen healthy, sedentary students from New York University was collected. These individuals completed a cardiopulmonary fitness test ($VO_2$ max test), a battery of neuropsychological tasks, and a series of self-reported mood and study strategies questionnaires at the beginning and end of one semester in which they maintained their sedentary lifestyles. At the beginning and end of the

following semester, they repeated these tasks, but increased their exercise regimens to include three or more aerobic exercise sessions per week lasting 45 minutes or longer. Grade point average (GPA) was obtained for each semester as well. Compared to the sedentary semester, the exercise intervention significantly increased cardiopulmonary fitness ($F(1,13)=15.246$, $p=0.002$), quality of life ($F(1,10)=4.771$, $p=0.05$), positive affect ($F(1,10)=5.662$, $p=0.039$), information processing speed as measured by the Eriksen Flanker Task (congruent trials $F(1,10)=9.093$, $p=0.013$; incongruent trials $F(1,10)=8.060$, $p=0.018$), short- and long-term memory as assessed by the Wechsler Logical Memory Test (immediate recall $F(1,7)=7.368$, $p=0.030$; delayed recall $F(1,7)=7.744$, $p=0.027$), and creative thinking as assessed by the Remote Associates Test ($F(1,12)=5.758$, $p=0.034$). In addition, those individuals who improved the most in their fitness showed the largest increases in semester GPA ($R=0.733$, $p=0.038$). These results suggest that even a single semester of exercise can improve cardiopulmonary fitness, mood, cognition, and school performance in previously sedentary, first-year college students.

**Disclosures:** **J.C. Basso:** None. **C. Crosta:** None. **M. Raskin:** None. **A. Wang:** None. **D. Kadakia:** None. **J. Choi:** None. **E. Milburn:** None. **R. Trivedi:** None. **W.A. Suzuki:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.04/UU63

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant MH105625

**Title:** Brain mechanisms of reactive and proactive control in children

**Authors: *W. CAI**[1], K. DUBERG[2], R. REHERT[2], J. CHEN[2], K. ZHANG[2], J. NICHOLAS[2], T. CHEN[2], B. PENNINGTON[3], S. HINSHAW[4], J. NIGG[5], V. MENON[6]
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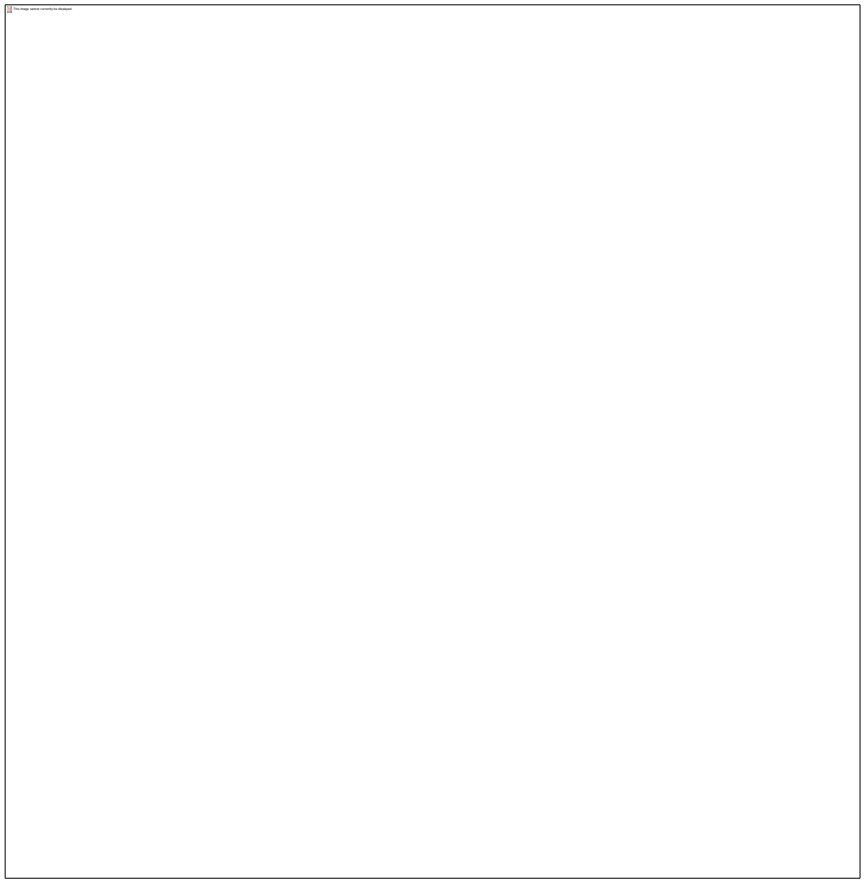
**Abstract:** Inhibitory control is crucial for cognitive development during childhood. Poor inhibitory control is the core deficit of ADHD, one of the most common neurodevelopmental disorders. Understanding brain development of inhibitory control in childhood is thus important for public health. The Dual Control model suggests that inhibitory control operates in two distinct modes: reactive and proactive control. However, little is known about the brain mechanisms underlying reactive and proactive control during childhood.
We acquired task fMRI data from 16 children (9-12 yrs, 5f/11m) who completed a standard and a conditional stop-signal task (SST & CSST) using a simultaneous multi-slice EPI sequence

(TR=490ms, TE=30ms). In SST, participants made responses to a Go signal or stopped response if a Go signal was infrequently followed by a Stop signal. In CSST, a MaybeStop cue (a stop signal might occur) or a NoStop cue (no stop signal will occur) was presented prior to Go signal. Reactive and proactive control were indexed by stop-signal reaction time (SSRT) and response slowing in MaybeStop vs NoStop Go, respectively. GLM contrasts of SuccStop vs. Go and MaybeStop vs NoStop Go were used to probe brain mechanisms in reactive and proactive control. A drift diffusion model was used to estimate decision boundary.
We found negative correlation between response slowing and stop-signal reaction time (SSRT) ($r$=-0.62, $p$=0.02), suggesting that children with better reactive control implement more proactive control. GLM analysis revealed that the right prefrontal-insula-parietal regions were significantly activated in reactive control, whereas dorsomedial prefrontal cortex and anterior insula were involved in proactive control ($p$<0.01, cluster size>133) (Fig. 1). Furthermore, activation in right IFG/MFG in reactive control was positively correlated with SSRT and activation in left thalamus in proactive control was negatively correlated with decision boundary change ($p$<0.01). Overall, similar but distinct brain mechanisms in reactive and proactive control are apparent in late childhood.



**Disclosures:** **W. Cai:** None. **K. Duberg:** None. **R. Rehert:** None. **J. Chen:** None. **K. Zhang:** None. **J. Nicholas:** None. **T. Chen:** None. **B. Pennington:** None. **S. Hinshaw:** None. **J. Nigg:** None. **V. Menon:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.05/UU64

**Topic:** H.02. Human Cognition and Behavior

**Support:** SONIMA FDN

**Title:** Neural mechanisms of response inhibition in children

**Authors: *K. T. DUBERG**, R. REHERT, S.-N. BOSTAN, S. QIN, A. PADMANABHAN, T. BRADLEY, O. ALTAMIRANO, Y. A. MARTIN, V. G. CARRION, V. MENON, W. CAI
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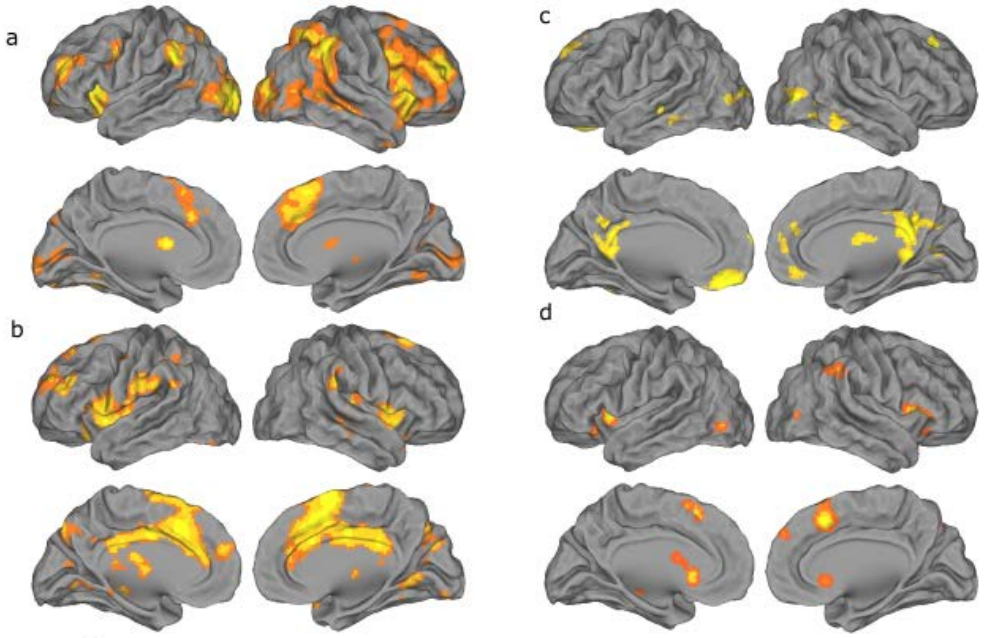
**Abstract:** Response inhibition, the ability to cancel or withhold inappropriate behavior, is an important feature of cognitive control. The development of cognitive control is particularly important in children since self control is critical to interpersonal and academic success. Although developmental trajectories of response inhibition have been well documented, little is known about brain mechanisms underlying response inhibition in children, since previous neuroimaging studies are limited by their small sample size and have resulted in inconsistent findings.

Our meta-analysis only revealed activation in fronto-insula-parietal regions at uncorrected threshold ($p<0.05$). Poor consensus of previous findings thus calls for a large-scale neuroimaging study. Here we collected behavioral and neuroimaging data from 40 children (9-11 yrs old, 16f/34m). Each participant completed 2 sessions of SST during which they made responses to green arrows, and, occasionally, withheld a response if the green arrows turned red after a short delay. Stop Signal Reaction Time (SSRT) was measured as behavioral index for inhibitory control. fMRI data was acquired on a 3T GE scanner using a T2* gradient echo-spiral in-out pulse sequence (TR=2000ms, TE=30ms).

Participants completed the task well (Go Accuracy: 94±4%, SSRT: 297±50ms). Brain analyses showed significantly greater activation in anterior insula, inferior, middle and superior frontal gyri, angular gyrus, and striatum in successful stop vs go trials. We also found stronger activation in anterior cingulate cortex in unsuccessful vs successful stopping. Interestingly, activation in right inferior temporal gyrus during successful stop trials was negatively correlated with SSRT. Furthermore, we found increased connectivity between right posterior parietal cortex and ventromedial prefrontal cortex in successful stop compared to go. All results were thresholded at $p<0.01$, with cluster size greater than 128 voxels. Together, our study highlights that a fronto-striatal-parietal network underlies inhibitory control function in children.




a) Activation during successful stop vs go trials
b) Activation during unsuccessful stop vs successful stop trials
c) Connectivity during successful stop vs go trials (seed: right Posterior Parietal Cortex)
d) Meta analysis (p<0.05)

**Disclosures:** **K.T. Duberg:** None. **R. Rehert:** None. **S. Bostan:** None. **S. Qin:** None. **A. Padmanabhan:** None. **T. Bradley:** None. **O. Altamirano:** None. **Y.A. Martin:** None. **V.G. Carrion:** None. **V. Menon:** None. **W. Cai:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.06/UU65

**Topic:** H.02. Human Cognition and Behavior

**Title:** Association between alcohol use, impulsivity and inhibitory control in adolescents from a Brazilian sample

**Authors: *A. R. WILLHELM**[1], A. S. PEREIRA[2], S. H. KOLLER[2], R. M. DE ALMEIDA[3]
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**Abstract:** Introduction: The last area to develop in the central nervous system is the prefrontal cortex (PFC), the area also linked to the ability to control impulses. This suggests that

adolescents tend to have more impulsive behaviors that can result in risky behaviors such as alcohol and drugs. The aim was compare adolescences between 13 to 16 years old with and without alcohol first use in the variables impulsivity and inhibitory control. Method: The sample was composed for 122 participants from private and public schools with age 13 to 16, divide in two groups: with (n=95) and without (n=27) first alcohol consumption. The instruments used were: Sociodemographic and alcohol and drugs use questionnaire, Barratt impulsiveness scale-youth, Go/No-go task and Five Digits test. It conducted a Student's t test to compare the two groups. Results: Statistically significant differences were observed in the three dimension of the impulsivity scale (motor: t = -3.161, p = .002; attention: t = -2.447, p=.016, non-planning: t = -2.398, p=0.18), the group that already consumed alcohol has higher mean scores in these variables. It was not found statistically significant differences in the tasks that evaluated inhibitory control between the two groups. In the group that already used alcohol, 40,42% (n=38) considerer that some relative have drinking problems, in other hand, 85,2% (n=23) of the group that never used alcohol considerer that anyone in their family have drinking problems. Discussion: In Brazil, the alcohol consumption is culturally acceptable, even for minors. Unlike the use of other substances, such as tobacco and drugs, the vast majority of the population does not consider the risks to health from the use of alcohol. Along with this, adolescence is the most susceptible stage to risky behaviors such as alcohol and drugs, by an immaturity of the PFC, which can result in greater impulsivity. Data from this study points to this direction, since the group that already tried alcohol showed higher average in the three measurements of impulsivity. It was observed that adolescents with higher levels of impulsivity tend to use alcohol, then performing this risky behavior described in the literature. This is not directly connected with inhibitory control, in which there were no significant differences. Cultural issues surrounding the use of this substance can explain data for the inhibitory control. The damage of alcohol consumption are minimized by the culture and the inhibitory control is characterize by interrupting or changing a behavior that is deemed inappropriate.

**Disclosures:** **A.R. Willhelm:** None. **A.S. Pereira:** None. **S.H. Koller:** None. **R.M. De Almeida:** None.

**Poster**

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**Program#/Poster#:** 619.07/UU66

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant University of Vermont - Subaward 65723992

**Title:** Neuroimaging correlates of smoking cessation in healthy adult cigarette smokers

**Authors: K. HUDSON**[1], A. IVANCIU[1], B. CHAARANI[1], P. SPECHLER[1], S. KUMAR[2], S. SAMIEI[3], D. WETTER[4], P. THOMPSON[5], S. HIGGINS[1], *H. GARAVAN[1]
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**Abstract: Objective:** Smoking is the leading cause of preventable death in the U.S. (CDC, 2005). Most individuals who attempt to quit relapse within two weeks. Maintaining abstinence in this period is a robust predictor of longer-term abstinence (Higgins et al., 2006). Thus, neurobiological changes that occur during the early abstinence period may yield critical predictors regarding successful abstinence or relapse. We examine neurocognitive processes implicated in relapse among smokers who are successful and unsuccessful in maintaining abstinence by assessing cognitive control and reward impulsivity. We aim to build upon initial findings by incorporating real-time stress and physiological assessments and ecological momentary assessment (EMA) data obtained during the period of smoking cessation. **Method:** Neuroimaging and behavioral data were acquired for 15 smokers and 15 non-smokers, matched on age, gender and SES. Participants were scanned twice: 1) following access to their regular cigarettes (smoked 15 minutes prior to scan), and 2) after overnight abstinence (CO verified; order counterbalanced). Brain activation maps of inhibitory control were generated from the Stop Signal Task. Brain perfusion maps were generated using 5 minutes of pCASL. **Results:** Smokers, when abstinent and satiated, demonstrated increased activation during successful inhibition in bilateral inferior frontal gyrus (IFG), relative to controls. Further, smokers, relative to controls, demonstrated lower cerebral blood flow (CBF) in overlapping bilateral IFG regions. Both the activation and perfusion effects were larger when smokers were abstinent. **Conclusions:** The results suggest functional differences in smokers in brain systems underlying inhibitory control. These differences may relate to relapse risk as both the perfusion and activation differences in these brain regions showed enhanced effects when abstinent. To assess the real-world significance of these results, an ongoing study employs mobile technologies to measure stress reactivity, nicotine craving, and EMAs. We aim to merge neuroimaging data that assess the functioning of these impulse control systems prior to abstinence with real-time physiological assessments and EMAs obtained during the smoking cessation period to generate predictive profiles for individuals at highest risk for relapse.

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**Poster**

**619. Cognitive Control and Performance**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** DFG SFB 779 TPA14N

**Title:** The influence of craving on cognitive control processes in cigarette smokers

**Authors: *S. E. DONOHUE**[1,2], K. LOEWE[1], J. A. HARRIS[1,2], J.-M. HOPF[1,2], H.-J. HEINZE[1,2], M. G. WOLDORFF[1,2,3], M. A. SCHOENFELD[1,2,4]
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**Abstract:** Cognitive control allows humans to flexibly switch between tasks, to inhibit responses when appropriate, and to deal with competing stimuli/responses. Such goals can be attentionally demanding, requiring trial-to-trial adjustments, which are subject to the waxing and waning of attention. In instances of impaired attentional allocation, these control processes are not always successfully implemented. Smokers who have not smoked for a period of time and are in a state of craving represents such a circumstance (i.e., one in which attention may be diverted from the task at hand). Although previous work from our group has shown that such craving can result in an overall increase in arousal, how craving may impact specific attentional control processes has yet to be determined. To address this, 20 smokers performed a task-switching paradigm, in which several cognitive-control processes were assessed in two sessions (i.e., one just after having smoked and another 'craving session' following a three-hour period of nicotine deprivation). The task entailed an instructional cue indicating the type of judgment to be made by the participant for a subsequent target. The prescribed judgment represented either a task switch, or a continuation from the previous trial. Targets were single digit stimuli, 80% of which required making a response, and 20% to withhold one. Participants received feedback following their response (or successful response inhibition), with fast and correct responses earning them the most points, and the amount of points determining the end of the experiment. Continuous EEG data were recorded for this task during both sessions, and we examined the ERP components to these various trial types as a function of craving. The ERP results were generally in line with previous observations for such tasks, with an increased CNV to the cue for switch vs. repeat trials, an N2/P300 enhancement for NoGo vs. Go targets, a frontal-central negativity for targets eliciting response conflict, and an enhanced P300 in response to the feedback on trials on which fewer points were earned. Interestingly, craving did not interact with all of the aforementioned processes, but showed a selective influence on only specific cognitive control-related processes. Craving had the greatest impact on the cue-elicited and feedback-elicited control processes, with the target processing being less affected. These results suggest that craving has a differential impact on different types of control processes, and indicate a complex interaction between craving and the attentional system.

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**Poster**

**619. Cognitive Control and Performance**

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**Support:** NIH P50MH094258

NSF BCS1554105

NIH R01MH110831

McKnight Endowment Fund for Neuroscience

NARSAD

**Title:** Single-neuron and filed-potential correlates of error monitoring in the human medial frontal cortex

**Authors: *Z. FU**[1], D.-A. J. WU[2], S. SULLIVAN[3], I. ROSS[4], J. M. CHUNG[3], A. N. MAMELAK[3], R. ADOLPHS[2], U. RUTISHAUSER[3]
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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. EEG source modeling suggest that the dorsal anterior cingulate cortex contributes to the ERN. However, it remains unknown how the ERN and the error-monitoring mechanism that the signal represents are reflected in the activity of single neurons.
We used intracranial electroencephalography (iEEG) and single unit recordings to investigate error monitoring in human subjects. 28 epilepsy surgery candidates with implanted intracranial depth electrodes performed 85 sessions of a speeded version of a color-word Stroop task. Subjects showed a robust Stroop effect (224.9 ± 20.8ms, $F(1,84) = 116.63$, $p < 10^{-10}$, mixed-effect one-way ANOVA model) and post-error slowing (71.9 ± 21.6 ms (mean ± s.e.m. across sessions), $F(1,214) = 24.66$, $p < 10^{-5}$, mixed-effect one-way ANOVA model).
We isolated 583 neurons in the dorsal anterior cingulate cortex (dACC) and 507 in pre-supplementary motor area (pre-SMA). 28% of all dACC neurons and 40% of all pre-SMA neurons changed their firing rate immediately after subjects made an error, but before feedback appeared. This suggested that the error signals were internally-generated, not triggered by external feedback. The onset of the neuronal error signal in pre-SMA preceded that in dACC by 67 ms.

Given the prominent post-error slowing effect (PES) and error signals, we hypothesized that dACC and pre-SMA are important components of the neuronal circuitry that implements the control processes that result in PES. Indeed, we found that 11% of dACC and 14% of pre-SMA neurons modulated their firing rate only in correct trials that followed an error.
We also simultaneously recorded a robust intracranial error-related evoked potential in both 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 was significantly higher in error trials than in correct trials. Here we again found that the iERN peaks in pre-SMA preceded those in dACC by 38ms. Finally, we found a trial-by-trial relationship between the ERN (a macroscopic signal) and the activity of error neurons (a microscopic signal): The amplitude of the ERN correlated robustly with error neuron firing rates and theta-band spike-field coherence between the two signals was larger during errors.
Together, our findings provide the first circuit-level description of the mechanism of error monitoring and post-error adjustment in human dACC and pre-SMA.

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**Poster**

**619. Cognitive Control and Performance**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** CIHR Foundation Scheme

NSERC Discovery Grant

**Title:** Sequential adjustments in cognitive control: Insights from simultaneous EEG-fMRI

**Authors: T. HINAULT**, K. LARCHER, N. ZAZUBOVITS, M. FERREIRA, J. GOTMAN, *A. DAGHER
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**Abstract: Introduction**: Optimal decision-making depends in part on the ability to inhibit the automatic processing of irrelevant information. Previous work has revealed conflict adaptation effects, with reduced interference on items that follow an incongruent stimulus compared to after a congruent stimulus. These studies on sequential modulation of control processes mainly relied on unimodal measures, which suffer from low spatial or temporal resolution. In this study, we simultaneously recorded EEG and fMRI to investigate the spatiotemporal dynamics of proactive

control.
**Methods**: Twenty-two participants performed a numerical Stroop task, in which they were presented visually with two numbers and were instructed to select the number with the higher numerical value, regardless of font size. We contrasted congruent items (i.e., the larger number in size is also the number with the higher numerical value) to incongruent items (i.e., one number is larger in size and the other number is larger in numerical value). To study sequential effects, we focused on the processing of incongruent items as a function of the congruency/incongruency of the preceding item. Participants were equipped with an MR-compatible 64-channel cap while they performed the task in a Siemens Trio 3T MRI. After separate pre-processing for EEG and fMRI data, we conducted a joint Independent Component Analysis (jICA; Calhoun et al., 2006) to couple event-related potential (ERP) time courses and fMRI spatial maps. Furthermore, network analyses were also performed, with Dynamic Causal Modeling (DCM) applied on both modalities.
**Results**: EEG and fMRI provided converging evidence to highlight the engagement of the anterior cingulate cortex and right dorsolateral prefrontal cortex (DLPFC) in reactive adjustments when no preparation was engaged, in a time course consistent with the N200 ERP component. Conversely, proactive adjustments yielded activations of DLPFC, left inferior frontal gyrus and inferior parietal lobule, both during the encoding phase and later with modulations of the conflict SP component. DCM results revealed a larger number of couplings, and higher coupling intensity within this network after the processing of an incongruent trial, in line with increased level of control. Finally, correlations were found between DCM couplings and behavioral adjustments.
**Conclusions**: Results contribute to a framework of the neural bases of proactive adjustments in performance by unraveling changes over time within the cognitive control network, both during the processing of a given item and as a function of the succession of items, and how this network relates to participants' performance.

**Disclosures:** **T. Hinault:** None. **K. Larcher:** None. **N. Zazubovits:** None. **M. Ferreira:** None. **J. Gotman:** None. **A. Dagher:** None.

**Poster**

**619. Cognitive Control and Performance**

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**Program#/Poster#:** 619.11/UU70

**Topic:** H.02. Human Cognition and Behavior

**Support:** MRC-Intamural program MC-A060-5PQ10

Gates Cambridge Scholarship

**Title:** Complexity and effort in the fronto-parietal and cingulo-opercular control networks

**Authors: *S. SHASHIDHARA**[1], Y. EREZ[1], D. J. MITCHELL[1], J. DUNCAN[1,2]
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**Abstract:** A specific set of regions identified as the multiple - demand (MD) network, encompassing well-defined frontal and parietal regions, is active during diverse cognitive functions. Results from resting - state functional magnetic resonance imaging (fMRI) suggest a split of the MD network into fronto-parietal and cingulo-opercular subnetworks. The literature gives some evidence of functional difference between these subnetworks: Fronto-parietal subnetwork activity has been linked to complex problem solving, while cingulo-opercular subnetwork activity has been associated with cognitive effort and autonomic arousal. In the current fMRI study, we used a maze task to examine the response of the two subnetworks to different aspects of task demand. In particular, we examined task complexity, manipulated by changing the number and nature of steps required to complete the maze (difficulty manipulation), and two variables, time stress and reward, aiming to alter the cognitive effort committed to solving a problem of fixed complexity. We manipulated time stress by comparing self-paced problem solving with problems visible only for a restricted time. On some of these trials, we also added the possibility of a substantial monetary reward. With these manipulations, we examined the activity profiles of fronto-parietal and cingulo-opercular subnetworks. Concurrently, we measured pupil size as a link to autonomic arousal. The behavioural data showed increased planning times for the difficulty manipulation and similar planning times for the time stress and reward manipulations, as compared to the baseline condition. Eye-tracking data showed larger pupil sizes for the difficulty and the reward manipulations. Whole-brain random effects analysis across participants, along with regions of interest (ROIs) contrasts, using MD network templates from previous studies, showed all ROIs of both subnetworks respond to all task manipulations. We found that, while the cingulo-opercular sub-network showed a modestly greater reward effect than the fronto-parietal, the difficulty and the time stress effects were similar across subnetworks. Despite strong anatomical differences, MD sub-networks show largely similar response to manipulations of task content and conditions.

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**Poster**

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**Title:** Cognitive and emotional stresses synergetically modulate cognitive control through the cingulo-striatal system: fMRI and EDA Studies

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**Abstract:** The dorsal part of the anterior cingulate cortex (dACC) is proposed to compute control signal, especially when a task difficulty is high. The region is also shown to be activated in response to pain or emotional stimulus with negative valence. Those findings indicate that activation of the dACC reflects a call for cognitive and behavioral alternation to resolve higher cognitive or emotional stress. In other words, the dACC is likely to respond to any kind of stress in order to produce control signal for a behavioral adjustment. Combining cognitive and emotional stress, the present study aimed to disclose a potential role of the dACC to integrate differential stresses for production of an adaptive control signal. Twenty-seven participants performed the emotional Stroop task that required a judgment of a target facial expression while ignoring congruent/incongruent distractors. Critically, we presented cognitive and emotional stress cues prior to the Stroop task in each trial. A cognitive stress cue indicated a time window given for a face judgment, which was presented for 1000ms. A high cognitive stress cue called for quick response, which was adjusted to individual key-press speed (500-850ms), whereas a low cognitive stress cue gave a time-window of 2000ms. Following to the cognitive cue, an emotional cue was presented for 1000ms, which indicated a presence/absence of pain punishment for incorrect responses. Given that the dACC integrates cognitive and emotional stresses, the region is predicted to produce greater activation in the condition with high cognitive and emotional stress cues. In order to measure stress level in each condition, we recorded electrodermal activity (EDA) of 11 participants outside a scanner. Performing a three-way repeated ANOVA, which included factors of the cognitive stress, emotional stress, and time-point, we found that the higher cognitive stress cue produced greater EDA across time-points, while the higher emotional stress cue showed gradual increase in EDA. Those results indicate that each cue successfully produced psychological stress. In the fMRI data of another 16 participants, we made a conjunction contrast that included main effects of the cognitive and emotional stresses, which allowed us to localize the brain areas showing significant activity in response to cue with high cognitive and emotional stresses. The dACC and the bilateral caudate nuclei showed significant activation, indicating that the dACC may integrate different type of stress in concert with the ventral striatal areas, and send the control signal toward the executive brain regions such as the lateral prefrontal cortex.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.13/UU72

**Topic:** H.02. Human Cognition and Behavior

**Support:** CIHR

**Title:** High trait self-control is associated with reduced engagement of executive and salience networks during voluntary suppression of food craving

**Authors: *J. HAN**, U. VAINIK, K. LARCHER, J. GUAN, A. DAGHER
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**Abstract:** Individuals with greater self-control report less desire for food, eat less and are more successful at losing weight. Previous neuroimaging studies implicate the dorsolateral prefrontal cortex (DLPFC) in dietary self-control. Activity in the DLPFC is greater during volitional suppression of food craving, and this predicts food intake and weight loss success. Multivariate analyses also implicate the fronto parietal executive control network, which include the DLPFC, and the salience network, largely anchored in the fronto-insula and dorsal anterior cingulate cortex, in dietary self-control, consistent with their known functions in attention and cognitive control. Indeed functional connectivity within these networks is altered in obesity and related to body mass index. Neuroimaging studies performed to date capture state self-control (self-control in action) and fail to consider the role of trait self-control. In order to identify neural mechanisms that underlie the relationship between trait self-control and eating-related behaviours, we classified young, healthy people as high and low self-controllers (n=28 and 20 respectively) based on the Reward-Based Eating Drive scale, which measures dietary self-control, satiety and preoccupation with food. Subjects underwent fMRI while they performed a food regulation task where they deliberately suppressed craving for comfort foods presented in pictures. After the scan, subjects were left alone and given a bowl of their favorite chips to eat. As expected, high self-controllers compared to the low ate fewer chips ($p<0.05$) and had lower body mass index ($p<0.05$). Voluntary control of food craving increased activity in the left DLPFC and pre-supplementary motor area, which also showed greater functional coupling with the ventromedial prefrontal cortex (FDR corrected $p<0.05$). Interestingly, food regulation-induced activity in the left DLPFC was significantly smaller in the high versus low self-control group ($p<0.05$). Moreover, independent component analysis identified components resembling the executive control, default mode and salience networks whose activity changed significantly during craving suppression ($ps<0.01$). The task-related activity in the salience network was greater in low self-controllers compared to the high ($p<0.05$) and correlated positively with the amount of chips consumed ($r=0.26$, $p=0.07$). The present study helps further characterize trait dietary self-control

by showing that high compared to low self-controllers eat less, have lower body mass index, and display less activation of the DLPFC and the salience network to suppress food craving.

**Disclosures:** **J. Han:** None. **U. Vainik:** None. **K. Larcher:** None. **J. Guan:** None. **A. Dagher:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.14/UU73

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant R01 MH109520

**Title:** Cognitive control networks contain a mixture of diverse connectivity patterns characteristic of predicted flexible hub mechanisms

**Authors: *T. ITO**, M. W. COLE
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**Abstract:** It is remarkable that task-relevant information distributed across diverse brain systems can be coordinated to perform complex tasks such as reading and interpreting this sentence. Applying the tools of network neuroscience (Rubinov and Sporns, 2010) to this problem, we sought to identify a basis for such inter-network coordination in the human brain's large-scale intrinsic functional connectivity architecture. We recently found evidence that task information is coordinated by flexible hubs - frontoparietal regions with widespread connectivity patterns that change according to task instructions (Cole et al., 2013). We hypothesized that flexible hubs require diverse intrinsic connectivity patterns to select from in order to code for the diverse tasks humans are capable of. We developed a new graph theoretical measure - integrated pattern diversity - to test whether intrinsic functional connectivity patterns are capable of providing such diversity for putative flexible hub networks. We found that the frontoparietal control network (FPN), which was recently identified as a likely flexible hub network based on task-evoked functional connectivity, had the highest integrated pattern diversity. We next characterized why the FPN exhibited such high integrated pattern diversity. Using community detection, we clustered similar connectivity patterns to split each functional network into subnetworks. We found that the FPN subnetwork organization had the highest modularity, suggesting that FPN had the greatest out-of-network connectivity pattern separation. These findings suggest FPN coordinates diverse functionality distributed throughout the brain. This is highly consistent with hypothesized flexible hub functionality and is likely important for coordinating performance of diverse task sets.

**Disclosures:** **T. Ito:** None. **M.W. Cole:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.15/UU74

**Topic:** H.02. Human Cognition and Behavior

**Support:** MOST 104-2410-H-431-006

MOST 105-2410-H-431-020

**Title:** An effective EEG neurofeedback training protocol to improve executive functions and to reduce depressive rumination

**Authors:** ***P.-Z. CHEN**, W.-L. LIN, S.-H. YU, C.-Y. TSENG
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**Abstract:** Depressive rumination is considered one of the important factors that foster the maintenance and relapse of depression. Recent studies have found this response style closely related to individuals' reduced executive functioning (EF), that is, greater difficulty in updating, switching, or inhibiting negative thoughts. Given that human's prefrontal cortex is where EFs located, the present study aimed at developing an effective EEG neurofeedback training (NFT) protocol to improve EFs and reduce depressive rumination. Previous studies have found that peak alpha frequency (PAF) in prefrontal cortex of healthy adults is normally 10-11 Hz. Thus, in this study, participants in the NFT group (n = 11) were trained to increase 10-11 Hz PAF power in prefrontal detected by auditory feedback for 20 training sessions, 30 min per each. Participants in the active control group (n = 11) did not receive neurofeedback training but instead were instructed to count the prepared sounds while their brainwaves were recorded. All participants were first screened as individuals with high rumination and high depression tendencies and received pre- and post-tests in
EFs tasks (updating, switching, and inhibition), depressive rumination and depression (BDI-II) assessments. The results showed a steady and significant increase of 10-11 Hz activity over 20 training sessions in the NFT group. The power of the rest of the alpha frequencies did not show significantly change. No change in both PAF and whole alpha frequency power were found in the active control group. These findings indicate the effectiveness and specificity of the training protocol applications. In addition, participants in the NFT group gained significant improvement in the EF task performances, while significantly decreasing in depressive rumination and depression symptoms assessments simultaneously. These findings contribute to uncovering the mechanism between EFs and depressive rumination, and provide implications in effectively preventing or intervening mood disorders.

Keywords: neurofeedback training, peak alpha frequency (PAF), executive function, depressive rumination

**Disclosures:** **P. Chen:** None. **W. Lin:** None. **S. Yu:** None. **C. Tseng:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

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**Program#/Poster#:** 619.16/UU75

**Topic:** H.02. Human Cognition and Behavior

**Support:** NINDS Grant NS0802069

NINDS Grant NS040813

NIMH Grant MH063901

**Title:** Causal evidence for lateral prefrontal cortex dynamics supporting cognitive control

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**Abstract:** The lateral prefrontal cortex (LPFC) is essential for higher-level cognition, but how its interactions support cognitive control remains elusive. Previously (Nee and D'Esposito, 2016), dynamic causal modeling (DCM) indicated that mid LPFC integrates abstract, rostral and concrete, caudal influences to inform context-appropriate action. Here, we use continuous theta-burst transcranial magnetic stimulation (cTBS) to causally test this model. cTBS was applied to three LPFC sites and a control site in counterbalanced sessions. Behavioral modulations resulting from cTBS were largely predicted by information flow within the previously estimated DCM. However, cTBS to caudal LPFC unexpectedly impaired processes presumed to involve rostral LPFC. Adding a pathway from caudal to rostral LPFC significantly improved the model fit and accounted for the observed behavioral findings. These data provide causal evidence for LPFC dynamics supporting cognitive control and demonstrate the utility of combining DCM with causal manipulations to test and refine models of cognition.

**Disclosures:** **D.E. Nee:** None. **M. D'Esposito:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.17/UU76

**Topic:** H.02. Human Cognition and Behavior

**Title:** Towards a multidimensional model of everyday cognitive failures: Integrating brain hemodynamics, cognitive functioning, mental health and personality in a sample of young adults

**Authors: *M. A. ROMANO-SILVA**[1], D. S. COSTA[2], D. M. MIRANDA[1], J. J. DE PAULA[2]
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**Abstract:** Background: Everyday cognitive failures are state-like difficulties in attention, memory or executive control which occurs in daily life. Previous studies correlated everyday cognitive failures with measures of mental health, personality, and cognitive functioning. There are however few studies investigating its biological underpinnings. There are also only a few studies which tried to integrate these previous factors. **Objective**: analyze how personality, mental health, cognitive functioning and brain fronto-parietal hemodynamics are associated with cognitive failures. **Methods**: we assessed 366 adults, with a mean age of 29±9 years. All answered the cognitive failures questionnaire (CFQ), questionnaires of mental health, personality and psychosocial measures. A subsample of 115 participants was submitted to cognitive assessment, including tests of language, visuospatial skills, memory, attention and executive functions. Another subsample of 28 subjects performed an experimental n-back test, a common neuropsychological measure of cognitive control during fNIRS, a brain imaging technique which allows the analysis of cortical hemodynamics. Regression and mediation models tested the association between all variables. **Results**: we found significant contributions of internalizing and externalizing symptoms with CFQ. CFQ scores were also associated with satisfaction with life, emotional stability, and conscientiousness. At the cognitive level, the main predictor's inhibitory control and cognitive flexibility. Higher left-frontal and lower right-parietal hemodynamics correlated with higher CFQ scores. Structural models suggest that measures of inhibition and cognitive flexibility were associated with mental health. Measures of mental health showed a direct effect in CFQ scores and indirect effects mediated by personality traits. Personality traits also were mediators of the association of CFQ scores and satisfaction with life. **Conclusion**: Brain hemodynamics during cognitive control are associated with cognitive failures, a relationship probably mediated by executive functions and mental health. Personality showed a major role in our model, acting as a mechanism for the association between mental health and cognitive failures and those with life satisfaction.

**Disclosures:** **M.A. Romano-Silva:** None. **D.S. Costa:** None. **D.M. Miranda:** None. **J.J. de Paula:** None.

**Poster**

**619. Cognitive Control and Performance**

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**Topic:** G.03. Emotion

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National Institute on Drug Abuse: DA027764 in US

**Title:** Brain dynamics involved in controlling physiological arousal improve task performance

**Authors:** ***N. WATANABE**[1,2,3,4], J. P. BHANJI[1], H. C. TANABE[3], M. R. DELGADO[1]
[1]Dept. of Psychology, Rutgers Univ., Newark, NJ; [2]Postdoctral Fellowship for Res. Abroad, Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Grad. Sch. of Informatics, Nagoya Univ., Nagoya, Aichi, Japan; [4]Natl. Inst. of Information and Communications, Osaka, Japan

**Abstract:** Successful execution of a difficult task is influenced by the ability to keep calm and carry on while preparing to perform. For instance, the incentive to win the game can cause excessive physiological arousal when a basketball player prepares to take a final free throw. The player must control this response to perform successfully under pressure. An intriguing question is how the brain controls arousal to improve performance. We investigated this question with a simple time-perception-motor task and simultaneous data acquisition of pupil dynamics and BOLD signal by fMRI (n = 22). We identified two brain regions that were recruited prior to task execution - when participants were in a “preparatory phase” - that corresponded with performance. The caudate nucleus correlated with trial-wise reward magnitude, with greater increases associated with trials that resulted in failure ($p < 0.05$ FWE corrected with nonparametric permutation test (NPPT)). The amygdala correlated with trial-wise physiological arousal characterized by pupil dilation, with a relative increase during trials resulting in failure compared to success ($p < 0.05$ FWE NPPT). These results suggested that an exaggerated response in the caudate and amygdala during the preparatory phase compromised performance success. Next, we probed the question of how other cortical regions may contribute to this circuitry to facilitate task performance by using brain-network analyses. First, a physio-physiological interaction functional connectivity analysis was conducted to identify brain regions associated with activation in the caudate and amygdala during the preparatory phase. We

identified a cluster in the orbitofrontal cortex (OFC; $p < 0.05$ FWE corrected). Then, to clarify the dynamics of this system, we conducted an effective connectivity analysis with dynamic causal modeling (DCM; Friston et al., 2003). Post-hoc Bayesian model optimization (Friston and Penny, 2011; Rosa et al., 2012) in DCM revealed the best network model characterized by: (1) driving signal inputs into the OFC; (2) bidirectional positive intrinsic connections between the OFC and amygdala and between OFC and caudate; and (3) the intrinsic positive connection from OFC to amygdala was negatively modulated only in success trials. The confidence level of this model was higher than other candidate models and the ratio of evidence for the best and second best was 164.38. These results are consistent with a potential role for the OFC in controlling and integrating information from amygdala and caudate nucleus during task preparation to improve performance during task execution.

**Disclosures:** **N. Watanabe:** None. **J.P. Bhanji:** None. **H.C. Tanabe:** None. **M.R. Delgado:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.19/UU78

**Topic:** H.02. Human Cognition and Behavior

**Support:** N000141612832

**Title:** Evaluating the relationship between activity in frontostriatal regions and successful and unsuccessful performance of a context-dependent rule learning task

**Authors: *A. E. CHANG**[1], Y. REN[1], A. S. WHITEMAN[1,2], C. STERN[1]
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**Abstract:** The goal of this study was to investigate the relationship between BOLD activity and successful performance in a context-dependent rule learning task. In particular, this study examined the contributions of prefrontal, medial temporal, and striatal regions to context dependent rule learning for successful and unsuccessful learners. Healthy participants (n = 31) learned to associate different object pairs with spatial contexts through trial and error during fMRI scanning. Our analyses sought to characterize the relationship between regional activity and behavioral performance in two ways. First, we examined how total average activity across the learning time series (324 trials) was correlated with individual cumulative accuracy. Second, we grouped participants into successful and unsuccessful learning groups and compared activity changes within PFC and striatal regions during early, middle, and late stages of the task. Preliminary analyses revealed strong positive correlations between total average activity and

individual cumulative accuracy in dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and striatum. Outside of the scanner, participants were given a forced choice retrieval task in which they were provided one half of the object pair presented during learning in a spatial location and were asked to identify the correct associate. We identified a similar positive relationship between accuracy on the subsequent retrieval task and activity during learning in our 3 regions of interest. At the group level, we found that activity in dorsolateral and ventrolateral cortex increased across time in successful learners but not in unsuccessful learners. Activity in these regions was found to be higher during late stages of the task in successful learners. These results suggest that the striatum and regions of the prefrontal cortex play an important role in the successful acquisition of context dependent rules during learning.

**Disclosures:** **A.E. Chang:** None. **Y. Ren:** None. **A.S. Whiteman:** None. **C. Stern:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

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**Program#/Poster#:** 619.20/UU79

**Topic:** H.02. Human Cognition and Behavior

**Support:** Wellcome Trust (104580/Z/14/Z)

ERC grant 313398 INTERACT

**Title:** Prefrontal cortical activation associated with pro-social prospective memory behaviour in a naturalistic setting

**Authors: *A. F. HAMILTON**[1], C. AICHELBURG[2], P. PINTI[2], A. MERLA[3], S. GILBERT[2], I. TACHTSIDIS[2], P. BURGESS[2]
[2]ICN, [1]UCL, London, United Kingdom; [3]UNIVERSITÀ DEGLI STUDI G.D'ANNUNZIO, Pescara, Italy

**Abstract:** Prefrontal cortex, especially area 10 plays a critical role in creating and maintaining delayed intentions, but it difficult to investigate social delayed intentions in a typical neuroimaging environment where the participant is isolated and limited in movement. In this experiment we used wireless fNIRS to examine prefrontal cortex activations during the maintenance and execution of intentions relating to social vs. non-social cues in a naturalistic environment. The experiment was conducted outside, on a typical London street. 19 participants undertook a prospective memory (PM) task in which they walked around Queen Square performing an ongoing task while also remembering to respond to PM targets. A typical ongoing task was counting particular words on street signs. Social targets were a confederate standing at predetermined locations around the square; non-social targets were parking meters. Participants

were instructed to approach and fist-bump each target. Prefrontal cortex activity was monitored using a 16-channel Wearable Optical Topography (WOT, Hitachi High-technologies Corporation, Japan) fNIRS system (sampling frequency=5 Hz). Data was analysed using NIRS-SPM with a design matrix modelling each task block and the PM events, which fitted for each data channel and signal type (oxyHb / deoxyHb) separately. Relative to baseline tasks, the PM blocks showed to significantly increased oxyHb/decreased deoxyHb in a wide number of medial and lateral PFC regions. There were more specific medial rostral PFC changes relative to the ongoing task only, which is broadly in accordance with findings from fMRI and PET studies of prospective memory. Most critically, the social PM condition saw a significant increase in oxyHb / decrease in deoxyHb in lateral prefrontal cortex regions. These results suggest that the behavioural advantage for pro-social delayed intentions is reflected in differences in activation within prefrontal cortex, and that this can be detected in rich naturalistic settings.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.21/UU80

**Topic:** H.02. Human Cognition and Behavior

**Title:** Stimulating the performance monitoring network: The long and short of it

**Authors: *N. A. PAQUETTE**[1], G. A. BUZZELL[2], P. J. BEATTY[1], F. BADER[1], P. SINCLAIR[1], K. CRAVEN[1], P. M. GREENWOOD[1], M. S. PETERSON[1], C. G. MCDONALD[1]
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**Abstract:** Longstanding research on the performance monitoring system has shown that error detection often serves as a signal to improve ongoing behavior. Increased activation of the Performance Monitoring Network (PMN) has been shown to lead to improved performance after errors when sufficient time is allotted between trials. However, recent findings show that under time constraints this improved performance following errors is not seen and that activation of the PMN can lead to markedly reduced sensory attention following error commission trials with short response-stimulus intervals (RSIs). To expand on these findings, we applied transcranial direct current stimulation (tDCS), a form of non-invasive brain stimulation, to the Medial-Frontal Cortex (MFC), a subdivision of the PMN. Following either sham or anodal stimulation, we then assessed post-error accuracy (PEA) after error trials with either a short or long RSI. Preliminary data are consistent with recent literature; a significant reduction in PEA was observed for short, compared to long RSI trials, regardless of the stimulation condition. This supports the notion that

the PMN impairs post-error behavior under time constraints. However, given that preliminary data did not demonstrate a significant effect of MFC stimulation on PEA, the data are consistent with the notion that a subdivision of the PMN other than the MFC is responsible for error-induced distraction (i.e. reduced PEA after errors with a short RSI). Data collection remains ongoing and the results will be discussed within the broader framework of the capacity limits of the performance monitoring system.

**Disclosures:** **N.A. Paquette:** None. **G.A. Buzzell:** None. **P.J. Beatty:** None. **F. Bader:** None. **P. Sinclair:** None. **K. Craven:** None. **P.M. Greenwood:** None. **M.S. Peterson:** None. **C.G. McDonald:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.22/UU81

**Topic:** H.02. Human Cognition and Behavior

**Support:** JSPS Grant-in-Aid for Young Scientists (B) #15K21414

**Title:** Influence of after-effects on preparatory attention depending on difficulty of tasks

**Authors: *M. TAKAYOSE**[1], R. KOSHIZAWA[2], K. OKI[3]
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**Abstract:** When the stop trial precedes the go trial in the stop-signal task, the executive function of the go trial is affected. Contingent negative variation (CNV) was measured to elucidate the relationship between the after-effects on preparatory attention and the difficulty levels of response pattern-dependent tasks.
In these tasks, the participants were required to press a key when a go-signal was presented; however, in the stop-signal task (SST) and change-signal task (CST), they were required to occasionally withhold this response when the go-signal was followed by a stop-signal and change this response when the go-signal was followed by a change-signal, respectively. In the multi-CST (m-CST), the participants were occasionally required to withhold or change their response when the go-signal was followed by a stop- or change-signal. To obtain the CNV, EEG recordings were taken from all participants during the tasks.
The response time (RT) just after stop and/or change trials was extended in all tasks. In the SST and CST, RT was decreased and the CNV amplitude was larger after successful trials than after trials with failed inhibition/change. In the m-CST, RT was increased and the CNV amplitude was decreased more than in the other tasks. Success with inhibition/change in relatively simple tasks

had little influence of aftereffects on preparatory attention. When the difficulty of the task was higher, its influence of after-effects was larger and may reduce preparatory attention.

**Disclosures:** **M. Takayose:** None. **R. Koshizawa:** None. **K. Oki:** None.

**Poster**

**619. Cognitive Control and Performance**

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**Program#/Poster#:** 619.23/UU82

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant P50 MH106435

NIH Grant R01 MH045573

**Title:** Corticostriatal networks reveal the role of the dorsal anterior cingulate cortex as a routing hub for cortical output integration

**Authors: *W. TANG**[1], S. N. HABER[2,1]
[1]Basic Neurosci. Div., McLean Hosp., Belmont, MA; [2]Univ. of Rochester, Rochester, NY

**Abstract:** Recent studies have found that cortical projections to the striatum form convergent zones. These special zones, conceptualized as hubs, are likely to facilitate network interactions among the corticostriatal pathways. Similarly, we have also identified a hub in the dorsal anterior cingulate cortex (dACC), which may facilitate corticocortical interactions. To further understand how the dACC hub and the striatum mediate different levels of functional integration, in this study we investigate the anatomical connectivity between the prefrontal cortex (PFC), the dACC hub and the striatal convergent zones.
Tract tracing was carried out in macaque monkeys. Bidirectional or anterograde tracers were injected into areas covering all divisions of the PFC and the dACC. We mapped out (1) the convergent PFC projections to the striatum, (2) the convergent PFC projections to the dACC hub, and (3) the dACC hub projections to the striatal convergent zones. The results showed a three-way connectivity pattern that links the dACC hub with the corticostriatal network: The dACC hub receives inputs from many different PFC areas; the same set of PFC areas form multiple striatal convergent zones. Moreover, the dACC hub projects to significantly more convergent zones than the other dACC regions outside the hub.
These results showed that the dACC hub is influenced by a variety of PFC areas. But, the hub also plays a role in mediating the output of these areas in the striatum. PFC areas directly influence striatal convergent zones while the striatum also receives integrated information from the dACC hub. This mechanism enables crosstalk between different functions, which can be key for flexible switching between behaviors.

**Disclosures:** **W. Tang:** None. **S.N. Haber:** None.

**Poster**

**619. Cognitive Control and Performance**

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**Program#/Poster#:** 619.24/UU83

**Topic:** G.03. Emotion

**Support:** NIH R21 MH109722-01A1

**Title:** Contributions of cortico-striatal pathways to the modulation of cognitive flexibility

**Authors:** ***A. E. REIMER**, M.-C. LO, M. F. MURILLO, M. R. MILAD, A. S. WIDGE
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**Abstract:** The involvement of cortico-striatal circuits has been implicated in a variety of cognitive and executive processes, including working memory, decision-making, inhibitory response control, and attentional set-shifting. Deficits in these processes are common in several psychiatric disorders such as schizophrenia, obsessive-compulsive disorder (OCD), and major depression (MDD). Considering that deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) is effective in reducing the symptoms of refractory OCD and MDD, we hypothesize that DBS may act by improving executive processes that lead to increased cognitive flexibility. Here, we investigated how DBS-like electrical stimulation of the dorsal portion of the ventral striatum (VS) in rodents affects cognitive flexibility in an operant set-shifting paradigm. Bipolar electrodes were implanted bilaterally aimed to the VS and biphasic DBS (200 μA, 0.1 ms pulse duration, 130 Hz, bipolar) was delivered continuously for 2 h prior to a set-shifting test. Additionally, in a different set of animals, electrolytic lesions were targeted to the same brain region. DBS improved animals' performance, demonstrated by faster reaction times (DBS OFF 2.28 ± 0.26, DBS ON 1.70 ± 0.24), and reduction in the number of errors (OFF 7.33 ± 1.16, ON 3.5 ± 3.17), and trials to complete the test (OFF 35.89 ± 5.27, ON 20.5 ± 7.79). On the other hand, electrolytic lesion impaired animals' performance, demonstrated by slower reaction times (pre-lesion 5 ± 2,00, post-lesion 54.25 ± 2.75) and increase in the number of errors (pre 3 ± 0.95, post 6.57 ± 2.35), and trials to complete the test (pre 18.33 ± 2.80, post 28.14 ± 8.80). Considering the importance of the prefrontal cortex (PFC) in cognitive flexibility, our data suggest that DBS may affect PFC functioning in ways that improve flexibility. This might occur by modulation of striatum/thalamus through efferent cortico-striatal projections, or by antidromically activating those same fibers and directly modulating PFC. Further experiments will clarify which PFC sites are most involved in this modulation.

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**Poster**

**619. Cognitive Control and Performance**

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**Program#/Poster#:** 619.25/VV1

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH DA026452

**Title:** Tonic cortical inhibitory control while withholding a specific action to relieve a strong urge

**Authors: *K. K. SUNDBY**, A. R. ARON
Dept. of Psychology, Univ. of California San Diego, La Jolla, CA

**Abstract:** A better understanding of urge is relevant for clinical disorders, daily experience, and theoretical issues in cognitive/motor control. It has been proposed that an urge has three core components: the experience of discomfort, the impulse to perform an action to relieve the discomfort, and the requirement to withhold the relieving-action itself. Here we tested if tonic cortical inhibitory control (of the action) is indeed a constitutive component of urge. We designed an experiment in which the subject experienced a strong impulse to press a button to relieve heat pain, but needed to resist pressing (over about 8 seconds) to avoid incurring a large loss. In 16 healthy young participants, we used paired pulse transcranial magnetic stimulation (TMS) over the motor cortex contralateral to the finger that could press to relieve the heat pain. With this paired-pulse TMS method we measured short intracortical inhibition (SICI) - which indexes GABAergic tone in M1. ANOVA was performed with finger (relevant, non relevant), and time point (baseline, timepoint 1 early, timepoint 2 late during the urge) with SICI as the dependent measure. There was a significant interaction between finger and timepoint $F(2,30) = 3.37$, $p<0.05$. Subsequent planned comparisons showed that SICI in the task-relevant finger alone was increased above baseline at timepoint 1( $p<.05$ ) and timepoint 2( $p<0.05$). This shows that tonic cortical inhibitory motor control is in place over many seconds during an urge. The result shows that this is specific for the effector that could move to relieve the discomfort - suggesting the result is unlikely a confound of arousal/stress per se. This is the first result to clearly demonstrate tonic suppression of action over multiple seconds.

**Disclosures: K.K. Sundby:** None. **A.R. Aron:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.26/VV2

**Topic:** H.02. Human Cognition and Behavior

**Support:** NINDS Grant NS065046

NIMH Grant MH099078

NIMH Grant MH111737

ONR MURI award N00014-16-1-2832

**Title:** Characterizing human prefrontal cortex representations with fMRI

**Authors: *A. BHANDARI**[1], M. RIGOTTI[3], C. GAGNE[4], S. FUSI[5], D. BADRE[2,6]
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**Abstract:** A central problem in cognitive neuroscience is understanding the nature and form of human prefrontal cortex (PFC) representations, which play a critical role in the expression of flexible, goal-directed behavior. An important property of such representations is their dimensionality. High-dimensional representation of task variables can support flexibility by enabling linear readouts of multiple, different conjunctions of task variables from the same representation. Indeed, in highly trained macaques, PFC representations of task variables approach maximum dimensionality, and this property predicts success on the task. Here we evaluate two proposed methods for estimating dimensionality from fMRI data. The first method involves enumeration of the number of arbitrary conjunctions of task variables that can be successfully decoded through multi-voxel pattern analysis (MVPA) of BOLD patterns. However, we demonstrate through a meta-analysis of published MVPA studies that the base rate for decoding information from PFC patterns is remarkably low, hampering the use of this method for estimating dimensionality. The second method involves estimating the similarity structure of the representation via cross-condition repetition suppression. This use of this method is complicated by the need to simultaneously estimate a large number of repetition suppression effects in the presence of low frequency fMRI noise. We evaluate the efficacy of a variety of fMRI designs, including isolated pair presentation and continuous carry over designs with deBruijn sequences for recovering representational dimensionality in simulations. Finally, we

evaluate this method empirically in an fMRI experiment for estimating dimensionality of prefrontal cortex representations.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.27/VV3

**Topic:** H.02. Human Cognition and Behavior

**Support:** Research Council of Norway, project number 240389

**Title:** The role of different prefrontal subareas in the inhibition of proactive interference during verbal working memory

**Authors: *A. LLORENS**[1], I. FUNDERUD[3], A. O. BLENKMANN[3], J. LUBELL[3], M. D. FOLDAL[3], T. R. MELING[2], A.-K. SOLBAKK[3,2], T. ENDESTAD[3], R. T. KNIGHT[4]
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**Abstract:** Verbal WM (vWM) can sometimes be disturbed by prior relevant information that is no longer needed. This is called proactive interference (PI). The inhibition of PI is fundamental for successful cognition. Imaging studies have found that the lateral prefrontal cortex, specifically on the left side, plays a role in the resolution of PI during vWM. Other studies show that the orbitofrontal cortex (OFC) is involved in WM operations, but its specific function during inhibition of PI is unknown. Our aim was to disentangle the role of prefrontal subareas in inhibitory mechanisms during vWM. We recruited a substantial cohort of patients with focal frontal lesions (17 bilateral OFC lesions, 7 right and 4 left lateral frontal) and compared performance between groups and against a matched healthy (age, education and IQ) control sample (n=16). Behavioral and EEG data were recorded while participants performed a Recent-Probes task. They briefly saw a list of 5 letters presented in succession, and were next asked whether a given letter was in the list. There were four conditions: the letter was (i) in the list, (ii) not in the list, (iii) in the list and in the previous trial's list, and (iv) not in the list but in the previous trial's list. In the last condition (iv), PI will be strongest. The memory trace from the previous list has to be inhibited in order to answer correctly. Behavioral results showed that when left and right lateral frontal lesion groups were merged, there was a significant group effect (p=.010) in the condition with highest PI (i.e: iv). Post hoc analysis revealed that the lateral patients were significantly slower than controls (+217 ms; p=.011) and the OFCs (+182 ms;

p=.035). There was no difference between the OFC and control groups. The main effect remained after splitting the lateral lesion group into left and right, (p=.017) for condition iv. Further analysis showed that the left lateral patients were significantly slower than controls (+294 ms; p=.03) and tended to be slower than the OFC patients (+258 ms; p=.066). Right lateral patients did not perform significantly differently than the other groups. These results indicate a differential role for specific prefrontal subareas during inhibition of PI, confirming that the left lateral prefrontal cortex is critically involved in inhibition of PI, but that the OFC is not. Our next step is to map patient lesion locations in conjunction with analysis of their EEG data recorded concurrently with task performance. We believe that the results will provide important data which are expected to elucidate the underlying electrophysiological dynamics of vWM inhibitory processes.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.28/VV4

**Topic:** H.02. Human Cognition and Behavior

**Support:** MRC-intramural program (MC-A060-5PQ10)

**Title:** Externally focused task switch activity in the “internally-directed” default mode network

**Authors: *V. SMITH**, D. J. MITCHELL, J. DUNCAN
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**Abstract:** A predominant finding in the literature is that the default mode network (DMN) shows decreased activity during externally-focused tasks compared to rest (Shulman et al., 1997). This finding has led to an emphasis in DMN research on internally-focused self-relevant thought processes including mind wandering, autobiographical memory, autobiographical planning, theory of mind and mental navigation. However, more recent research has questioned this emphasis on internal cognition (Baldassano et al., 2016; Chen et al., 2017). Most relevant to our study, Crittenden et al. (2015) found increases in DMN activity related to large changes in externally-focused tasks compared to small task changes or stay trials. Our study aimed to enhance our understanding of the function of the DMN during task switching. Using functional magnetic resonance imaging, we scanned 24 participants whilst switching between different tasks and brief rest periods. As well as replicating DMN task-switch effects, we also found large DMN increases for brief rests and task restarts after rest. Furthermore, multivoxel pattern

analysis showed distinct activity patterns during a task cue period for task types of different stimulus domains. Our findings are difficult to explain using theories of DMN function strictly linked to internal or self-directed cognition. In line with principal results from the literature, we propose that the DMN encodes scene, episode or context, by integrating spatial, self-referential and temporal information. Context encoding, we propose, plays a direct role in implementation and control of current cognition, be it internally or externally focused. Specifically, re-reference to the context is involved in large cognitive switches, from task to rest, rest to task, or between very different task domains.
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Crittenden, B. M., Mitchell, D. J., & Duncan, J. (2015). Recruitment of the default mode network during a demanding act of executive control. *Elife, 4*, e06481.

**Disclosures: V. Smith:** None. **D.J. Mitchell:** None. **J. Duncan:** None.

**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.29/VV5

**Topic:** H.02. Human Cognition and Behavior

**Title:** Structural correlates of executive function measures in older adults

**Authors: *H. L. LINDROTH**[1], R. MOHANTY[2], P. ROWLEY[3], V. A. NAIR[4], V. PRABHAKARAN[5], R. D. SANDERS[6]
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**Abstract: Objective**
To investigate the structural correlates of older adults performance with measures of executive function and verbal fluency.
**Methods**
Prospective cohort study of 29 older adults (≥65yo; mean age 72.9±4.7 years, 14 male)

undergoing an elective noncardiac surgery with a projected length of stay of two or more days. Recruitment is ongoing. Demographics including age, gender and education level were collected through interviews. Participants underwent preoperative MRI structural imaging and comprehensive neuropsychological testing including Trail Making Test B (TMT-B) and Controlled Oral Word Association Test (COWAT). MRI sequences were processed using FreeSurfer software, OSX v.6.0. The ENIGMA-3 protocol was applied for extraction of cortical measures (www.enigma.ini.usc.edu)**.** Statistical analysis was conducted using SPSS, $p \leq 0.05$ was considered significant. Descriptive statistics followed by stepwise regression were performed to identify significant structural predictors of test performance. All brain regions (68) were entered into a stepwise regression equation for model-building. $R^2$ values and statistical significance are reported. Cluster-wise analyses were conducted using Freesurfer Qdec. Age, gender, left and right cortical thickness averages were used as covariates.

**Results**

Twenty-nine participants were analyzed. The mean score on TMT-B was 96.41 seconds (56.86SD) and COWAT-Total 33 words (11SD). The stepwise regression model most predictive of TMT-B performance demonstrated an adjusted $R^2$ of 0.713 ($p$-0.0001) and included left temporal pole, left posterior cingulate, left parstriangularis (inferior frontal gyrus), right parsorbitalis (inferior frontal gyrus) and right paracentral lobule. The regression model most predictive of COWAT-Total demonstrated an adjusted $R^2$ of 0.220, ($p$-0.006) and included left superior temporal gyrus. Age, gender, left and right cortical thickness averages were not significantly correlated with these identified regions and were not selected in the stepwise regression model. Cluster-wise analysis corrected for multiple comparisons revealed a significant correlation ($p$=0.04) between COWAT-Total and the left superior temporal gyrus. TMT-B did not demonstrate a significant correlation in cluster-wise analysis.

**Conclusion**

In this cohort, structural differences in select frontal and temporal regions were predictive of performance on TMT-B and COWAT-Total tests, independent of age and gender. The identified predictive regions are consistent with prior findings in neuroimaging studies of executive function and language.

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**Poster**

**619. Cognitive Control and Performance**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 619.30/VV6

**Topic:** H.02. Human Cognition and Behavior

**Title:** Functional connectivity and morphometric correlates of cognitive outcomes in elderly subjects using machine learning-based predictive model

**Authors: *R. MOHANTY**[1], P. ROWLEY[2], H. LINDROTH[2], V. A. NAIR[1], V. PRABHAKARAN[1], R. D. SANDERS[2]
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**Abstract:** Objective: Healthy aging is associated with changes in cognitive domains in the human brain. This study examines how resting-state functional connectivity and thickness of cortical regions correlate to two measures of executive control function assessed during neuropsychological tests, namely, the Trail Making Test-B (TMTB) and Controlled Oral Word Association Test (COWAT) in a group of elderly subjects with the help of a machine learning regression model.
Methods: This study included pre-operative data from 29 subjects aged over 65 years (mean age = 72.9±4.6 years, 15 female/14 male) who underwent non-cardiac surgery. Subjects were scanned for resting-state functional MRI and structural MRI. Additionally, all subjects underwent a battery of neuropsychological assessment that recorded their performance to TMTB (mean score = 96.4±56.8 seconds) and COWAT (32.3±11.1 words) which are typically associated with the executive control function. Seed-based functional connectivity was computed spanning 268 distinct brain regions. Cortical thickness measures were derived from structural MRI over 68 distinct cortical regions. A machine learning support vector regression model was implemented to perform a linear-kernel multiple regression by utilizing both functional and cortical information as input features to predict the two outcomes.
Results: Multiple measures from, both, functional connectivity as well as cortical thickness were involved in predicting TMTB ($R^2 = 0.7283$; $p < 0.001$) and COWAT ($R^2 = 0.7038$; $p < 0.001$) scores. For TMTB, the highly weighted predictors included functional connectivity involving left inferior parietal lobule and left postcentral gyrus, and cortical thickness of left pars triangularis. Among the strong predictors for COWAT were connectivity between left superior parietal lobule and left precuneus, and cortical thickness of left pars triangularis. Additionally, functional and cortical predictors from similar regions of the brain, such as left lingual gyrus, left middle temporal gyrus, etc. were found among the predictors of both outcomes.
Conclusion: Machine learning-based regression model shows potential for identifying specific functional and morphometric correlates of TMTB and COWAT performances. No impact of age and gender was found in either prediction. Emerging predictors involving left pars triangularis of inferior frontal gyrus, left superior parietal lobule are in line with findings in neuroimaging studies of the executive control function.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.01/VV7

**Topic:** H.02. Human Cognition and Behavior

**Title:** Effects of paced breathing on convergent and divergent thinking

**Authors: *A. K. HALT**[1], B. M. KILLE[1], B. J. FERGUSON[3], D. R. DRYSDALE[1], *B. E. SUTTON[1], B. HERRIOTT[2], D. Q. BEVERSDORF[4]
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**Abstract:** Previous studies have shown that propranolol, a beta adrenergic antagonist, can reduce the negative effects of stress on problem solving abilities. Furthermore, propranolol can improve performance on problem solving for the most challenging of tasks without the presence of stress. We aimed to determine whether non-pharmacological approaches could yield comparable effects. For example, the use of paced breathing, a technique used to slow breathing in an experimental setting, has been shown to increase heart rate variability, an indication of increased parasympathetic nervous system activation. This may give rise to increased problem solving ability, especially in terms of convergent and divergent thinking based task performance. The goal of this project is to better understand if paced breathing can be implemented to improve problem solving in addition to convergent and divergent thinking. We hypothesized that paced breathing—as compared to normal breathing—will decrease an individual's cardiovascular response to stress, as measured by electrocardiogram (ECG) and improve performance on convergent and divergent thinking tasks. Using a repeated measures design, participants engaged in counterbalanced paced breathing and normal breathing sessions. In both the paced and normal breathing sessions, cognitive tasks administered include the alternate uses task (scored for originality, flexibility, and elaboration), semantic fluency task (scored for originality and semantic distance), and letter fluency task (scored for originality and elaboration). We expect that scores on originality, flexibility, elaboration, and semantic distance will be higher for paced breathing than normal breathing sessions due to increased cognitive flexibility attributed to decreased cardiovascular response to the stress of cognitive testing. If relaxation techniques are as effective as propranolol in affecting problem solving, it can benefit those who suffer from cognitive or problem solving deficits and cognitive impairments related to stress.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.02/VV8

**Topic:** H.02. Human Cognition and Behavior

**Support:** KAKENHI (26120732)

**Title:** Neural mechanisms for deciding with predicting others in human brain

**Authors: *N. MA**[1], N. HARASAWA[1], K. UENO[2], N. ICHINOHE[3], M. HARUNO[4], K. CHENG[5], H. NAKAHARA[1]
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**Abstract:** In social environments, we make decisions, considering what others would do. Specifically, even when we make choices for our own reward, the expectation and associated decision-making often needs to depend on others' behavior. However, it is still poorly understood how humans incorporate the prediction of others' behavior into self-oriented decision in the neural and computational processes. To address this issue, we devised a novel behavioral task under value-based decision-making paradigm in human fMRI, combined with computational modeling. In our task, there are main trial and two types of control trial, self and other trials. Main trial required the subject to choose an option, using the prediction of others' choice, because a set of options to choose depended upon the other's choice. Self and other trials examined each component in the main trials: making ordinary, probabilistic self-value-based decisions and plainly predicting others' decisions themselves, respectively. Probing their behavior, first, we confirmed that the subject's behavior followed self-value difference and predicted-others' value difference in the self and other trials, respectively. In the main trials, the subject chose the option, governed by a balance of the two possible self-value differences that would be generated by two different predictions of others' choices (predicted-others' value difference), and the relative contribution of the two self-value differences varied along with the difficulty of predicting others' choices or the magnitude of predicted-others' value difference. Imaging results showed distinct neural circuits for predicting other’s choices between the other and main trials. In the other trials, the prediction significantly correlated the activities in anterior insula and right temporoparietal junction (TPJ), and also in ventromedial prefrontal cortex (vmPFC). In the main trials (and also self trials), the vmPFC activations were significantly correlated with the subject's own choices but not with the prediction. The prediction had

significant activations in dorsomedial prefrontal cortex and posterior insula across the subjects, and the rTPJ activations were rather correlated with the degree of performance in the mail trials. These results together indicate that additional neural circuits, and also differential roles of respective brain regions, are recruited for making use of prediction others' in self-oriented decisions.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.03/VV9

**Topic:** H.02. Human Cognition and Behavior

**Support:** KAKENHI26120732

KAKENHI16H06570

**Title:** Neural mechanisms for converting social value into one's own decision value

**Authors: *H. FUKUDA**[1], N. MA[1], S. SUZUKI[2], N. HARASAWA[1], K. UENO[1], J. L. GARDNER[3], N. ICHINOHE[4], M. HARUNO[5], K. CHENG[1], H. NAKAHARA[1]
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**Abstract:** The neural signals of internal reward valuation process comprise a self-oriented currency for decision-making. In social setting, our decisions are influenced by social concerns such as reward to others. However, it is poorly understood how social values converges into self reward valuation in the neural and computational process. Here, we demonstrate the social value conversion process, using a novel behavioral paradigm in human fMRI with computational modeling. The task is consisted of 3 types of trials. Standard trials access a basic valuation process, in which the subject chooses between two options, each of which is associated with one's own probabilistic outcome (standard reward). Other trials examine social value conversion, in which a number in an option additional to standard reward indicates an additional reward to others (donation to a pre-selected charity), while bonus trial is a control in which an additional number indicates an additional reward to the self (bonus), respectively. We indeed found a significant modification on the choice behavior by the others' reward, although the extent is weaker than that by bonus given the same face amount. We hypothesized three key elements for the neural process of social value conversion into self-own decisions: (1) other-value in offer, (2)

the effective influence by the offer on choices, as the effect (even with the same offer) may vary dependent on standard value difference and (3) the final decision value that accounts for both of the standard and additional rewards. The offered other-value was encoded in the right temporoparietal junction (rTPJ), and also in the left dorsolateral prefrontal cortex (ldlPFC), wherein the offered bonus value was encoded not in the rTPJ but in the ldlPFC. The effective other-value was reprsented in right anterior insula (rAI) activation and the final decision value in ventromedial prefrontal cortex (vmPFC). Psychophysiological interaction analysis lent a support for a staged processing of our hypothesis, from the offer to the effective influence on choices and then to decision; the rTPJ and ldlPFC responses significantly modulated the rAI responses that, in turn, influenced decision signals in vmPFC responses. By contrast, the ldlPFC responses had a direct impact on the vmPFC responses for bonus value. Further, we found that these characteristics of social neural conversion underlie different socio-behavioral isotypes, demonstrating the variability in the conversion between prosocial and selfish subjects: emphasis of rAI and ldlPFC coupling to vmPFC responses, respectively. These findings identified primary neuro-computational processes for social value conversion.

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**Poster**

**620. Decision Making and Reasoning**

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**Program#/Poster#:** 620.04/VV10

**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH grant MH112541

**Title:** Ambiguity seeking versus avoidance during probabilistic decision making: Effects of outcome type (financial loss or reward)

**Authors:** *C. GAGNE[1], ***C. GAGNE**[1], E. L. A. LAWRANCE[2], A. G. E. COLLINS[1], S. J. BISHOP[1]
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**Abstract:** Computational psychiatry provides a promising avenue for translating basic psychological and neuroscientific knowledge into the clinic (Huys 2016). Within this new field, there is increasing interest in computationally modeling individual differences in decision making and associating them with psycho-pathologically relevant traits. This approach has successfully linked specific decision making deficits to trait impulsivity (Gillan 2016) and trait depression (Huys 2012). However, to-date, little computational work has been done to

characterize the aberrant decision making observed in anxiety. Anxious individuals are known to find uncertainty aversive (Mitte 2007). We have previously shown trait anxiety to be linked to impoverished use of environmental volatility to update learning about aversive outcomes (Browning et al., 2015). Preliminary data from our lab also indicates that anxious individuals are ambiguity avoidant when making decisions about primary aversive outcomes (see poster by E Lawrance). In the current study, we investigate whether healthy volunteers show ambiguity seeking versus avoidance behaviors when making probabilistic decisions and investigate whether this varies as a result of psychopathy related traits including trait anxiety and trait depression. Participants performed a probabilistic decision-making task under conditions of reward and loss. The two options chosen between varied in probability and magnitude of outcome. For 50% of trials, information required to calculate probability of outcome was missing; level of missing information was manipulated in a continuous fashion. Preliminary data (n=120) reveals that participants are predominantly ambiguity averse under reward, but switch to be ambiguity seeking under loss ($t=-7.49$, $p<0.001$). This categorical effect was attenuated as choices became less ambiguous, with many individuals becoming ambiguity-neutral at low levels of ambiguity. Our preliminary data also suggests that in contrast to situations with primary aversive outcomes, trait anxiety is not significantly associated with increased ambiguity aversion under conditions of financial reward gain or loss ($r(118)=0.15$, $p=.20$; $r(118)=0.12$, $p=.27$). The specificity of anxiety-related ambiguity aversion to conditions involving potential future primary aversive outcomes fits well with existing characterizations of anxiety.

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**Poster**

**620. Decision Making and Reasoning**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.05/VV11

**Topic:** H.02. Human Cognition and Behavior

**Support:** ERC grant 260932

**Title:** Probabilistic decision-making under ambiguity when outcomes are aversive: Behavioral and brain correlates of trait anxiety

**Authors: E. L. A. LAWRANCE**[1], J. O' REILLY[1], J. BIJSTERBOSCH[1], C. GAGNE[2], T. E. J. BEHRENS[1], *S. J. BISHOP[2]
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**Abstract:** There is ambiguity associated with many choice options we encounter. For people with clinical or subclinical anxiety, intolerance of uncertainty and decision making difficulties are key psychological features, negatively interfering with daily life. It has been proposed that anxious individuals are also strongly ambiguity averse. Here we use a computational decision making framework to interrogate how anxiety influences probabilistic decision making under conditions of ambiguity about aversive future events.
Within the computational decision making literature, categorical and parametric ambiguity have been examined by manipulating, respectively, the presence or level of missing information about action-outcome contingencies. In the current study, we investigated how individual differences in trait anxiety influenced participants' performance on a probabilistic aversive decision making task while fMRI data were acquired. The experimental design used allowed us to separate out effects of categorical and parametric ambiguity. On each trial, the two options presented varied in probability and magnitude of outcome. For 50% of trials, information required to calculate the probability of outcome for one of the two options was obscured; level of missing information was manipulated in a continuous fashion. Our findings indicate that both categorical and parametric ambiguity aversion are present across our participant cohort, in line with previous demonstrations of ambiguity aversion. Across participants, both categorical and parametric ambiguity aversion varied positively with trait anxiety levels. Increased blood oxygen level dependent (BOLD) responses to categorical ambiguity were observed in dorsal anterior cingulate cortex (dACC) and inferior frontal sulcus (IFS). BOLD responses in these regions were also modulated by parametric ambiguity, with increased response to higher levels of missing information. The representation of missing information was also apparent in rostrolateral prefrontal cortex for subjects with high trait anxiety. A further model separating trials by choice revealed that high trait anxious subjects primarily show an increased IFS and dACC response to ambiguity on trials where they go on to choose the ambiguous option. This is consistent with high trait anxious participants requiring increased recruitment of these regions to overcome their heightened propensity to ambiguity aversion.

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**Poster**

**620. Decision Making and Reasoning**

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**Program#/Poster#:** 620.06/VV12

**Topic:** H.02. Human Cognition and Behavior

**Support:** Wellcome Trust

**Title:** Information seeking under stress and anxiety

**Authors: *C. J. CHARPENTIER**[1,2], M. GÄDEKE[2], T. SHAROT[2]
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**Abstract:** Information gathering is an integral part of learning and decision-making. Yet we know relatively little of how people implement the decision to know or remain ignorant. Classic theories focus on the instrumental utility of information. However, human behavior does not always fit nicely with those models, as in the case of patients at risk who avoid medical screening even when treatment is available. Here, we examine the hypothesis that information gathering is modulated by affect. In two studies we manipulate the expected valence of information and the affective state of the participants. We then record their willingness to pay (WTP) in order to receive or avoid information about their earnings in a financial market task. The information was non-instrumental; it could not be used to alter rewards and did not influence the earnings participants received at the end of the task. Our results reveal that participants were willing to pay more (1) to receive good news and *avoid* bad news and (2) in volatile markets. We next examined whether these effects were related to the affective state of the participants. We found that the influence of volatility, but not valence, on the desire for information was related to anxiety. In Study 1 more anxious individuals exhibited a stronger effect of market volatility on WTP for non-instrumental information. In Study 2 we induced anxiety using an acute stress manipulation. Consistent with the results of Study 1, individuals whose anxiety levels increased the most showed the strongest effect of market volatility on WTP for non-instrumental information. Our results highlight the tight relationship between affect and information seeking and suggest that anxious individuals attempt to reduce the burden of uncertainty by gathering (non-instrumental) information.

**Disclosures: C.J. Charpentier:** None. **M. Gädeke:** None. **T. Sharot:** None.

**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.07/VV13

**Topic:** H.02. Human Cognition and Behavior

**Title:** Mood and personality traits have minimal influence on temporal discounting behavior

**Authors: S. SPIVACK**[1], S. J. PHILIBOTTE[2], N. H. SPILKA[2], I. J. PASSMAN[2], *P. WALLISCH[3]
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**Abstract:** We are interested in the influence of mood and personality traits on temporal choice behavior. Previous research suggests that there is a link (Hirsh et al., 2010; Koff & Lucas, 2011;

Manning et al., 2014; Whiteside & Lynam, 2011). However, this research was underpowered. Here, we exposed 600 participants to stimulus material - music - known to induce changes in mood. Using this high powered sample, we can show that music does influence mood in predictable ways, but the effect of music on temporal choice behavior is modest, on the order of 1/25 of a standard deviation. In addition, personality traits such as extraversion or neuroticism did not predict temporal choice behavior. Our results put a low ceiling on the influence of music on temporal choice behavior and suggest that individuals are more impervious to deviate from rational choice due to incidental circumstances than commonly believed.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 620.08/VV14

**Topic:** H.02. Human Cognition and Behavior

**Title:** Vicarious subjective value representation in the human brain: An fMRI investigation

**Authors: *M. R. PIVA**[1], K. VELNOSKEY[2], R. JIA[1], I. LEVY[3], S. W. CHANG[2]
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**Abstract:** When making economic decisions, people are usually biased to the present moment. When asked if they would rather receive $5 today or $6 in 5 days, most would choose $5 today. However, when asked to choose between $5 in 180 days or $6 in 185 days, people are much more likely to choose the delayed higher amount. Thus, people seem to discount future rewards in a time-inconsistent manner when making choices that impact their own well-being. Extensive previous research cutting across economics, psychology, and neuroscience has indicated that decay of subjective value with increasing delay follows a hyperbolic rather than exponential decay function and is computed by a defined set of neural structures (e.g., Kable and Glimcher, 2007). By contrast, the behavioral and neural processes underlying how people make decisions that directly impact another individual remain largely unclear. Exploring this additional dimension of decision-making is both highly relevant to everyday life, in which making decisions for or about others is exceedingly common, and to further elucidating the computation of value representation in the brain. To address this issue, we designed a study in which participants were asked to make choices between an immediate monetary reward and various monetary rewards of greater values delivered following a delay. In half of trials, participants made decisions for themselves (“Self” trials), while in the other half of trials, participants made decisions for a randomly selected other participant (“Other” trials). We hypothesize that

ventromedial prefrontal cortex (vmPFC), ventral striatum (VS), and posterior cingulate cortex (PCC) encode subjective value in Self trials, while an overlapping but distinct set of neural structures encode vicarious subjective value in Other trials. Preliminary behavioral findings based on data from 15 participants indicate that Other choices, like Self choices, follow the expected hyperbolic rate of decay rather than exponential decay. We also found that participants displayed a higher rate of discounting in Self trials than in Other trials ($t(14) = 2.52$, $P = 0.025$, two-tailed paired *t*-test). Finally, choice consistency before and after each participant's determined indifference point was significantly higher in Self trials than in Other trials ($t(14) = 3.58$, $P = 0.003$, two-tailed paired *t*-test). These behavioral and upcoming univariate and multivariate analyses of concurrent BOLD signals acquired during fMRI sessions can provide additional insight into the shared as well as distinct neural activations underlying decision-making for self and other.

**Disclosures: M.R. Piva:** None. **K. Velnoskey:** None. **R. Jia:** None. **I. Levy:** None. **S.W. Chang:** None.

**Poster**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant MH005286

Yale Faculty of Arts and Sciences (FAS) Imaging Fund

National Science Foundation Graduate Research Fellowship

**Title:** Activity in the temporoparietal junction (TPJ) tracks dynamic changes in uncertainty when observing goal directed action

**Authors: *K. R. VELNOSKEY**, S. W. C. CHANG, G. MCCARTHY
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**Abstract:** The temporoparetal junction (TPJ) is a critical region for processing goal directed behavior (Allison, Puce, & McCarthy, 2000), yet the mechanism by which this occurs remains unknown. While some suggest that the TPJ is organized in domain-specific social modules (i.e. Deen et al., 2015), others suggest that it serves a domain general function (Geng & Vossel, 2013; Lee & McCarthy, 2016). In social contexts, this general function may be critical for modeling predictions about expected behavior. We propose that the TPJ is involved in dynamically updating such a model, and, in particular, that TPJ activation tracks changes in neural parameters for generating predictions over time.

We tested this hypothesis using a novel fMRI task in which participants (N=21) viewed a dot traversing a complex maze containing two possible goals. Participants continuously expressed their certainty about the goal that the dot was trying to reach by moving a horizontal slider. Mazes were either barrier free (High Uncertainty condition) or included some barriers to constrain the dot's path and reduce subjective uncertainty about the goal (Low Uncertainty condition). Critically, the dot's path was matched in both conditions. We examined how TPJ activity was modulated by these conditions using whole-brain general linear models (GLM) including a regressor derived from participants' slider positions for estimating subjective changes in goal target uncertainty over time.
The High Uncertainty v. Low Uncertainty contrast revealed significant activation clusters in bilateral STS, bilateral insula, left primary motor cortex, and SMA. However, only bilateral TPJ and insula were parametrically modulated by participants' own changes in subjective uncertainty over time. The correlation of participants' subjective uncertainty with activation in left and right TPJ ROIs revealed a significant main effect of TPJ ROI, $F(1,14)=19.66$, $p<0.001$, with higher correlations (*r)* in right TPJ, $M=0.090$, $SD=0.131$, than in left TPJ, $M=0.003$, $SD=0.093$. Analyses of task dependent connectivity between STS/TPJ and other brain regions using psychophysical interaction (PPI) are being conducted to examine how uncertainty modulates network level responses, and computational modeling is being applied to further specify the relationship between STS/TPJ activity and goal observation with respect to uncertainty estimation. The current results suggest that STS/TPJ responses to goal directed behavior are in part explained by uncertainty in modeling the external world.

**Disclosures: K.R. Velnoskey:** None. **S.W.C. Chang:** None. **G. McCarthy:** None.

**Poster**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NIDA/NIH Grant R01DA042065.

**Title:** Latent cause inference in social biases

**Authors: *Y. SHIN**[1], Y. NIV[2]
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**Abstract:** When making decisions in a social environment, how do we form impressions about a group of people whose members are diverse? If the majority of members are similar to one another with few members who are dissimilar from other people in the group, would experiences with those rare members influence the overall impression? Here, we explore how seemingly

irrational biases where rare events gain prominence in overall estimation may result from normative inference of latent causes-causal structures of the world that generate a set of observations. We hypothesized that sparsity of events may lead to inference of unique latent causes for such events. This tendency to separate rare events to small latent causes, while grouping common events in large latent causes that explain multiple events, can cause overweighting of rare events in learning, if averaging is across latent causes rather than individual events. We tested this hypothesis by manipulating sparsity of non-overlapping event distributions. We first simulated the inference process, and showed the predicted effects of our theory. We then tested these predictions empirically in four decision-making experiments. Subjects observed a sequence of coin donations and were subsequently asked to estimate the average donation. As predicted by the latent-cause model, average estimation was biased toward sparse distributions (Exp 1 and 2). This bias was not explained by correctly averaging log-transformation of the donations (Exp 3), and disappeared when we interrupted the latent cause inference process by introducing step-by-step average estimation (Exp 4). These results suggest that social biases that have been found in empirical social cognition research may be the results of a semi-rational Bayesian latent cause inference process. Our theory also applies to formation of impressions about an individual on the basis of multiple interactions, and not only to evaluations of groups of people.

**Disclosures:** **Y. Shin:** None. **Y. Niv:** None.

**Poster**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** National Eye Institute, R01 EY11378

Howard Hughes Medical Institute

Wellcome Trust

**Title:** How to build a bias: A role for confidence in belief formation

**Authors: *A. ZYLBERBERG**[1], D. M. WOLPERT[2], M. N. SHADLEN[3]
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**Abstract:** Humans and other animals can extract and exploit the statistical regularities of an environment to make better decisions. A case in point is the learning of prior probability in decision making. The prior is often ascertained from observations (e.g., base rates). This is a

straightforward process if the observations that inform the prior are certain and countable (e.g., in Laplace's rule of succession). However, such observations are often derived from sequences of decisions whose outcomes are unaccompanied by explicit feedback. Without clear feedback about decision outcome, the prior must be estimated through a process resembling probabilistic reasoning. Just as a single decision is informed by a sequence of samples of evidence of unknown reliability, so too is the belief in a base rate informed by a sequence of decisions whose veridicality is only known to a degree of certainty (i.e., confidence). We hypothesized that in the absence of explicit feedback, decision confidence guides the apprehension of prior probability. Five human participants viewed a patch of randomly moving dots and decided if the net direction of motion was rightward or leftward. Within a block of 15 to 42 trials, one direction of motion was more likely, but which direction was more likely (and by how much) was unknown to the participant. Participants made four responses on each trial. They first reported the perceived direction of motion and the confidence that the decision was correct ("choice and confidence about motion direction", CCMD). They next reported whether they believed the bias of the block favored rightward or leftward and the confidence in this belief ("choice and confidence about bias", CCB). They received no feedback after the trial. Consistent with previous studies, we found that the block's bias influenced CCMD. This implies that subjects could rapidly learn the bias of the block and use this knowledge to make more accurate decisions. Further, participants used CCMD to revise their prior. This was reflected by the incorporation of the prior in the perceptual decisions as well as in the CCB reports. A bounded evidence accumulation model explained the decisions about motion by incorporating an estimate of the bias in the accumulation. In turn, the bias was updated based on the likelihood that the motion was rightward or leftward. The model predicted the dynamics of belief about the direction bias over the block. The findings expose a role for confidence in belief updating, suggesting that the brain maintains probabilistic representations over decision hierarchies and time scales: direction over one trial and bias over many trials. These probabilities are accessible for explicit report.

**Disclosures:** **A. Zylberberg:** None. **D.M. Wolpert:** None. **M.N. Shadlen:** None.

**Poster**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** Wellcome Trust (091593/Z/10/Z)

**Title:** Information processing dynamics predict extreme political beliefs

**Authors: *M. ROLLWAGE**[1,2], R. J. DOLAN[1,2], S. M. FLEMING[1]
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**Abstract:** Holding extreme and inflexible political beliefs is commonplace, and often leads to negative consequences including "gridlock" in public policy. While societal drivers of this effect have long been posited, we were concerned as to whether extreme beliefs might have unsuspected links with characteristics of basic information processing that govern insight into the correctness of one's own beliefs (metacognitive ability) as well as the degree of integration of new evidence. In a large, web-based sample (N=379), participants were asked to discriminate patches of flickering dots, which was either directly followed by a confidence rating or by additional evidence presentation before the confidence rating. Metacognitive ability was measured as the correspondence between confidence ratings and actual performance on decisions without additional evidence, whereas post-decision evidence integration is characterized by a change in confidence due to additional evidence. Following correct choices, additional evidence should normatively increase participants' confidence (due to integration of confirmatory evidence) whereas for incorrect choices, additional evidence should lead to a decrease in confidence (due to integration of disconfirmatory evidence). A factor analysis of political attitudes resulted in a two-factor solution with factors of orientation (liberal/conversative) and extremity/dogmatism of belief. We show that metacognitive ability predicts individual's sensitivity to post-decision evidence. Critically, dogmatism was negatively predicted by metacognitive ability and also by disconfirmatory evidence integration, while political extremeness was associated with reduced integration of disconfirmatory evidence. Our results indicate that low-level information processing dynamics predict dogmatic world views, potentially contributing to a pervasive resilience of extreme political beliefs.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

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**Program#/Poster#:** 620.13/VV19

**Topic:** H.02. Human Cognition and Behavior

**Title:** Decoding representation learning in the orbitofrontal cortex during dynamic attention to relevant dimensions

**Authors: *N. DRUMMOND**[1], A. GEANA[2], N. SCHUCK[2], Y. NIV[3]
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**Abstract:** In order to make effective decisions we must learn what aspects of the environment are relevant to procuring reward and avoiding punishment. Learning and basing decisions on only the environmental dimensions that are relevant to the current task improves performance, speeds learning and simplifies generalization to future situations. To study how we learn to attend to relevant dimensions, Niv et. al (2015) developed the "Dimensions Task," a stochastic and dynamic version of the Wisconsin Card Sorting Task in which on each trial subjects are asked to choose one of three stimuli in order to obtain rewards. Each stimulus has a color, a shape and a texture, but only one of these dimensions determines reward. *It is still not clear how exactly feedback on a certain trial affects attention on the next trial, that is, what is the computational and neural mechanism for learning from experience what to attend to.*
A main hurdle in the way of understanding how attention changes trial to trial, is that we do not know what the subject is attending to on each specific trial. In the computational framework of reinforcement learning, the information relevant for making a decision is called the "state," and decision-making occurs by comparing values of different actions at each state. In the case of the Dimensions Task, the state can be thought of as the dimension relevant for reward. As the subject focuses attention on the relevant dimension, they are effectively in the "state" of the task relevant for reward. Specifically, based on a wide range of findings, we have hypothesized that the orbitofrontal cortex (OFC) contains a moment-by-moment representation of the current state of the task.
Combining these two lines of work, here we use MVPA fMRI decoding methods to track, trial by trial, the evolution of state information in the OFC as subjects play the Dimensions Task. Directly measuring how experience affects task states in an attention learning task allows us to elucidate the fundamental process by which we learn what to attend to in the service of optimal decision making by tracking the learning process. Moreover, our results provide additional support for the theory that the OFC contains dynamic state information.

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**620. Decision Making and Reasoning**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant R01MH107513

**Title:** Hyperscanning during natural dialogue between two individuals with high socioeconomic disparities

**Authors: *O. DESCORBETH**[1], X. ZHANG[2], J. A. NOAH[2], S. DRAVIDA[3], J. HIRSCH[2,4,5,6]
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of Med., New Haven, CT; [6]Dept. of Med. Physics and Biomed. Engin., Univ. Col. London, London, United Kingdom

**Abstract:** Neural substrates and mechanisms that mediate online[1] social cognition are poorly understood largely due to challenges of neuroimaging in natural conditions. However, recent developments in functional near-infrared spectroscopy (fNIRS) enable simultaneous neuroimaging (hyperscanning) of dyads during live social interactions. Prior behavioral findings suggest that social disparities signal “in” and “out” group memberships, although neural encoding of social disparities during dynamic interpersonal interactions has not been investigated. In this study, 84 individuals (19 high and 23 low disparity dyads) of mixed gender, race, and age were scanned during natural dialogues using an 84-channel fNIRS system (Shimadzu, LABNIRS) with 42 channels covering both hemispheres of each participant. A post-scan self-report survey indicated that high disparity dyads were more anxious than low disparity dyads; however, acoustic analysis of spoken narratives revealed no evidence for a difference in total spoken words between groups. We hypothesized that a contrast difference would be observed in the right temporal-parietal junction based on prior associations with social behavior; however, results did not support this hypothesis. Rather, increased neural activity based on deoxyhemoglobin signals was observed in the left dorsolateral prefrontal cortex (DLPFC) of high disparity dyads relative to low disparity dyads using SPM voxel-wise and discrete channel-wise analyses ($p<0.05$). Both analysis approaches suggest that executive planning and speech production mechanisms are more engaged during verbal interactions between individuals from disparate socioeconomic groups. We further hypothesized that cross-brain coherence between partners would vary as a function of disparity. Consistent with contrast findings, increased cross-brain coherence of fNIRS signals was found between DLPFC and premotor cortex for high disparity dyads. However, for low disparity dyads, cross-brain coherence increased between fusiform gyrus, the subcentral area, and somatosensory cortex, consistent with reciprocal signaling of face information. This is the first demonstration of a neural basis for altered dynamic interactions during social communication between pairs of individuals from highly disparate socioeconomic backgrounds, and advances a theoretical framework for such interactions by suggesting an extended role for executive planning mechanisms in real-life conversations.
1) Schilbach, L. (2014). On the relationship of online and offline social cognition. *Frontiers in Human Neuroscience, 8*, 278. https://doi.org/10.3389/fnhum.2014.00278

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant R01MH107513

**Title:** Frontal EEG theta oscillation differences during two-person, live, eye-to-eye contact compared to picture gaze

**Authors: *J. A. NOAH**[1], Y. ONO[5,1], X. ZHANG[1], S. DRAVIDA[2], J. HIRSCH[1,3,4,6]
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**Abstract: Background.** Human eye-to-eye contact is a primary source of social cues and communication. Functional neuroimaging studies (fMRI and fNIRS) have suggested that brain responses to viewing actual faces compared to pictured faces are dependent on the gaze of the eyes. Distributed face-selective regions, including the fusiform gyrus, amygdala, superior and middle temporal gyrus, and orbitofrontal cortices are more sensitive to direct than to indirect gaze.
**Aim:** Our goal here was to test the hypothesis that the eye-to-eye contact effect is represented in specific frequency bands of the EEG signal.
**Methods.** Twenty-six adults (13 pairs, 22+/- 6 years old, 15% female, 100% right-handed (Oldfield, 1971)) participated in a paradigm that cued dyads of partners to look at each other or at a fixation object placed ten degrees to the side of their partner in block design in which three seconds of eye viewing was interleaved with three seconds of fixation object viewing. Subjects also participated in an identical paradigm in which both individuals concurrently looked at the eyes of a calibrated photograph instead of their partner using the same block design. EEG recordings were obtained at a sample rate of 256/sec from electrode positions at F3, F4, F7, F8, C3, C6, PO7, and PO8 according to the 10-20 standard EEG layout. Gaze data was also obtained using a two-person eye-tracking system. EEG signals were averaged and smoothed using a median filter with a 0.10 s window, effectively a 7 Hz low-pass filter.
**Results and Discussion.** Gaze data confirmed the statistically comparable eye behavior between the eye-to-eye and eye-to-picture conditions. Point-by-point t-tests of event-related potential (ERP) indicated altered frontal activity 2s after onset of the active eye-to-eye contact relative to the eye picture gaze. In comparison, there was no difference between the two waveforms during the resting baseline ERPs. Wavelet decomposition analysis shows ERP results are specific to theta band when comparing direct eye-to-eye contact versus eye-to-picture conditions ($p = 0.01$). Other frequency bands were not differentiated by this condition. Findings extend models of eye-to-eye contact in live social situations to include theta-specific oscillations.

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NIH Grant R01MH107513

**Title:** Neural correlates of a smile: An fNIRS investigation

**Authors: *J. PARK**[1], J. A. NOAH[2], X. ZHANG[2], S. DRAVIDA[3], J. HIRSCH[2,4,5,6]
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**Abstract:** A naturally generated smile is a significant affective social cue that often conveys intention to communicate. However, the associated neural correlates are not well understood largely due to the challenges of movement artifacts in fMRI. Because of the salient social significance of the smile expression, we hypothesized that the underlying network will include neural systems known to be involved in social cognition in addition to the motor systems necessary to form the facial expression. Here we employ functional near-infrared spectroscopy (fNIRS), a neuroimaging technology with head-mounted optodes providing 105 channels distributed over both hemispheres of each participant (Shimadzu, LABNIRS). This system is tolerant of limited movement and was employed to determine the neural correlates of a natural smile generated by expression-inducing visual stimuli. Twenty-two healthy participants viewed intermixed neutral and smile-inducing images during 15 sec epochs that each included 3 images with 5 sec duration. The picture epochs alternated with 15 secs of baseline epochs for a total of four minutes. A video recording and facial classification system, GE Sherlock, was synchronized with the fNIRS signal acquisitions, and automatically identified the smile expression in addition to rating the magnitude from 0 – 1 based on facial landmarks such as the size and shape of the mouth opening. The automated classification system was validated by manual ratings. The deOxyHb signal, a proxy for neural activity similar to the BOLD signal, was analyzed based on both the task and rest periods and on continuous smile classification ratings as a regressor using conventional GLM methods. The observed neural activity based on the smile magnitude included: visual association cortex (V3), pre- and supplementary motor cortex, bilateral supramarginal gyrus (SMG), left superior temporal gyrus (STG), and bilateral frontopolar area ($p<0.05$). Consistent with the social cognition hypothesis, these novel findings provide evidence that the production of a smile is associated with processes within social and communicative neural systems in addition to expected visual and sensory motor systems.

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**Poster**

**620. Decision Making and Reasoning**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** The School of Pharmacy, The University of Auckland

**Title:** Do cannabis users show differences in decision-making with risk- and reward-related processing?

**Authors: *L. E. CURLEY**[1,2], C. B. C. MCNABB[1,2], M. AL-ATTAR[1], P. BINT[1], T. BOWERS[1], J. HINTON[1], A. SIRAJ[1], R. HESTER[4], B. RUSSELL[5], I. KIRK[3,2], R. KING[3], J. COCHRAN[6]
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**Abstract:** Cannabis is one of the most commonly used illicit substances worldwide. In unintoxicated long-term users, evidence has shown associated deficits across many cognitive domains, especially in verbal learning, memory, and attention. There are some domains where the evidence remains mixed, including decision-making and reward-related processing. Some studies have shown that long-term cannabis use is associated with increased risk-taking behaviour, however this behaviour has not been found in other studies. One task proposed to simulate real-life decision-making that involves risk, reward and punishment is the Iowa Gambling Task (IGT). The IGT consists of four decks of cards for subjects to choose from. Each card selection causes monetary gain or loss. Two of these decks are disadvantageous, yielding greater gains but even greater and more frequent losses, resulting in a net loss. The other two decks are advantageous, giving only small gains but smaller and less frequent losses, resulting in a net gain. This study aimed to determine if long-term cannabis users (n=16) show differences in reward-related behaviour in comparison to non-users (n=17) using the IGT. The IGT, programed in Psychtoolbox, allows participants to choose from four decks of cards over the course of 100 trials, with a feedback screen after each card selection. Each card selection causes monetary gain or loss with subsequent feedback. Behavioural data were separated into five bins of twenty responses and compared using an ANOVA in SPSS. Despite some trends in behaviour whereby cannabis users chose the riskier decks over the advantageous decks, there were no significant differences found ($p=0.749$). These results are in line with previous studies including those in chronic heavy cannabis users, which found no difference in the IGT. Findings from other literature have suggested that financial risk-taking may be less sensitive than other types of risk-

taking behaviour, whereby studies investigating behaviours associated with risk-based decision-making have not typically dissociated probability of risk from magnitude of reward. Furthermore research into assessing how cannabis users evaluate and respond to feedback has been sparse. Future research should target risk-based decision-making that differentiates risk from magnitude of reward and how participants respond to feedback for future decisions.

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**Poster**

**620. Decision Making and Reasoning**

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**Topic:** H.02. Human Cognition and Behavior

**Support:** NSF EPSCoR (RII Track-2 FEC)

**Title:** Influence of attentional modulation on the construction of reward value

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**Abstract:** Over the past four decades, prospect theory has been successfully used to capture choice under risk. An often-neglected aspect of this theory is the editing phase which can facilitate decision making by simplifying the comparison between gambles with many possible outcomes. Editing is especially important for evaluating real-life options which involve many possible outcomes. However, most experiments testing prospect theory involve gambles with only two possible outcomes (reward and no reward, gains and losses). Here, we designed an experiment to examine the construction of subjective value for more complex gambles and to test prospect theory for capturing choice between such gambles. In the first session of the experiment, human subjects selected between a sure option and simple gambles which yielded variable-size reward or no reward with specific probabilities. Each simple gamble was presented with a rectangle consisting of two portions with different colors. The color of each portion indicated the magnitude of possible reward outcome, and the size of each portion signaled the probability of each outcome. The choice behavior from this session was used to estimate the utility and the probability weighting functions for each subject, and to tailor pairs of equally preferable mixed gambles (gambles with three possible outcomes). This was done to allow detection of additional mechanisms involved in the construction of subjective value. The subjects selected between these pairs of tailored gambles during the second session of the experiment. We used various models to fit subjects' choice behavior in the second session in order to identify

additional mechanisms involved in the construction of subjective value. More specifically, we extended prospect theory to include the possibility that alternative gamble outcomes could be weighted differently before they are combined to form the overall gamble value. Our results supported several assumptions of prospect theory. Subjects constructed values using a concave utility function and an inverted S-shape probability weighting function. We also found, however, that most subjects assigned greater weight to the outcome with the largest reward magnitude compared to the other two outcomes. This differential weighting of possible outcomes could be instantiated via attentional modulation and enabled subjects to more easily choose between gambles with similar subjective values. Overall, our results reveal additional mechanisms involved in the evaluation of mixed gambles and highlight the role of attention in the construction of reward value.

**Disclosures: M. Moradi Spitmaan:** None. **E. Chu:** None. **A. Soltani:** None.

**Poster**

**620. Decision Making and Reasoning**

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**Title:** Tonic activity at striatal dopamine 2/3 receptors encodes subjects' confidence in their policies

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**Abstract:** Objective
'Active inference' is a computational decision-making model based on the premise that perception and action maximise Bayesian model evidence - or minimise prediction error. Under

active inference, dopamine is thought to encode the precision (inverse variance) of an agent's beliefs that its policies will achieve its goals.
This precision plays a similar role to the inverse temperature parameter in standard (softmax) decision models - i.e. it affects the stochasticity of decision-making - but, unlike inverse temperature, it is updated in a Bayes-optimal fashion according to the context. In this way, when the agent is confident that a policy will achieve its goal, its choice of a policy becomes less stochastic and more deterministic.
Previous work has shown that dynamic updates to this precision during a task correlate with BOLD activation in the dopaminergic midbrain during fMRI (Schwartenbeck et al., 2014). We wished to demonstrate a relationship between dopamine receptors themselves and precision using positron emission tomography (PET). We hypothesized that tonic dopamine signalling in the associative striatum encodes a subject's prior on this precision.
Given tonic signalling activates dopamine 2 receptors (D2Rs), we predicted: i) Prior precision, estimated using an active inference model of a Go NoGo task, would correlate with D2R availability, estimated using [11C]PHNO PET imaging. ii) Given both total D2R concentration and tonic D2R occupancy by dopamine affect D2R availability, the relationship between prior precision and D2R availability would be quadratic.
Methods
43 healthy participants performed a Go NoGo task (Guitart-Masip et al., 2012) and also underwent [11C]PHNO PET scanning to measure striatal D2/3R availability (BPnd). Participants' prior precisions were estimated using an active inference model.
Results
Prior precision (Go NoGo task) had a quadratic relationship with D2/3R BPnd ($r^2$=0.12, p=0.035) in the associative striatum ROI. When subjects who performed the cognitive task >3 weeks apart from the PET scan were excluded (n=20 remaining), more variance was explained ($r^2$=0.23, p=0.021).
Conclusions
These results indicate that tonic dopaminergic signalling in the striatum may encode subjects' prior beliefs about the precision of their policies; i.e. their initial confidence that their actions will achieve their goals, and hence the degree of stochasticity of subsequent actions.

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**Poster**

**620. Decision Making and Reasoning**

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**Program#/Poster#:** 620.20/VV26

**Topic:** H.02. Human Cognition and Behavior

**Title:** Shifts in belief encoded in the dopaminergic midbrain and ventral striatum depend on dopamine-2/3 receptor availability: A PET-fMRI study

**Authors: *M. M. NOUR**[1,2], T. DAHOUN[2,3], R. A. ADAMS[4,5], P. SCHWARTENBECK[6,7,8], C. COELLO[9], O. D. HOWES[1,2]
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**Abstract:** Dopamine dysfunction is central to psychotic disorders, which are characterized by cognitive and perceptual abnormalities. An intriguing hypothesis is that dopamine may be critical for processing epistemic value, or the meaningful information content of observations, which drives adaptive belief updating about the (hidden) states of the world. To test this hypothesis, 39 healthy subjects (mean age 26y, 22 male) performed an fMRI task that allowed us to decompose sensory information into information-theoretic surprise (reflecting unexpected but not necessarily meaningful information content) and Bayesian surprise (reflecting actual shifts in beliefs due to meaningful information). Trial-wise regressors for information-theoretic and Bayesian surprise were generated by fitting a computational model based on Bayesian belief updating to individual subjects' behavior (model $R^2$= .67). 36 subjects also had a [$^{11}$C]-(+)-PHNO PET scan to quantify dopamine 2/3 receptor (D2/3R) availability in the substantia nigra/ventral tegmental area (SN/VTA) and ventral striatum (VS). 17 subjects had a second PET scan 3 hrs following 0.5mg/kg oral amphetamine, to quantify striatal dopamine release capacity. In line with previous findings, Bayesian surprise was encoded in the SN/VTA and VS, putatively reflecting dopaminergic activity. The SN/VTA BOLD signal effect size correlated negatively with SN/VTA baseline D2/3R availability (rho = -.43, P=0.009), consistent with studies indicating that D2/3R act to inhibit midbrain dopamine neurons. VS activation correlated negatively with VS amphetamine-induced dopamine release (rho = -.66, P=0.005), indicating that subjects with high dopamine release capacity (as is also found in schizophrenia) showed blunted striatal activation in response to belief-changing information. Information-theoretic surprise was encoded by a network including the supplementary motor cortex and anterior insula, and showed no association with D2/3R availability or dopamine release. Our results indicate that the dopaminergic SN/VTA and VS are involved in processing the meaningful information (epistemic value) of observations, as reflected in Bayesian belief updating. They provide further evidence that (unsigned) belief updates depend on dopaminergic activity, at variance with classical interpretations of dopamine function in terms of (signed) reward prediction errors. This is highly relevant for theories of aberrant belief formation in psychosis, which posit that mesostriatal dopamine dysfunction results in the formation of false beliefs about the causes of sensory information, leading to hallucinations and delusions.

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**Poster**

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**Program#/Poster#:** 620.21/VV27

**Topic:** H.02. Human Cognition and Behavior

**Title:** Emergent oscillations in the subthalamic nucleus of a simulated basal ganglia

**Authors: *T. M. SHEA**, J. J. RODNY, A. S. WARLAUMONT, D. C. NOELLE
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**Abstract:** The subthalamic nucleus (STN) has been shown to support conflict resolution during perceptual and cognitive decisions by indirectly inhibiting motor responses. Excitatory efferent synapses of the STN modulate the overall inhibitory output of the internal segment of the globus pallidus (GPi). During a high conflict perceptual decision (e.g. flanker task) or an interrupted response (e.g. stop signal task), local field potentials (LFP) recorded from the STN exhibit increased power in the theta and beta bands and increased theta-locked neuronal firing. However, the mechanisms contributing to conflict-related oscillations measured in the STN remain unclear. We propose low signal-to-noise ratio stimuli (high conflict) fail to suppress noise-evoked activity, leading to widespread neuronal synchrony.
We simulated a thalamic-cortical-basal ganglia network with 6,000 spiking neurons and ~500,000 synapses. Connectivity in the model was based on linear topology, such that neurons in the GPi performed off-center, on-surround signal selection of the associated thalamocortical loop. To our knowledge, this is a novel use of topological connectivity in a spiking neural network to explain conflict-related oscillations. We injected Gaussian-shaped “bump attractor” stimuli into the model thalamus and randomly varied the stimulus signal-to-noise ratio while recording the ratio of stimulus-evoked action potentials to noise-evoked action potentials.
We identified two distinct classes of response in our results, corresponding to low and high conflict stimuli. The threshold between low and high conflict stimuli was not built into the model, but emerged from the interactions between neuronal populations. During low conflict stimuli, the model exhibited robust noise suppression and weak oscillations. For high conflict stimuli, noise suppression was delayed or inadequate. High conflict stimuli induced greater power in the simulated LFP of STN in theta and beta bands. These oscillations emerged when the injected stimulus was insufficiently strong to overcome inhibition via the hyperdirect pathway. In simulations with the GPi output disabled, both low-conflict noise suppression and high-conflict oscillations were eliminated.
These results indicate a potential mechanism for the emergence of conflict related oscillations due to the topology of the direct, indirect, and hyperdirect pathways. This mechanism supports recent work on neuronal ‘communication through coherence’ and entrainment to STN

oscillations. Importantly, the model demonstrates that oscillations emerge without intrinsic neuronal dynamics (e.g. bistability) or local recurrent circuits.

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**Poster**

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**Title:** Impulsive or indecisive: Effects of altered excitation-inhibition balance on decision making in a cortical circuit model

**Authors: *N. H. LAM**[1], T. BORDUQUI[3], J. HALLAK[3], A. C. ROQUE[4], A. ANTICEVIC[2], J. H. KRYSTAL[2], X.-J. WANG[5], J. D. MURRAY[2]
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**Abstract:** Disruption of the synaptic balance between excitation and inhibition (E/I balance) in cortical circuits is a leading hypothesis for pathophysiologies of neuropsychiatric disorders, including schizophrenia and autism. Disrupted E/I balance is generally linked to arising cognitive deficits, such as impaired decision making. However, a mechanistic understanding is lacking for how E/I disruptions at the synaptic level may induce cognitive deficits at the behavioral level.
To investigate these issues, we studied how altered E/I ratio may impair perceptual decision making in a biophysically-based computational model of an association cortical circuit. We perturbed E/I balance in the model bidirectionally through hypofunction of NMDA receptors at two sites: on inhibitory interneurons (elevating E/I ratio via disinhibition), or on excitatory pyramidal neurons (lowering E/I ratio). We found that both elevated and lowered E/I ratio can similarly impair decision making, as assessed by standard psychometric performance, following an inverted-U dependence.
Nonetheless, these distinct E/I perturbations differentially alter how perceptual evidence is

accumulated over time in the circuit. Under elevated E/I ratio, decision making is impulsive: evidence early in time is weighted much more than late evidence. Under lowered E/I ratio, decision making is indecisive: evidence integration and winner-take-all competition between options are weakened overall. The time course of evidence accumulation can be characterized using multiple psychophysical task paradigms, which provide dissociable predictions at the behavioral level.
These results are well captured by an extended drift-diffusion model, which is modified so that integration is imperfect via a self-coupling term: impulsive decision making under elevated E/I ratio is captured by unstable integration, and indecisive decision making under lowered E/I ratio is captured by leaky integration. This link to the psychological drift-diffusion model provides theoretical insight and allows fitting to experimental results.
In general, our findings characterize critical roles of cortical E/I balance in cognitive functions, and the utility of timing-sensitive psychophysical paradigms. Our model makes specific predictions for behavior and neural activity that are testable in humans or animals under causal manipulations of E/I balance and in disease states. Furthermore, they provide a testbed for Computational Psychiatry demonstrating that neural circuit models can play a translational role between basic neurophysiology and clinical applications.

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**Poster**

**620. Decision Making and Reasoning**

**Location:** Halls A-C

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**Topic:** H.02. Human Cognition and Behavior

**Support:** This work was supported by a faculty startup grant from UT Dallas and the Dallas Foundation.

**Title:** Action selection under uncertainty: fMRI evidence in a novel task

**Authors:** ***V. G. FIORE**[1], J.-C. YU[2], C. C. TATINENI[2], A.-C. V. GUERTLER[2], X. GU[2]
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**Abstract:** In series of recent models we have targeted the neural dynamics of cortico-striatal circuits and analysed the way their multistable dynamics are affected by aberrant learning (as e.g. in addiction). These models predict that the amount of information required to change policy or

action strategy, adapting to a changing environment, can be used as a measure of the transient dynamics characterising cortical striatal circuit. We hypothesize that the ventral cortico-striatal circuit is involved in conditions of greater uncertainty, as it is dedicated to forward planning. To test this hypothesis, we have designed a novel *3-option continuous bead task, based on* a well-established decision making task (the bead task), to allow a precise manipulation of conditions of uncertainty and evidence accumulation. In each trial, a coloured bead is extracted from one of three jars containing beads of three colours (visible ratio of 80%-10%-10%). The participants have to decide from which jar each bead is extracted from. No feedback is provided after each decision, but the participants can estimate the source of each bead based on the last few extractions, which are shown on screen. This design was used to compare *low uncertainty* (3 or more repeated extraction of beads of the same colour), *moderate uncertainty* (two repeated extractions) and *high uncertainty* (an new colour extracted after a series of 3 or more). We assessed this task in an fMRI study with healthy volunteers. First, we found significant activity in nucleus accumbens and ventromedial prefrontal cortex, when contrasting trials with moderate uncertainty vs. trials with low uncertainty. Second, we found a trend highlighting increased activity in the insula and nucleus accumbens when comparing trials of high uncertainty vs trials with low uncertainty. These results suggest a single trial of confirming or conflicting evidence has clear-cut effects in terms of neural activity. We hypothesise the activity of the ventral circuit drops as a direct function of uncertainty because confirming information can be largely ignored in forward planning. Conversely, unexpected information is mediated by insula and nucleus accumbens, to signal mismatch in predicted information and saliency, even in the absence of a reward. We hypothesise these systems jointly signal a new cycle of evidence has to be taken into account. In the continuous sequence of confirming and conflicting information offered by the task, the ventral circuit is only reactivated by accumulating confirming evidence (i.e. two beads of the same colour), which allow planning future actions rather than performing random choice selections.

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**Poster**

**620. Decision Making and Reasoning**

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**Program#/Poster#:** 620.24/VV30

**Topic:** H.02. Human Cognition and Behavior

**Support:** Social Science Matrix, UC Berkeley

**Title:** Toward a proof of concept for neuroimaging-based financial-system regulation: Near-infrared spectroscopy (NIRS)-recorded lateral neocortical activity in lab markets with monotonically decreasing or peaked fundamental values

**Authors: *J. L. HARACZ**
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**Abstract:** Objective: To build an experimental design that could yield a proof of concept for neuroimaging-based financial-system regulation, the present review aims to: 1) find evidence that near-infrared spectroscopy (NIRS) could detect low lateral neocortical activity as an asset-price bubble biomarker and 2) seek a lab-market paradigm that could model the uncertainty that financial-system regulators have about whether rising asset prices represent a bubble.
Methods: A systematic literature review focused on NIRS and lab-market studies to develop the above experimental design.
Results: NIRS-recorded prefrontal cortical activity was higher in the "cold" deliberative version of the Columbia Card Task (CCT) compared to the "hot" affective CCT version (Holper & Murphy, 2014). These results suggest the use of NIRS to detect low lateral neocortical activity as a bubble biomarker, consistent with the hypothesis that evolutionarily ancient or new neurocircuitry, respectively, drives decision making during bubble or non-bubble periods of financial market activity (Haracz, 2013). A proof of concept for using this biomarker in a financial-system regulatory setting would be enabled by designing a lab-market paradigm that reliably generates asset-price increases during either bubble or non-bubble market periods, thereby modeling the above regulators' uncertainty. Two versions of a well-studied continuous double auction paradigm (Smith et al., 1988) could be used: assets in standard (S) lab markets would have a monotonically decreasing fundamental value that typically yields price bubbles (Smith et al., 1988), whereas assets in peaked (P) markets would have a fundamental value that peaks (i.e., rises in early periods of asset trading and falls in later periods [Noussair & Powell, 2010]). In P markets, asset prices tend to closely track fundamental value, so no bubble is yielded typically. This tendency could be strengthened by adjusting subjects' cash and asset endowments. Price peaks represent a bubble in S markets, but not in P markets. A subject acting as a regulator could observe computer-displayed NIRS-recorded lateral neocortical activity from asset-trading subjects along with ongoing asset prices and trades, but the omission of fundamental values would blind the regulator to whether markets are S or P. Therefore, the regulator would be trained to use NIRS data (i.e., low lateral neocortical activity) to detect bubbles and implement asset-holding caps (Lugovskyy et al., 2014) to deflate bubbles, thereby yielding a proof of concept for neuroimaging-based financial system regulation.
Conclusion: NIRS and lab market studies could yield the above proof of concept.

**Disclosures: J.L. Haracz:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.01/VV31

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Single molecule fluorescence *In situ* hybridization in the mouse retina

**Authors: *M. THOMSEN**[1,2], M. VISWANATHAN[2], H. ZHAO[2], S. HATTAR[1]
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**Abstract: Purpose** High-throughput transcriptomic data sets often yield hundreds of interesting candidate genes for further study, but confirmation of this data in vivo often relies on methods of in situ hybridization that are time-consuming and technically challenging. To facilitate rapid, robust screening of gene targets from transcriptomic studies, we have adapted a protocol for single molecule fluorescent in situ hybridization (smFISH) for use in whole-mount mouse retinas. smFISH requires less than half the time of conventional in situ hybridization protocols and permits single transcript resolution and quantitation. **Methods** We used computational methods to design a library of 20-30mer oligonucleotide probes that tile >100 mRNA sequences of interest with minimal off-target binding. Each probe was flanked by universal primer sequences and gene-specific orthogonal priming sequences that permit amplification of probe sets for a single gene from the library. The total library of >12,000 oligos was chemically synthesized and suspended in a small volume of TE buffer. Probe sequences for each gene were amplified from the library using several rounds of PCR with gene-specific primers. Following amplification of probes for each gene, antisense fluorescent oligonucleotide probes were generated by PCR with fluorophore-coupled primers. Freshly dissected mouse retinas were quartered and lightly fixed in 4% PFA then permeabilized in 0.5% TritonX-100/PBS. Following permeabilization retinas were incubated overnight with fluorescent probes in hybridization buffer. After hybridization, retinas were washed several times in 10% formamide/2XSSC, mounted, and imaged immediately. **Results** smFISH robustly labeled transcripts in the ganglion cell layer (GCL) of mouse retinas with little background fluorescence. Individual transcripts for more than 20 genes were visualized and quantified. **Conclusions** We have optimized a method of smFISH for rapid and reliable detection of individual mRNA transcripts in the mouse retina. This method has significant advantages over conventional in situ hybridization including shortened protocol time, single transcript resolution, and improved reliability.

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**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.02/VV32

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Visualization of circular RNA expression at single-cell resolution in C57Bl/6J developmental mouse brain using BaseScope™ technology

**Authors: *A. LAEREMANS**, N. LI, E. PARK, X.-J. MA, N. SU
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**Abstract:** Recently, a universal class of endogenous noncoding RNAs that is generated by "head-to-tail' splicing has emerged as a new promising type of RNA with biomarker potential. This particular spicing event results in the covalent linkage of the 5' end of one exon with the 3' end of another exon and creates stable single-stranded circular RNA molecules (circRNA) in eukaryotes. These highly conserved circRNAs are characterized by tissue- and developmental stage-specific expression patterns. More specifically, it was recently shown that circRNAs are particularly enriched in the brain where they are often derived from genes that code for synaptic proteins, and with expression dynamics independent of the linear mRNA transcripts. Although abundantly expressed in the nervous system, their function remains largely unknown. Also, evidence has emerged across different fields that pinpoints a role for circRNAs in various diseases, including cancer, possibly by regulating gene expression levels through interaction with other molecules (eg. miRNAs). Therefore, the accurate detection and localization of circRNAs is a key factor to elucidate their functions especially given the fact that they could serve as putative clinical biomarkers. BaseScope™ is a novel *in situ* hybridization technology that allows the visualization of specific exon-exon and exon- (retained) intron junctions in a highly specific and sensitive manner in cells and tissues. Besides the robust identification of splice junctions, BaseScope™ *in situ* hybridization also provides high resolution single-cell spatial information within the morphological tissue context. To illustrate the strength of this BaseScope™ application for the intriguing field of circular RNAs, we present the specific anatomical localization and cellular distribution of various brain plasticity-related targets including the Alzheimer's disease-related *Dlgap1* gene. For these targets their circRNA and linear mRNA counterpart are visualized supplemented with the corresponding quantification in P1, P10 and P30 C57Bl/6J mouse brains.

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**Poster**

**621. RNA and Gene Expression Techniques**

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**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH Grant EY024694

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NIH Grant EY026344

**Title:** Sequencing and proteomic approach illuminates mRNA and protein isoform diversity and their contributions to neural development and disease

**Authors: *T. RAY**[1], K. J. COCHRAN[2], W. J. SPENCER[3], G. ALEXANDER[4], N. SKIBA[5], J. N. KAY[6]
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**Abstract:** Alternative splicing and alternative promoter usage are two mechanisms by which single genes can encode multiple protein isoforms. These processes are capable of exponentially increasing the functional capacity of the genome, because they can generate protein variants with distinct interaction partners and hence distinct functions. For these reasons alternative splicing of cell-surface receptors is an appealing mechanism to pursue how neurons communicate during development to set up highly specific synaptic connections. Despite mounting evidence that isoform diversity is critical to neural circuit formation, this diversity is still widely disregarded in gene expression and protein function studies, leading to inaccurate characterizations of gene function. One reason for this problem is the lack of tools for cataloging isoforms at the mRNA and protein level. While high throughput RNA sequencing has become commonplace, the short reads generated in typical RNAseq create an incomplete representation of the transcriptome, making subsequent gene isoform analyses dependent on computational assembly of transcripts—a problematic approach. Consequently, incomplete cataloguing of gene isoforms results in incomplete predicted protein libraries, thereby hindering protein identification using mass spectrometry.
Here we describe an experimental and bioinformatic workflow to overcome these limitations. First, we analyzed standard RNAseq data and identified cell-surface receptors with unannotated high isoform diversity in developing mouse retina. Of these, 31 were chosen for targeted single molecule PacBio sequencing, which yields long reads and therefore permits full-length isoform identification. We sequenced developing and adult retina to identify isoforms that correlate with key neural developmental hallmarks. Bioinformatics tools (Iso-Seq, ToFU) were used to

catalogue isoforms, and molecular tools were used to verify temporal (RNAseq, qPCR) or spatial (BaseScope in situ hybridization) expression of isoforms in the retina. The catalog of full-length PacBio-sequenced isoforms was used to generate an in silico protein library of unannotated "dark" isoforms (i.e. isoforms absent from RefSeq based libraries). Then we performed mass spectrometry on cell-surface protein preparations from retinal tissue,and searched for novel protein isoforms using our library. This pipeline proved robust in identifying novel cDNA isoforms that are translated into protein. We found a surprising number of "dark" isoforms, many of which are positioned to diversify the functions of genes known to influence retinal development and disease.

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**Poster**

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**Program#/Poster#:** 621.04/VV34

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** AIHS Polaris Award

**Title:** Mapping behaviourally induced Homer1a expression along the septotemporal axis of CA1

**Authors: *S. T. DUBE**, M. J. ECKERT, V. LAPOINTE, A. M. DEMCHUK, L. MESINA, B. L. MCNAUGHTON
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**Abstract:** The rodent hippocampus is generally described as being functionally segregated along the septotemporal axis. However, the specific delineations used to define subregions along this axis are not consistent, and current anatomic, genetic, and physiological data propose varying categorizations. In this study, we used the neural activity dependent immediate-early gene (IEG) *Homer1a* (*H1a*) to map the behaviourally induced putative activity of neurons along the septotemporal axis of CA1.
While electrophysiological studies suggest a continuously decreasing gradient in the proportion of active cells from septal to temporal areas, our IEG expression data indicate that hippocampal modules can be classified as a domain with no gradient in the septal half of CA1, an abrupt drop in IEG expressing cells in intermediate CA1 and an substantial increasing gradient from intermediate to temporal CA1. The increase in the temporal direction corresponds roughly to amygdalar input to CA1, with increasing IEG expression corresponding to increasing amygdalar input in temporal areas; however, further experiments will be needed to determine the basis for this pattern of functional topography.

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**Program#/Poster#:** 621.05/VV35

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH NINDS (1F31NS095465-01A1)

**Title:** Optimized cortical inhibitory neuron targeting using microRNA-guided neuron tags (mAGNETs) in the rodent brain

**Authors: *T. TA**, M. K. KEAVENEY (SAYEG), X. HAN
Biomed. Engin., Boston Univ., Boston, MA

**Abstract:** Genetic modifications to specific neural subsets allow for the discovery of their functional role(s) in the brain. We recently developed microRNA-guided neuron tags (mAGNETs) as a novel strategy for targeting viral transgene expression to neuronal subtypes by exploiting endogenous microRNA (miRNA) regulation. We previously demonstrated that the mAGNET technique is capable of targeting inhibitory cells in the cortex of mice at ~82% targeting rate, when packaged in lentivirus. Since this preliminary study, we have been able to improve the targeting rate in mice to >90% by incorporating a different promoter, and >95% by packaging in adeno-associated virus (AAV). We additionally tested these GABA targeting mAGNETS in rats, and found that they successfully targeted inhibitory neurons in the cortex, although not as efficiently as in the mice. Altogether, these optimized mAGNETs provide a novel method of virally targeting cortical inhibitory neurons in the rodent brain.

**Disclosures:** **T. Ta:** None. **M.K. Keaveney (Sayeg):** None. **X. Han:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.06/VV36

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH Grant 1DP2NS082126

**Title:** Adult neurogenesis enhances hippocampal dependent performance via influences on bilateral networks and age dependent synaptic integration

**Authors: *S. BENSUSSEN**[1], K. CHING[2], H. GRITTON[2], J.-M. ZHUO[2], X. HAN[2]
[2]Biomed. Engin., [1]Boston Univ., Boston, MA

**Abstract:** Adult neurogenesis supports performance in many hippocampal dependent tasks. Recent studies have demonstrated that adult born neurons exhibit heightened excitability and plasticity during a critical developmental period, which may be essential for their contribution to certain tasks. However, it remains largely unknown how these unique biophysical and synaptic properties may translate to networks that support behavioral function. Recently, we transiently silenced adult born neurons at different ages using optogenetics while mice were performing a location discrimination task. We discovered that adult-born neurons promote location discrimination during early stages of development, but only if they undergo maturation during task acquisition. Optogenetic silencing of young adult-born neurons also produced changes extending to the contralateral hippocampus, detectable by both electrophysiology and fMRI measurements, suggesting young neurons may modulate location discrimination through influences on bilateral hippocampal networks. To further explore how synaptic integration of young neurons influences their network and behavioral impact during the critical period, we developed novel optogenetic tools to tag synapses, and tracked synaptic integration of adult born neurons throughout their maturation period.

**Disclosures: S. Bensussen:** None. **K. Ching:** None. **H. Gritton:** None. **J. Zhuo:** None. **X. Han:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.07/VV37

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH 5R01MH094705-04 to ZJH

R01MH109665-01 to ZJH

Robertson Neuroscience Fund to Z.J.H

NARSAD/BBRF Postdoc fellowship to AP

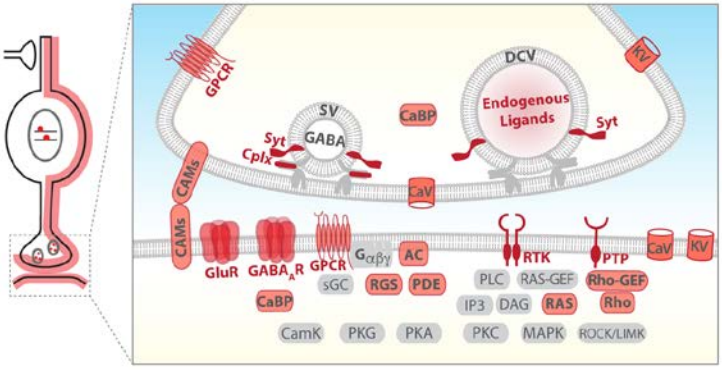
T. and V. Stanley to MC

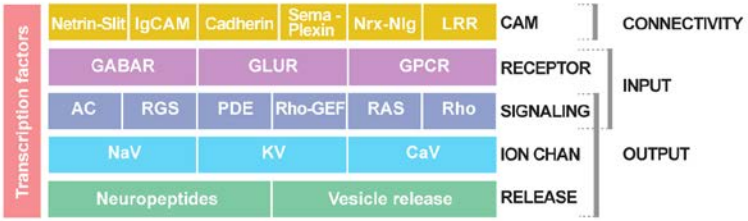
T. and V. Stanley to JG

**Title:** Transcription architecture of synaptic connectivity and signaling underlies cortical GABAergic neuron identity

**Authors: *A. PAUL**[1], M. CROW[2], J. GILLIS[2], Z. HUANG[3]
[1]Cold Spring Harbor Lab., Cold Spg Hbr, NY; [2]Stanley Inst. for Cognitive Genomics, Cold Spring Harbor Lab., Woodbury, NY; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

**Abstract:** Understanding the biological basis of neuronal identity is necessary to decipher brain cell diversity towards discovering the organization logic of neural circuits. Here, through single cell transcriptome analysis of phenotype-defined cortical GABAergic neurons and a computational genomic screen for transcription profiles that jointly distinguish them, we have discovered the transcriptional architecture of cardinal neuron types and subpopulations. This genetic architecture encodes neuronal connectivity and input-output properties and consists of functionally congruent gene families that include cell adhesion molecules, transmitter-modulator receptors, ion channels, membrane-proximal signals protein, neuropeptides and vesicular release components, and transcription factors. Combinatorial and coordinated expression of select members across these families shapes a multi-layered and coherent molecular architecture along cell membrane, thereby customizing the patterns and properties of synaptic communication. This molecular genetic framework of core neuronal identity integrates, explains and predicts cell phenotypes along multiple axes and provides a foundation for cell type discovery and classification.




**Disclosures:** **A. Paul:** None. **M. Crow:** None. **J. Gillis:** None. **Z. Huang:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.08/VV38

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Using pattern detection in large datasets to identify "switched" transcripts in brain regions of adult mouse brain

**Authors: *R. S. NOWAKOWSKI**[1], L. M. DICARLO[3], K. XU[4], N. BROWNSTEIN[2], J. FAN[5], C. VIED[6]
[1]Biomed. Sci., [2]Behavioral Sci. and Social Med., FSU Col. of Med., Tallahassee, FL; [3]Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL; [4]Sch. of Big Data, Fudan Univ., Shanghai, China; [5]Departments of Statistics and Finance, Princeton Univ., Princeton, NJ; [6]Florida State University, Col. of Med., Tallahassee, FL

**Abstract:** We set out to identify candidate genes that are either “controllers” of region specific gene expression in the adult mouse brain or that are proximally controlled by “controllers”. For this identification we used a large dataset consisting of transcriptomic (RNA-seq) data from 4 brain regions, cerebellum, hypothalamus, hippocampus and neocortex, with an n of 12 for each brain region. As a first step towards identifying the “controller” genes, we hypothesize the existence of “switched” genes, defined as genes which exhibit an on/off pattern of expression. We specify that on/off transcripts have only two levels, an “off” state that is indistinguishable from zero expression and an “on” state that is statistically greater than the “off” state and which also is not different statistically among the brain regions that are not “off”. Note that this on/off definition delimits only a small proportion of the transcripts that are differentially expressed. For example, we define hi/lo transcripts as having a non-zero low state, i.e., the low state is significantly greater than zero expression, and many transcripts have more than two levels of expression. We identified on/off switched genes using ANOVA/GLM (with Benjamini-Hochberg FDR corrections) followed by all 6 possible pair-wise post-hoc tests and also with genome wide criteria for specifying the “zero level” of expression”. This is possible because of the small number of brain regions analyzed and the power contributed by the 12 replicates. With only 6 pair-wise combinations a pattern matching approach is both feasible and exhaustive, i.e., statistical criteria can be specified that allows all transcripts in the dataset to be assigned to the “on/off” switched type or not. For the transcripts with only 2 levels of expression regression analysis of the minimum expression level vs the maximum expression level for each transcript shows that there is an inverse relationship ($r=-0.69$; $p<10^{-100}$) between the step-size (i.e., the difference between the on-state expression and the off-state expression) and the minimum level of expression. Some of the transcripts with the lowest off-state expression levels showed increases of over 1,000-fold in the on-state. A pattern analysis of the post-hoc comparison results

shows that on/off switched transcripts exist for all possible combinations of the 4 brain regions analyzed, i.e., on/off in 1/3, 2/2, or 3/1 tissues. Approximately 25% of the on/off transcripts are transcription factors. A preliminary evaluation of the tissue specificity of the on/off transcripts using the Allen Brain Atlas indicates that they are not only brain region specific but also cell type specific.

**Disclosures: R.S. Nowakowski:** None. **L.M. Dicarlo:** None. **K. Xu:** None. **N. Brownstein:** None. **J. Fan:** None. **C. Vied:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.09/VV39

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** Howard Hughes Medical Insititute

**Title:** Quantitative mrna imaging throughout the entire *Drosophila* brain

**Authors: *X. S. LONG**[1], J. COLONELL[1], A. WONG[1], R. H. SINGER[1,2,3], T. LIONNET[1,4]
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Dept. of Anat. and Structural Biol., [3]Dominick P. Purpura Dept. of Neuroscience, Gruss Lipper Biophotonics Ctr., Albert Einstein Col. of Med., Bronx, NY; [4]Inst. for Systems Genetics, Dept. of Cell Biol., New York Univ. Langone Med. Ctr., New York, NY

**Abstract:** The expression of select genes at specific times in appropriate neurons is required for brain development and function. *Drosophila* has emerged as an important model system to identify the gene networks underlying neuron types and functions. However, existing approaches that quantify gene expression using sequencing methods mainly rely on isolated cell populations, in which neurons are extracted from their native context. RNA fluorescent *in situ* hybridization (FISH) is a powerful technique that allows measuring mRNA levels with subcellular resolution within preserved tissue, but its application to adult *Drosophila* brains has proved difficult due to limited probe penetration and sample autofluorescence. Here, we describe a FISH method that permits detection of the localization and abundance of single mRNAs in cleared whole-mount adult *Drosophila* brains. The approach is specific, rapid, quantification with subcellular resolution on a standard confocal microscope. We further demonstrate single-mRNA detection across the entire brain sample using a custom Bessel Beam-Structured Illumination microscope (BB-SIM). We anticipate this method will constitute an important tool for addressing the cellular and molecular basis of brain function.

**Disclosures:** **X.S. Long:** None. **J. Colonell:** None. **A. Wong:** None. **R.H. Singer:** None. **T. Lionnet:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.10/VV40

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH grant R01 ES024064

**Title:** Overexpression of miR-200a in the regulation of Keap1 < Nrf2 mRNA expression > spinal cord motor neuron cell lines

**Authors: *D. WIWATRATANA**[1], W. D. ATCHISON[2]
[1]Comparative Med. and Integrative Biol., Michigan State Univ., East Lansing, MI; [2]Dept Pharmacol & Toxicol, Michigan State Univ. Dept. of Pharmacol. and Toxicology, East Lansing, MI

**Abstract:** One pathogenic mechanism occurring in several neurodegenerative diseases is associated with the perturbation of cellular oxidative stress and antioxidants. Nuclear factor-erythroid-2-related factor 2(Nrf2) is a transcription factor responsible for inducing several cellular antioxidants. The functional activity of Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1(Keap1) which sequesters Nrf2 in the cytoplasm and undergoes Nrf2-ubiquitin proteasome degradation. To defend against oxidative stress, reducing the Keap1 protein could be a potential target to enhance Nrf2 activity. A small non-coding RNA called microRNA plays a role in the regulation of mRNA expression by complementarily binding to target mRNAs. It has been reported that miR-200a regulates Nrf2 and Keap1 expression in human breast cancer cell lines. In this study, the miR-200a, regulated by tetracycline promotor, was transduced by lentivirus into the neuroblastoma spinal cord cell line 34 (NSC34), a motor neuron cell line. A treatment of 1µg/ml of doxycycline (Dox) was introduced to regulate the expression of miR-200a. The majority of the NSC34 clones (5 out of 7) indicated the upregulation of miR-200a after 48h of 1µg/ml Dox application. The expression of Keap1 and Nrf2 mRNAs were inconsistent among these 7 clones. However, the clone with the highest level of miR-200a expression (3.14 fold of induction) indicated the down regulation of Keap1 (0.9 fold of induction) and upregulation of Nrf2 mRNA (1.4 fold of induction). The discrepancy of Keap1 and Nrf2 mRNA expression between these clones could be due to, 1) the location of the miR-200a integration into the chromosome, 2) the timing of miR-200a regulation of the Keap1 mRNA expression and 3) the timing to regulate Nrf2 activity after Keap1 knock down. This research is supported by NIH grant R01 ES024064.

**Disclosures:** **D. Wiwatratana:** None. **W.D. Atchison:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.11/VV41

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** Advance Queensland Research Fellowship (JPK)

Rebecca L. Cooper Medical Research Grant (JPK)

Australian Research Council (RS)

**Title:** Targeting dopamine progenitors with *In utero* electroporation in mice

**Authors: *J. P. KESBY**[1,2], R. SUÁREZ[1], D. W. EYLES[1,3]
[1]Queensland Brain Inst., St. Lucia, Australia; [2]UQ Ctr. for Clin. Res., Univ. of Queensland, Brisbane, Australia; [3]Queensland Ctr. for Mental Hlth. Res., Wacol, Australia

**Abstract:** Schizophrenia is a chronic psychiatric disorder with a poorly understood aetiology. Altered brain function and psychotic symptoms present prior to disease onset suggesting a developmental origin. Dopamine is a neurotransmitter that has been implicated in the cause and treatment of schizophrenia and thus represents a core developmental drug target in schizophrenia. Understanding the consequences of altered dopamine neuron development requires the use of techniques that allow for the specific manipulation of dopamine progenitors in the embryonic brain. We are developing a protocol using in utero electroporation that will allow for the transfection of small interfering RNA (siRNA) to dopamine neurons in mice. A reporter plasmid encoding a yellow fluorescent protein was injected into the mesencephalic ventricle of e11 mice embryos. Electroporation was accomplished using a triple electrode configuration. The head of each embryo was held between two positive circular electrodes, positioned laterally but ventral to the floor plate, with a third negative electrode held dorsal to the embryo head to generate a ventral electrical field vector that transfects the plasmid into the ventral mesencephalon. We will present evidence using immunofluorescence microscopy that demonstrates the specific transfection of dopamine progenitors in the ventral mesencephalon. These data suggest the targeting of developing dopamine neurons early in development is possible with the use of in utero electroporation. Furthermore, this work demonstrates the feasibility of future experiments transfecting siRNA against dopamine differentiation factors in the embryonic mouse brain. When combined, the use of targeted in utero electroporation and siRNA will allow us to gain a better insight into the role of specific differentiation factors in dopamine neuron development.

**Disclosures:** **J.P. Kesby:** None. **R. Suárez:** None. **D.W. Eyles:** None.

**Poster**

**621. RNA and Gene Expression Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 621.12/VV42

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** NIH R01NS081054

NIH R01MH091850

Rett Syndrome.Org

The Pew Foundation

**Title:** Biotin tagging MeCP2 reveals contexual insights into the Rett syndrome transcriptome

**Authors: B. JOHNSON**[1], Y. ZHAO[1], M. FASOLINO[1], J. LAMONICA[1], Y. J. KIM[2], G. GEORGAKILAS[1], K. WOOD[1], D. BU[1], Y. CUI[1], D. GOFFIN[1], G. VAHEDI[1], T. H. KIM[2], *Z. ZHOU[1]
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Texas at Dallas, Dallas, TX

**Abstract:** Mutations in *MECP2* cause Rett syndrome (RTT), an X-linked neurological disorder characterized by regressive loss of neurodevelopmental milestones and acquired psychomotor deficits. However, the cellular heterogeneity of the brain impedes understanding of how *MECP2* mutations contribute to RTT. We therefore developed cell type-specific biotin tagging of MeCP2 in mice bearing RTT-associated mutations and profiled their nuclear transcriptomes. Although most gene expression changes are largely specific to each mutation and cell type, lowly expressed cell type-enriched genes are preferentially disrupted by MeCP2 mutations, with upregulated and downregulated genes reflecting distinct functional categories. Subcellular RNA analysis in MeCP2 mutant neurons further reveals reductions in the nascent transcription of long genes and uncovers widespread post-transcriptional compensation at the cellular level. Finally, we overcame cellular mosaicism in female RTT models and identified distinct gene expression changes between neighboring wild-type and mutant neurons, altogether providing contextual insights into RTT etiology that support personalized therapeutic interventions.

**Disclosures:** **B. Johnson:** None. **Y. Zhao:** None. **M. Fasolino:** None. **J. Lamonica:** None. **Y.J. Kim:** None. **G. Georgakilas:** None. **K. Wood:** None. **D. Bu:** None. **Y. Cui:** None. **D. Goffin:** None. **G. Vahedi:** None. **T.H. Kim:** None. **Z. Zhou:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.01/VV43

**Topic:** I.03. Anatomical Methods

**Title:** Neuronal diversity of the zebrafish habenulo-interpeduncular pathway revealed by CRISPR/Cas9 genome editing

**Authors: J.-H. CHOI**, E. DUBOUÉ, J. CHANCHU, *M. E. HALPERN
Carnegie Instn for Sci., Baltimore, MD

**Abstract:** The bilaterally paired dorsal habenulae (dHb) are part of an evolutionarily conserved pathway that connects telencephalic nuclei to the midbrain interpeduncular nucleus (IPN). In zebrafish, the left and right dHb differ in their size, molecular properties, neuroanatomical organization, and connectivity. Previous studies have demonstrated that the dHb contain a number of specialized neuronal populations, including those producing acetylcholine, substance P and somatostatin. Connections between dHb neuronal subtypes and subregions of the IPN have not been well-defined. To produce a precise dHb-IPN connectivity map, we are generating transgenic lines that label specific neurotransmitter and neuropeptide cell populations using CRISPR/Cas9-mediated integration. We have introduced the QF transcription factor of *Neurospora crassa* into genes whose expression demarcates specialized dHb cell types. These lines can be used to drive expression of a variety of QUAS- regulated reporter and effector transgenic lines, enabling tracing of neuronal processes, optogenetic activation or calcium imaging. For example, we identified a small, cholinergic neuronal cluster in the right dHb that is preferentially innervated by bilaterally projecting olfactory neurons. This distinct cluster of dHb neurons projects to a sub-region of the ventral IPN. Identification of the olfactory cues that modulate these right dHb neurons will help elucidate the function of this asymmetric neural circuit.

**Disclosures: J. Choi:** None. **E. Duboué:** None. **J. Chanchu:** None. **M.E. Halpern:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.02/VV44

**Topic:** I.03. Anatomical Methods

**Support:** NIMH (ZIAMH002498)

**Title:** The issue of cross-contamination with Affymetrix's ViewRNA© *In situ* hybridization histochemistry procedure: Work around and other tips

**Authors: *S. YOUNG**, J. SONG
Natl. Inst. of Mental Hlth., Bethesda, MD

**Abstract:** The advent of sensitive non-radioactive in situ hybridization histochemistry (ISSH) procedures has enabled rapid and sensitive exploration of gene expression in the brain and other tissues (Young et al., 2015). Two, in particular, are being used based on the same original patent employing multiple pairs of probes to reduce background upon which the amplification is built: ACD's RNAscope© and Affymetrix's ViewRNA©. These procedures also enable duplex and even multiplex detection of transcripts in the same sections.
We began to use ViewRNA© to perform duplex ISHH to explore expression of various genes in the CA2 area of the hippocampus. The ViewRNA© duplex technique on tissues uses two type of probes that visualize alkaline phosphatase deposition: Type 6 that are developed using Fast Blue followed by Type 1 that are developed using Fast Red. These reagents generate blue visible and far red fluorescent colors followed by red visible and red fluorescent colors, respectively. We became concerned that the Fast Blue color was being deposited inappropriately on the Type 1 probes in addition to the appropriate Type 6 probes. We assured ourselves that this was not a problem with our fluorescent filter cube sets (TRITC: EX->511-551, EM->573-613; Far Red: EX->610-640, EM->750-810) as single probe ISHH show no bleed-through of Type 6 blue development into the TRITC channel and vice versa.
We discovered that if, instead of following the manufacturer's recommendation to develop the Type 6 probe before the Type 1 probe, we reversed the order, we had no cross-contamination (on either probe type). The resultant duplex ISHH looked like the expected summation of the two individual ISHH. We have seen this phenomenon now with several different Type 6/Type 1 pairs. We will present speculations as to the cause of this cross-contamination as well as other tips to get the most out of this powerful technique.
References: 1. Young WS, Song J, & Mezey E. (2016). Hybridization Histochemistry of Neural Transcripts. *Current Protocols in Neuroscience. Ed: Crawley JN, et al., 75*, 1.3.1-1.3.27. http://doi.org/10.1002/cpns.9
This research was supported by the intramural research program of the NIMH (ZIAMH002498).

**Disclosures: S. Young:** None. **J. Song:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.03/VV45

**Topic:** I.03. Anatomical Methods

**Support:** U01MH105971

**Title:** Methodology for building Nissl stain and cell type-based brain atlases for vertebrate species

**Authors: *R. MUÑOZ CASTAÑEDA**, K. UMADEVI VENKATARAJU, M. BOTA, P. OSTEN
Cold Spring Harbor Lab., Cold Spring Harbor, NY

**Abstract:** We consider cell types to be the elementary building blocks of the brain, as they determine the properties of local circuits that can be built to serve the area-specific brain functions. While, there is a growing knowledge of cell type-specific cell anatomy in the mouse brain, there are virtually no data on quantitative, brainwide cell-type distribution in any other vertebrate species. Yet, it seems clear that knowing cell type distributions across brains of a broad range of species would greatly advance our understanding of how neuronal populations combine to form local and long-range circuits that give rise to structure-specific and, in some cases, species-specific brain functions.
Here we describe a set of anatomical tools, initially developed in our laboratory for the study of mouse brain anatomy, which form a versatile methodological pipeline for the generation of Nissl stain-based 3D brain atlases and tissue autofluorescence-based 3D reference brain volumes for data registration and quantification. The creation of the Nissl stain-based brain atlases is based on modifications of the iDISCO+ protocol (Renier et al. 2016). Thereafter, the samples are imaged by Serial Two Photon Tomography (STPT) (Ragan et al 2012), which generates complete brain volumes with minimal tissue distortions. The tissue autofluorescence-based brain volumes are generated by STPT and light sheet fluorescence microscopy (LSFM), resulting in the generation of brain volumes with detailed anatomical features that can be used for automated and precise data registration. Finally, the cell type maps are generated using iDISCO+ immunostaining with antibodies against cell type-specific protein markers. This methodology is applicable for brain atlasing and cell type mapping across a broad range of vertebrate species.

**Disclosures: R. Muñoz Castañeda:** None. **K. Umadevi Venkataraju:** None. **M. Bota:** None. **P. Osten:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.04/VV46

**Topic:** I.03. Anatomical Methods

**Support:** Simons Foundation SCGB 350789

NIH 5RO1NS073129

NIH 5RO1DA036913

Simons Foundation 382793/SIMONS

IARPA

**Title:** Interrogating the logic of neuronal projections using *In situ* barcode sequencing

**Authors: *X. CHEN**[1], J. M. KEBSCHULL[1,2], H. ZHAN[1], G. M. CHURCH[3,4], J. H. LEE[1], A. M. ZADOR[1]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Watson Sch. of Biol. Sci., Cold Spring Harbor, NY; [3]Wyss Institute, Harvard Med. Sch., Boston, MA; [4]Dept. of Genet., Harvard Med. Sch., Boston, MA

**Abstract:** Cortical neurons in a single area project to diverse targets in the mammalian brain. These neuronal projections form the structural basis for information flow within and out of the cortex. Several major classes of cortical projection neurons project to various targets, but individual neurons project to only a subset of the targets predicted by the classes. Single-neuron tracing experiments can resolve projection patterns at cellular resolution, but the throughput of most methods is too low to allow for the statistical analysis of the organization of projections. To infer the logic behind the organization of cortical projections of single neurons, we therefore developed a highly-multiplexed technique that maps single-cell projections *in situ*. We have previously presented MAPseq, which allows for massively parallel neuronal projection mapping at single neuron resolution (Kebschull et al., 2016). In MAPseq, each neuron expresses a unique RNA sequence (barcode) that fills both the soma and the axons. By dissecting out the brain areas in interest and sequencing the barcode, we can thus read out the projection patterns of single neurons. MAPseq, however, does not preserve information about the precise spatial location of the neurons. To overcome this challenge, we have adapted *in situ* sequencing to efficiently read out RNA barcodes *in situ*. Combining *in situ* sequencing with MAPseq (*in situ* MAPseq), we were able to interrogate the projections and locations of thousands of neurons in mouse auditory cortex at cellular resolution. Each experiment requires about two weeks. We used hierarchical clustering to find clusters that correspond to known classes of projection neurons. The laminar

distribution of these classes was consistent with previous results. To look for subclasses beyond those that have previously been identified, we decomposed single cell projection patterns into linear combinations of groups of correlated projections, which we denote projection “modules”. The spatial distribution of neurons expressing certain modules was spatially more compact than neurons without these modules, indicating that modules also show laminar preference. Unlike the clusters, however, modules were non-exclusive, so that a given neuron can express more than one module. These results suggest that cortical projections are hierarchically organized with different rules at each layer. The methods presented here can be applied to any brain region, and will allow for the systematic analysis of projection patterns and spatial organization throughout the brain.

**Disclosures:** **X. Chen:** None. **J.M. Kebschull:** None. **H. Zhan:** None. **G.M. Church:** None. **J.H. Lee:** None. **A.M. Zador:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.05/VV47

**Topic:** I.03. Anatomical Methods

**Support:** NIH R01DC008983

David and Lucile Packard Foundation

NIH R01EY0190491

NIH R01EY025722

NIH F31DC015185

**Title:** Dissection of neural pathways using anterograde transsynaptic transfer of AAV1

**Authors: *B. ZINGG**[1], X.-L. CHOU[1], Z.-G. ZHANG[2], L. MESIK[1], F. LIANG[2], N. K. ZHANG[3], H. W. TAO[3], L. I. ZHANG[3]
[1]Neurosci. Grad. Program, [3]Zilkha Neurogenetic Inst., [2]USC, Los Angeles, CA

**Abstract:** To decipher neural circuits underlying brain functions, it is essential to map the input and output connectivity of specific neuronal populations, which is facilitated by applying viral tracers. Although retrograde transsynaptic viruses are widely used for identifying the presynaptic sources of transduced neurons, analogous anterograde transsynaptic tools for tracing neural pathways downstream of postsynaptically targeted neurons remain under development. Here, we explored the potential application of adeno-associated virus (AAV) for anterograde transsynaptic

mapping of brain circuits. AAV1-Cre from transduced presynaptic neurons effectively drove Cre-dependent transgene expression in selected postsynaptic neuronal targets, and thus allowed the tracing and functional manipulation of axonal projections from the latter input-defined neuronal population. Application of this tool in superior colliculus (SC) revealed that SC neuron subpopulations receiving different corticocollicular projections specifically drove different types of defense behavior. This anterograde transneuronal tagging is thus useful for the forward screening of distinct functional neural pathways embedded in complex brain circuits.

**Disclosures: B. Zingg:** None. **X. Chou:** None. **Z. Zhang:** None. **L. Mesik:** None. **F. Liang:** None. **N.K. Zhang:** None. **H.W. Tao:** None. **L.I. Zhang:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.06/VV48

**Topic:** I.03. Anatomical Methods

**Support:** Max Planck Society

**Title:** The whole mouse brain-on-tape for volume electron microscopy and cellular connectomics

**Authors: *S. MIKULA**
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**Abstract:** The development of methods enabling the mapping of all synaptic connections between all neurons comprising an individual mammalian brain would lead to brain-wide circuit reconstructions that precisely define the neuronal networks underlying and responsible for generating the diverse behavioural repertoire for that individual. Recent advances in mouse whole-brain electron microscopic (EM) sample preparation (Mikula & Denk, 2015) and multi-beam scanning electron microscopy (mSEM, Kemen et al., 2015) have brought us closer to a complete mouse whole-brain cellular connectome.
Several obstacles remain, however. Here, I report on an approach using mouse whole-brain ultramicrotomy and serial-section SEM.
For serial sectioning, it is convenient to collect automatically cut sections on a tape conveyor belt (e.g., automated tape-collecting ultramicrotomy, or ATUM). Different tape substrates were tested for mouse whole-brain section collection, including Kapton, carbon nanotube-coated PET, and copper, iron and aluminum foils. Of these, aluminum foil was chosen due to its intrinsic electrical conductivity, low cost, low atomic number (which results in better EM contrast), thin passivation layer and long-term stability.
However, commercial ATUM systems damage aluminum foil during the course of section

collection and thus a custom section collection system was designed specifically to accommodate the collection of mouse whole-brain ultra-thin sections on aluminum foil. A working prototype consisting of two DC rotary motors, which actuated source and target tape reels, collected more than 5000 serial sections of mouse mid-sagittal sections at 80 nm thickness.
Serial section SEM indicates that sample membrane contrast and section thickness are sufficient for identifying matching neurites across adjacent sections. Synapses can be readily identified. Quantitative assessments of neurite traceabilty and synapse detection across the whole-brain serial sections are ongoing. Preliminary results suggest that the whole mouse "brain-on-tape" will be a useful tool for mouse whole-brain volume electron microscopy and cellular connectomics.
References:
Kemen, T., Malloy, M., Thiel, B., Mikula, S., Denk, W., Dellemann, G., & Zeidler, D. (2015). Further advancing the throughput of a multibeam SEM (Vol. 9424, p. 94241U-94241U-6). https://doi.org/10.1117/12.2188560
Mikula, S., & Denk, W. (2015). High-resolution whole-brain staining for electron microscopic circuit reconstruction. Nature Methods, 12(6), 541-546. https://doi.org/10.1038/nmeth.3361

**Disclosures: S. Mikula:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.07/VV49

**Topic:** I.03. Anatomical Methods

**Title:** Network cloning using DNA barcodes

**Authors: *S. SHUVAEV**, B. BASERDEM, A. ZADOR, A. KOULAKOV
Cold Spring Harbor Lab., Cold Spring Harbor, NY

**Abstract:** The connections between neurons determine the computations performed by both artificial and biological neural networks. Recently, we have proposed SYNseq, a method for converting the connectivity of a biological network into a form that can exploit the tremendous efficiencies of high-throughput DNA sequencing. In SYNseq, each neuron is tagged with a random sequence of DNA-a "barcode"-and synapses are represented as barcode pairs. SYNseq addresses the analysis problem, reducing a network into a suspension of barcode pairs. Here we formulate a novel and complementary synthesis problem: How can the suspension of barcode pairs be used to "clone" or copy the network back into an uninitialized tabula rasa network? Although this synthesis problem might be expected to be computationally intractable, we find that, surprisingly, this problem can be solved efficiently, using only neuron-local information. We present the "one barcode one cell" (OBOC) algorithm, which forces all barcodes of a given

sequence to coalesce into the same neuron, and show that it converges in a number of steps that is a power law of the network size. Rapid and reliable network cloning with single synapse precision is thus theoretically possible.

**Disclosures:** **S. Shuvaev:** None. **B. Baserdem:** None. **A. Zador:** None. **A. Koulakov:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.08/VV50

**Topic:** I.03. Anatomical Methods

**Support:** NIH-TRA 1R01NS092474

Allen Institute for Brain Sciences

**Title:** Automated antibody screening via probabilistic synapse detection

**Authors:** ***A. K. SIMHAL**[1], K. D. MICHEVA[2], J. S. TRIMMER[3], F. C. COLLMAN[4], R. J. WEINBERG[5], S. J. SMITH[6], G. SAPIRO[1]
[1]Electrical Engin., Duke Univ., Durham, NC; [2]Molec Cell. Physiol, Stanford Univ. Sch. Med., Stanford, CA; [3]Neurobiology, Physiol. & Behavior, Univ. of California, Davis, Davis, CA; [4]Allen Inst. for Brain Sci., Seattle, WA; [5]Cell Biol. & Physiol., Univ. North Carolina, Chapel Hill, NC; [6]Synapse Biol., Allen Inst. For Brain Sci., Seattle, WA

**Abstract:** Deeper exploration of the brain's vast networks requires new tools for high-throughput structural and molecular profiling of the diverse populations of synapses that connect those networks. Immunofluorescence array tomography allows both structural and molecular discrimination, but depends critically on the availability of high-quality antibodies that are rigorously validated for this specific application. Evaluating large numbers of antibodies against the same target antigen for effective and specific immunolabeling of array tomography samples is a difficult and labor-intensive process that requires manual inspection of the images. Furthermore, determining which antibody is best suited for this purpose is a subjective process, with varying levels of agreement between different experts.
We propose instead to use an automatic query-defined probabilistic synapse detection algorithm to quantify the results of antibody screens. For each test, the user controls synaptic markers and marker puncta sizes to consider. This model-based algorithm incorporates fundamental biological knowledge of synapses and how they are manifested in the immunofluorescence data by the selected antibody being tested [1]. Using this algorithm, we consider the automatically-detected synapse densities and the background noise levels (both the noise floor and the number of false positive blobs) to evaluate the efficacy of each antibody.

We tested this approach on several different antibody datasets. Each dataset consisted of multiple antibodies against the same synaptic protein, imaged alongside one or more known synaptic protein antibodies. The proposed method correctly identified the top antibody each time, and correctly picked the top three antibodies 75% of the time (as determined manually by an expert). This approach opens the door for high-throughput antibody screening analyses, as well as for antibody quality control. All data, code, and data derivatives will be made available after publication.

[1] Simhal, Anish K., et al. "Probabilistic Fluorescence-Based Synapse Detection." PLoS Computational Biology, May 2017.

**Disclosures:** **A.K. Simhal:** None. **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC; Stanford Univ.. **J.S. Trimmer:** None. **F.C. Collman:** None. **R.J. Weinberg:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC; Stanford Univ.. **G. Sapiro:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.09/VV51

**Topic:** I.03. Anatomical Methods

**Support:** NIH Grant 1R01NS092474

**Title:** Comparing mouse and human synapses with automated probabilistic synapse analysis

**Authors: *K. D. MICHEVA**[1], A. K. SIMHAL[2], J. T. TING[4], A. L. KO[6], W. W. SEELEY[7], E. F. CHANG[8], A. NANA LI[9], E. LEIN[10], F. C. COLLMAN[11], D. V. MADISON[12], R. J. WEINBERG[13], S. J. SMITH[5], G. SAPIRO[3]
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**Abstract:** Mouse models are widely used in biomedical research, but do they provide a satisfactory model for Homo sapiens? Much of our current understanding of the molecular basis

of neuropsychiatric diseases is based on studies in rodents, but surprisingly little is yet known about the similarities and differences between the synapses of mice and men, though this knowledge will be a crucial step toward developing new drugs directed at synaptopathies. The introduction of array tomography enables acquisition of hyperspectral proteometric data, allowing identification of multiple synapse subtypes in both mouse and human tissue, critical data that can allow this comparison.
We had previously developed an automated query-defined probabilistic synapse detection algorithm and validated it in mouse brain tissue [1]. We now show that this algorithm performs equally well on human tissue obtained from brain surgeries, and use it to quantify similarities and differences between human and mouse synapses, with a focus first on excitatory glutamatergic synapses in neocortex.
Our results show that excitatory synapse sizes and densities differ between mouse and human neocortex, with human synapses being larger and sparser. However, many of the correlations between the size of synapse postsynaptic densities and their AMPA and NMDA receptor content, previously documented in rodents, also hold true for human cortical synapses. We further refine this comparison by including variables such as the type of vesicular glutamate transporter present in the presynaptic bouton, the abundance of mitochondria, and the type of postsynaptic target. This computational approach opens a door to thorough comparison between human and animal model synapses, as well as to a data-driven discovery of novel synaptic arrangements in human brain. The methods developed for this application are readily applicable to other datasets. All data, code, and data derivatives will be made available after publication.
[1] Simhal, Anish K., et al. "Probabilistic Fluorescence-Based Synapse Detection." PLoS Computational Biology, May 2017

**Disclosures:** **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC. **A.K. Simhal:** None. **J.T. Ting:** None. **A.L. Ko:** None. **W.W. Seeley:** None. **E.F. Chang:** None. **A. Nana Li:** None. **E. Lein:** None. **F.C. Collman:** None. **D.V. Madison:** None. **R.J. Weinberg:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC. **G. Sapiro:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.10/VV52

**Topic:** I.03. Anatomical Methods

**Support:** VR 2015-04198

VR 2016-00968

**Title:** SMLocalizer, a CUDA based ImageJ plugin for PALM/STORM data analysis

**Authors: *H. BRISMAR**, K. BERNHEM, L. WESTIN
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**Abstract:** Single molecule localization microscopy (SMLM) has in the last decade been developed as an important technique for diffraction unlimited microscopy. Super resolution imaging is here achieved by a combination of switching fluorophore molecules and mathematical analysis software. A challenge for the non-expert user is the complexity of the computational analysis with a multitude of input parameters. Many algorithms and methods have been developed, often specific for a particular variant of SMLM.
Here we present a software solution, SMLocalizer, which reduces the complexity in SMLM analysis by automatically obtaining most of the input parameters iteratively from the data and requires minimal user input for accurate analysis. We demonstrate the performance of the algorithms and make a comparison to other software in terms of localization error and detection probability as function of signal to noise ratio on synthetic and real imaging data. The software can be used to analyze 2D and the majority of established 3D modalities. The algorithms are implemented as a plugin for the well-established ImageJ toolkit and provide a significant speed-up of analysis by use of GPU acceleration. We demonstrate the application of the algorithms in a study of co-distribution of the neuronal specific alfa3 isoform of Na,K-ATPase and NMDAR in hippocampal neurons.

**Disclosures: H. Brismar:** None. **K. Bernhem:** None. **L. Westin:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.11/VV53

**Topic:** I.03. Anatomical Methods

**Support:** KAKENHI JP26460388

KAKENHI JP16KT0134C1

**Title:** Brain amine neurotransmitters are comprehensively visualized by matrix-free laser desorption/ionization imaging mass spectrometry using a unique photocleavable derivatizing agent

**Authors: *T. MATSUDA**[1], H. FUKANO[1,2], M. WAKI[1,2], S. TAKEI[1,2,3], F. ETO[1,2], M. SETOU[2,3,4,5,6], T. MAKI[7], I. YAO[1,3]

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**Abstract:** In mammals including human, cognitive function such as memory and attention depends on various brain neurotransmitters. Neurotransmitters are mainly divided into 3 groups; peptides, monoamines, and amino acids. Especially, amino acids represented by glutamate, gamma-aminobutyric acid (GABA), and serotonin play a pivotal role in central nervous system and closely involve severe neurological disease such as Alzheimer's disease, depression, and epilepsy. The amine neurotransmitters are considered to indirectly interact with each other to maintain brain functions, and the impairment of the homeostatic balance contributes to pathological states in brain. Conventionally, activities or expressions of related enzymes and amount of single neurotransmitter have been measured by biochemical assays and microdialysis, but these traditional approaches have not enabled us to directly analyze abundance and distribution of multi-neurotransmitters on brain tissues. Here, we conducted a comprehensive analysis of cerebral amine neurotransmitters by a recently-developed method, laser desorption/ionization imaging mass spectrometry (LDI-IMS) against mouse brain samples. In this study, firstly we prepared mouse brain sections on indium tin oxide (ITO)-coated slides using 6-8 weeks old male C57BL/6 mice. Secondly, we sprayed a unique reagent, 4-[3,5-Dimethyl-4-(4-nitrobenzyloxy)phenyl]-4-oxobutyric acid succinimidyl ester (DNPO-SE) on the sample slides, and derivatized amino acids in brain tissues. Finally, we detected the ionized products derived from derivatized amino acids and comprehensively visualized amine neurotransmitters in brain by imaging mass spectrometry (IMS) with iMScope (Shimadzu). As a result, we successfully detected and visualize the distribution of the eight amines simultaneously; alanine, serine, taurine, methionine, GABA, cysteine, glutamate, and serotonin in the mouse brain tissues. Interestingly, each amine showed a different spatial distribution in the brain. Notably, serotonin characteristically located in the raphe nucleus that contains numerous serotonin-producing neurons. In conclusion, utilizing a derivatizing reagent DNPO-SE enables us to inclusively analyze amine neurotransmitters in mouse brain tissues by matrix-free LDI-IMS. The newly-developed approach may have applicability to other reactive amines, and it may be a simpler and quicker experimental technique than conventional matrix-assisted IMS methods. The matrix-free IMS using DNPO-SE make it possible for us to simultaneously investigate the group of amine neurotransmitters in normal status and pathologic conditions in brain.

**Disclosures:** **T. Matsuda:** None. **H. Fukano:** None. **M. Waki:** None. **S. Takei:** None. **F. Eto:** None. **M. Setou:** None. **T. Maki:** None. **I. Yao:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.12/VV54

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Title:** Detection of 2-hydroxyglutarate in IDH-mutant gliomas by 3-tesla magnetic resonance spectroscopy

**Authors: *M. NATSUMEDA**[1], H. IGARASHI[2], M. OKADA[1], K. MOTOHASHI[1], T. NAKADA[2], Y. FUJII[1]
[1]Neurosurg., [2]Ctr. for Integrated Brain Sci., Brain Res. Institute, Niigata Univ., Niigata, Japan

**Abstract:** *Introduction.* Accumulation of 2-hydroxyglutarate (2HG) is known to occur in isocitrate dehydrogenase (*IDH*)-mutant gliomas. Detection 2HG is possible by 3.0-tesla single voxel magnetic resonance spectroscopy (SVMRS), thus making non-invasive diagnosis of *IDH*-mutant gliomas possible. We set out to determine whether reliable detection of 2HG is feasible in *IDH*-mutant World Health Organization (WHO) grade 2 through 4 gliomas. *Methods.* A total of 110 patients harboring WHO grade 2 through 4 gliomas underwent preoperative MRS evaluation to detect 2HG and other metabolites. MRI/$^{1}$H-MRS analysis was performed using a 3.0-tesla system (Signa LX, General Electric). First, proton density images (Fast Spin Echo; TR/TE = 5000/40; FOV: 20 x 20 mm; matrix: 256 x 256; slice thickness: 5 mm; inter slice gap: 2.5 mm) were taken. The slice with the largest depiction of tumor on proton density images was selected for SVMRS. A point-resolved spectroscopic sequence (PRESS), with chemical-shift-selective water suppression was used with the following parameters: (TR: 1.5 s; TE: 30 ms; data point 512; spectral width 1000 Hz; number of acquisitions: 128 -196; volume of interest (VOI): 12-20 x 12-20 x 12-20 mm). Presence of *IDH*-mutations was determined by IDH1 R132H immunohistochemical analysis and DNA sequencing of surgically obtained tissues. *Results.* Thirty seven out of 66 (56.1%) grade 2/3 gliomas and 6 out of 44 (13.6%) GBs were *IDH*-mutant. *IDH*-mutant gliomas exhibited significantly higher accumulation of 2HG (median 5.029mM for grade 2/3 gliomas, 3.191 mM for GBs vs. 0.000 mM for *IDH*-wildtype grade 2/3 and GBs, both $p < 0.0001$, Mann-Whitney test). Also, lower levels of Glx (the sum of Glutamine and Glutamate) were detected in *IDH*-mutant grade 2 through 4 gliomas and lower levels of Glutathione in *IDH*-mutant grade 2/3 gliomas. A cutoff of 2HG = 1.489 mM achieved 100% sensitivity and 68.4% specificity and 83.3% sensitivity and 92.6% specificity in determining *IDH*-mutation in grade 2/3 gliomas and GBs, respectively. Grade 2/3 gliomas with high 2HG accumulation had significantly longer overall survival than those with low 2HG accumulation ($p = 0.02$, Log Rank test). *Discussion.* Non-invasive and reliable detection of 2HG in *IDH*-mutant grade 2 though 4 gliomas was possible by 3.0-tesla SVMRS.

**Disclosures:** **M. Natsumeda:** None. **H. Igarashi:** None. **M. Okada:** None. **K. Motohashi:** None. **T. Nakada:** None. **Y. Fujii:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.13/VV55

**Topic:** I.01. Molecular, Biochemical, and Genetic Techniques

**Support:** IMIR PF KU Leuven

FP7 Inmind

**Title:** Synthesis and preclinical evaluation of [11C]BA-1, a PET tracer for brain CSF-1R

**Authors:** ***G. M. BORMANS**[1], B. ATTILI[2], M. AHAMED[2], S. CELEN[2]
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**Abstract:** **Introduction** Positron emission tomography (PET) provides a sensitive non-invasive imaging technique to study receptors and enzyme expression and occupancy. A radiolabelled ligand with high affinity for a receptor which is overexpressed during neuroinflammation is highly desired as an imaging agent for neuroinflammation.[1] The colony stimulating factor-1 receptor (CSF-1R) appears to play a significant role in neuroinflammation. Since, it is profoundly expressed in microglia, the CSF-1R can be a potential target for visualization of neuroinflammation.[2] No specific radiotracer is available for visualizing CSF-1R with PET yet. Herein, we describe the synthesis and preclinical evaluation of [$^{11}$C]BA-1 for imaging of CSF-1R. **Materials and Methods** Synthesis of reference and precursor were done according to literature methods[2]. [$^{11}$C]-methyl triflate reacts with the precursor in presence of a base at room temperature for 2 minutes providing carbon-11 radiolabeled [$^{11}$C]BA-1 which was purified by reversed-phase high pressure liquid chromatography (RP-HPLC). Baseline biodistribution and blocking study performed in mice. Baseline and blocking microPET imaging studies were performed on a Focus™ 220 microPET scanner with female rats and non-human primate. **Results** Biodistribution study demonstrates a high tracer uptake in the brain (4% ID at 2 min post injection). Baseline microPET scans in rats suggest good uptake of tracer into the brain with SUV 1.2. MicroPET studies with non-human primates confirm high tracer uptake in the cortical regions in line with literature, and this uptake was reduced by pretreatment with BLZ-945, a CSF-1R inhibitor. Therefore, the present findings indicate that the developed tracer could be a suitable molecule for the in vivo imaging of CSF-1R. **Conclusion** We successfully synthesized, optimized radiochemistry and preclinical evaluation was done with [$^{11}$C]BA-1 tracer as a selective for brain CSF-1 receptor imaging. **References** 1. Dieter Ory et al., (2014). PET Radioligands for in Vivo Visualization of Neuroinflammation, Current Pharmaceutical Design,

No. 17, 5897-5913 2. Carl R Illig et al., (2008). Discovery of novel FMS Kinase inhibitors as anti-inflammatory agents, Bioorganic & Medicinal Chemistry Letters, 18, 1642-1648.

**Disclosures:** **G.M. Bormans:** None. **B. Attili:** None. **M. Ahamed:** None. **S. Celen:** None.

**Poster**

**622. Connectomics: Molecular Techniques**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 622.14/VV56

**Topic:** F.02. Behavioral Neuroendocrinology

**Support:** FAPESP: 2014/24113-1

**Title:** Systematic screening of the molecular markers of leptin signaling in the hippocampus: role of pi3k pathway

**Authors: *B. DE CARVALHO BORGES DEL GRANDE**[1], M. B. CELANI[1], A. G. SILVA[1], J. ANTUNES-RODRIGUES[1], J. DONATO JR[2], L. L. K. ELIAS[1]
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**Abstract:** Leptin plays a pivotal role in the control of energy balance through its action via LepR in hypothalamic nuclei. The LepR is also expressed in the hippocampus, a structure involved in learning and memory processing. Leptin administration into the hippocampus suppresses feeding and appetitive behaviors. However, which signaling pathway is recruited in the hippocampus by leptin has not been defined. Our study aims to identify molecular markers of leptin signaling pathways in the hippocampus as well as to systematically screen the changes in transcript levels following leptin treatment, using a qPCR array (PAMM-030ZA-6, Qiagen). Intraperitoneal injection of saline (0.15M NaCl, in 5µl/g) or leptin (2.5µg/g, in 5µl/g) was performed in adult male C57BL/6 mice. As studies have extensively suggested that STAT3 pathway is the main pathway by which leptin promotes its hypophagic effects in the hypothalamus, we assessed the phosphorylation of STAT3 in response to leptin in the hippocampus. In contrast to the robust leptin-induced STAT3 phosphorylation in the hypothalamus, leptin promotes no STAT3 phosphorylation in the hippocampus. To visualize the LepR expressing neurons, we crossed the LepR-Cre, a knock-in strain that coexpresses Cre-recombinase with the *Lepr* gene with the R26-tdTomato mouse, which induces the expression of a red fluorescent reporter protein. To evaluate neuronal activation in response to leptin we assessed c-Fos expression and c-Fos/LepR-Cre tdTomato colocalization in the hypothalamus and hippocampus. A higher number of c-Fos/LepR-Cre tdtomato expressing cells in the hypothalamus, but not in the hippocampus, was found in response to leptin. Because the hypophagic effect of leptin depends in part on the activation of PI3K and ERK signaling pathways in the hypothalamus, we further investigated

ERK and PI3K signaling in the hippocampus. There was no leptin-induced changes in pERK1/2 expression in the hippocampus. However, we found that leptin stimulates the PI3K pathway in the hippocampus of wild type mice, characterized by an increased expression of total AKT protein and an increased phosphorylation of AKT at S473 residue. Phosphorylated AKT in the hippocampus was shown to colocalize with LepR-Cre tdTomato expressing cells. Our data suggest that leptin signaling pathways in the brain are site specific. In the hypothalamus, c-Fos, STAT3 and ERK pathways mediate leptin actions. On the other hand, remarkably, the PI3K-AKT pathway is recruited by leptin in the hippocampus. Molecular markers downstream PI3K-AKT may underlie the mechanisms involved in the role of leptin in the hippocampus which affects feeding behaviors and cognitive processes.

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**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.01/VV57

**Topic:** D.07. Vision

**Support:** NIH MH103479

NIH EY023336

**Title:** Stimulus sensitivity of narrowband gamma oscillations in human visual cortex

**Authors: *B. L. FOSTER**, W. BOSKING, M. BEAUCHAMP, D. YOSHOR
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**Abstract:** Grating stimuli (e.g. Gabor patches) are commonly used to study neural responses in visual cortex. Across several species, grating stimuli reliably elicit narrowband gamma oscillations in the local field potential that peak around 30-70 Hz. Recordings from macaque V1 have shown that increases in the visual contrast of presented gratings shifts the peak frequency of induced gamma oscillations rightward (e.g. grating-contrast:gamma-frequency = 25%:38Hz; 50%:44Hz; 100%:53Hz; Ray & Maunsell, 2010). More recently, human intracranial V1 recordings have suggested that narrowband gamma oscillations are uniquely driven by grating stimuli, and not naturalistic images (e.g. faces or houses; Hermes et al., 2015). This sensitivity to stimulus attributes has important implications for the role of gamma oscillations in perception, and the mechanisms of neural circuit rhythmicity. We therefore sort to replicate these observations of stimulus dependence in human visual cortex using intracranial recordings from 7 subjects viewing grating (n = 7) and object (n = 4) stimuli. To improve the number of visual

recording sites, hybrid micro/macro electrocorticography (ECoG) strip electrodes were employed. Electrode location in visual cortex was verified through MRI/CT imaging and receptive field mapping. During Experiment 1 large field static grating stimuli (sine-wave; 1 cycle/deg.) were presented randomly at 20%, 50% and 100% Michelson contrast. Stimuli were presented for 500 ms, with a 200 ms pre-stimulus fixation cue and a random inter-stimulus interval between 500-1500 ms. During Experiment 2 grayscale object images (faces, bodies, cars, houses, limbs, numbers, letters, scrambled) were randomly presented for 500 ms with a random inter-stimulus interval between 500-1500 ms. Spectral analysis revealed large increases in narrowband gamma power in response to grating stimuli. In addition, the peak frequency of gamma oscillations was parametrically increased with higher contrast levels, as was the power of response (e.g. grating-contrast:gamma-frequency = 20%:28Hz; 50%:40Hz; 100%:48Hz). Consistent with non-human primate recordings (Ray & Maunsell, 2010) the specific peak frequency of gamma oscillations, and their contrast dependence, was variable between subjects but typically shifted through a range of ~25-65 Hz. Consistent with human recordings (Hermes et al., 2015) object stimuli did not induce similar narrowband gamma oscillations, but did produce clear broadband spectral responses. These findings provide a strong replication of stimulus dependent gamma oscillation frequency in primate visual cortex.

**Disclosures: B.L. Foster:** None. **W. Bosking:** None. **M. Beauchamp:** None. **D. Yoshor:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.02/VV58

**Topic:** I.04. Physiological Methods

**Support:** Universidad de Guanajuato

**Title:** Modulation of postmenopause and premenopause on resting state electroencephalographic power in women

**Authors: *E. G. GONZÁLEZ-PÉREZ**, S. SOLÍS-ORTIZ
Med. Sci., Univ. De Guanajuato, León, Mexico

**Abstract:** Hormonal changes that characterize postmenopause may influence cortical electrical activity. This study investigated whether postmenopause influences resting-state electroencephalogram (EEG) power in the frontal, central, parietal and occipital cortical regions compared with premenopausal women who have regular menstrual cycles. EEG recording were conducted in the resting state in twenty early postmenopausal healthy women between 48 and 60 years and twenty premenopausal healthy women between 40 and 45 years. The resting EEG spectral absolute power in the frontal, central, parietal and occipital cortical regions was

analyzed during eyes closed condition. The frequency bands considered were the delta, theta, alpha1, alpha2, beta1 and beta2 and were compared among participants. Postmenopausal women compared to premenopausal women showed theta power increased in frontal regions, alpha1 power increased in the frontal region, alpha2 power increased in the frontal and central regions and beta1 power increased in the frontal, central and parietal regions. Delta and beta2 bands did not show changes among participants. These findings suggest that suggest that the hormonal status of women may impact on brain function leading to states of low and high cortical activation, which may affect behavior and cognition.

**Disclosures: E.G. González-Pérez:** None. **S. Solís-Ortiz:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.03/VV59

**Topic:** I.04. Physiological Methods

**Support:** Great-West Life

**Title:** Sensory gating alterations in major depressive disorder, and their relationship to clinical symptoms

**Authors: *S. DE LA SALLE**[1], M. BIRMINGHAM[2], P. BLIER[2], V. KNOTT[2]
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**Abstract:** The use of electroencephalography has shown promise in detecting cortical dysfunction in major depressive disorder (MDD). Cognitive impairments in MDD are evidenced across all functional domains, including pre-attentive auditory sensory and attentional processes. Physiological measures that can be used to index these impairments include the P50 and N100 event-related potentials (ERPs). Reduced P50 gating is thought to index impaired filtering of irrelevant sensory information, while reduced N100 gating may signify impaired attention. However, differences in the P50 and N100 ERPs between patients and healthy controls and the relationship to clinical symptoms requires further study. The main objective of this study was to investigate differences in sensory gating and attention between medication-free depressed individuals and healthy controls. The study also aimed to determine whether P50 and N100 processes were related to the severity of clinical symptoms. 20 healthy non-patient male and female controls were matched, based on age, sex, smoking status and handedness, to a population of 20 outpatient volunteers with a documented diagnosis of DSM-IV MDD. All participants were administered the Beck Depression Inventory, the Stress Reactive Rumination Scale, and the Dysfunctional Attitudes Scale. Following the questionnaires, the auditory paired-

click paradigm was administered and electrophysiological responses were acquired using a 32-electrode array. P50 and N100 peak amplitudes (μV), latencies (ms), and gating indices (ratio [rP50] and difference scores [dP50]) were quantified and statistically analyzed using a two-tailed t-test for comparison between control and depressed groups. Finally, correlations between physiological measures and clinical symptoms were carried out. The depressed group exhibited reduced sensory gating and attentional processes compared to the healthy control group. Significant correlations between clinical symptoms and P50, N100 measures were observed. These findings provide additional support for sensory and attentional impairments in MDD.

**Disclosures: S. De La Salle:** None. **M. Birmingham:** None. **P. Blier:** None. **V. Knott:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

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**Program#/Poster#:** 623.04/VV60

**Topic:** I.03. Anatomical Methods

**Support:** NIH NINDS NS075321

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Barnes Jewish Hosptial Foundation

American Parkinson Disease Association

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Oertli Fund for Parkinson Disease Research

**Title:** Head angle during image acquisition impacts interpretation of DBS electrode position

**Authors: *S. A. NORRIS**[1], M. MILCHENKO[2], A. Z. SNYDER[2], M. C. CAMPBELL[1], M. USHE[1], J. L. DOWLING[3], K. M. RICH[3], J. S. PERLMUTTER[4]
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**Abstract: Background:** Deep brain stimulation (DBS) in the region of the subthalamic nucleus (STN) produces variable motor, psychiatric and cognitive effects in patients with Parkinson disease. This variation may be due to differences in electrode location relative to targeted areas of STN. Thus, the ability to accurately localize electrodes and their movement during the post-operative period is crucial for ongoing research trying to determine regional effects of DBS.

**Objective:** To quantitatively assess post-operative DBS electrode displacement, accounting for head position during image acquisition. **Methods:** We studied 56 patients with bilateral implantation of STN DBS electrodes who underwent immediate pre-operative MRI (DBS-MRI) and post-operative head computed tomography (DBS-CT) in supine position and delayed head CT (DEL-CT) with variable degrees of head tilt. We determined electrode locations using a novel method involving three-stage maximization of CT signal integrated over an analytic model of DBS implant. DBS-MRI, DBS-CT and DEL-CT images were co-aligned by optimizing a 6-parameter rigid body transform. In addition, DBS-MRI were affinely aligned to standard (MNI152) space, and CT images were also resampled to this space using a combination of computed transforms. We compared electrode location in standard space between DBS-CT and DEL-CT and computed the correlation coefficients *r* between the magnitude of displacement between scans versus the time between scans, volume of postoperative subdural air (median = 3.9 $cm^3$), and delta head angle between scans. **Results:** On average, electrode displacement from DBS-CT to DEL-CT in MNI152 space occurred in the rostral-ventral-lateral direction (right: $x$ = -0.12, $y$ = 0.43, $z$ = -0.83; left: $x$ = 0.14, $y$ = 0.44, $z$ = -0.89) with increased curvature (average radius = 366 mm vs 223 mm), each independently different from zero. Magnitude of displacement and degree of curvature was independent of elapsed time between CTs. Delta head angle significantly correlated with electrode displacement ($p$=1e-10, $r$=0.46 for both sides); a weaker correlation with subdural air on DBS-CT was observed ($p$=1e-2, $r$=0.21 and 0.25 for left and right sides, respectively). **Conclusion:** These data support a role for post-operative electrode movement, with head angle at the time of image acquisition significantly contributing to electrode movement. Accurate interpretation of post-operative DBS electrode position must account for the angle of image acquisition.

**Disclosures: S.A. Norris:** None. **M. Milchenko:** None. **A.Z. Snyder:** None. **M.C. Campbell:** None. **M. Ushe:** None. **J.L. Dowling:** None. **K.M. Rich:** None. **J.S. Perlmutter:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.05/VV61

**Topic:** I.03. Anatomical Methods

**Support:** NIH NINDS NS075321

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NIH NINDS NS058714

Barnes Jewish Hosptial Foundation

American Parkinson Disease Association

Barbara and Sam Murphy Fund

Oertli Fund for Parkinson Disease Research

**Title:** 7T mri probabilistic stn atlas for use with 3T mri

**Authors: M. MILCHENKO**[1], S. A. NORRIS[2], A. Z. SNYDER[1], K. L. POSTON[5], M. C. CAMPBELL[2], *M. USHE[3], J. S. PERLMUTTER[4]
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**Abstract: Background.** Deep brain stimulation (DBS) of the subthalamic nucleus (STN) effectively reduces motor symptoms in most patients with Parkinson disease (PD), yet also may produce untoward effects. Investigation of DBS effects requires accurate localization of the STN which can be difficult to unambiguously identify on magnetic resonance images (MRI) collected with clinically available 3T scanners. Objective: To develop a high quality STN atlas using a 7T scanner that can be applied to standard 3T images.
**Methods**. We created a high definition probabilistic STN atlas derived from seven elderly subjects imaged at 7T. This atlas was nonlinearly registered to a standard template representing 56 patients with PD imaged at 3T. This process required development of novel methodology for non-linear multi-modal image registration. STN in individuals can be localized by registration of a 3T image to this 3T template.
**Results.** We demonstrate mm-scale STN localization accuracy by comparison with another publicly available 7T atlas. We also demonstrate less agreement with an earlier histological atlas. STN localization error in the 56 patients imaged at 3T was less than 1 mm on average.
**Conclusions.** Our methodology enables accurate STN localization in patients imaged at 3T. The STN atlas is freely available to the research community. This new image registration methodology may be generally applicable to other datasets.

**Disclosures: M. Milchenko:** None. **S.A. Norris:** None. **A.Z. Snyder:** None. **K.L. Poston:** None. **M.C. Campbell:** None. **M. Ushe:** None. **J.S. Perlmutter:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.06/VV62

**Topic:** I.03. Anatomical Methods

**Title:** Pharmacological functional MRI analysis of dopamine D1 receptor interventions with 11.7 T high field MRI scanner

**Authors: Y. KIMURA**[1], S. NAKAZAWA[1], Y. MORI[2], K. NISHIGORI[1], *M. YAMANAKA[1], J. ICHIHARA[1], Y. YOSHIOKA[2]
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**Abstract:** Dopamine D1 receptor (D1R) is an important therapeutic target associating with schizophrenia, Parkinson's disease, attention deficit hyperactivity disorder and attracting attention. Pharmacological-fMRI (phfMRI) is useful to investigate the neurophysiological function of particular receptors and to evaluate pharmacological interventions. However, the neurophysiological function of D1R is largely unknown. In this study, we investigated the effects of D1R interventions on the BOLD effects using phfMRI with a preclinical high-field MRI scanner. All MRI experiments were conducted using an 11.7-T vertical-bore Bruker Avance II imaging system and a volume radio frequency coil for transmission and reception. During the MRI session, 8-week-old male Wistar rats (final N = 21) were anesthetized with 1-2% isoflurane, via a face-mask. Structural and fMRI data were obtained by multi-slice rapid acquisition with relaxation enhancement (RARE) sequence. After 60-minute sequential scan (pre-treatment scan), SKF82958 (3 mg/kg, an agonist of D1-like receptors), SCH39166 (1 mg/kg, an antagonist of D1-like receptors) and saline (as control) were subcutaneously administrated for each group, respectively. The 90-min follow-up scan (post-treatment scan) was conducted immediately after those treatments. We used SPM12 for the slice realignment, co-registration, and spatial normalization. SPM also generated parametric maps of statistical significance of BOLD effects with a general linear model. In this study, we found a significant increment of the BOLD signal intensity in the striatum, thalamus, and cerebellum by SKF82958 treatment ($p<0.05$, FWE-corrected). In contrast, SCH39166 showed a significant decrease in similar regions where we found signal increment with SKF82958 treatment ($p<0.05$, FWE-corrected). Temporal analyses revealed that the peak effect was found at 30-60 min after the SKF82958 treatment, whereas that was found at post 60-90 min after SCH39166. In summary, phfMRI detects D1R-modulated BOLD effects in the specific brain regions, including the striatum which contains the high D1R distribution. Thus, we successfully visualized D1R-derived neural activation in various brain regions. PhfMRI can contribute to the *in vivo* pharmacological assessment of targeting dopamine receptors.

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**Poster**

**623. Neurophysiology: Humans**

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**Program#/Poster#:** 623.07/VV63

**Topic:** I.03. Anatomical Methods

**Support:** Brain Initiative RFA-MH-15-200

**Title:** Non-invasive methods for estimating brain network latencies

**Authors: *S. PAJEVIC**[1], A. V. AVRAM[2], A. BERNSTEIN[3,7], R. COPPOLA[4], M. J. CURRY[3,8], R. D. FIELDS[5], M. HALLETT[6], T. HOLROYD[4], G. LEODORI[6], A. C. SIMMONS[3], N. THIRUGNANASAMBANDAM[6], Z. NI[6], P. J. BASSER[3]
[2]NIBIB, [3]NICHD/SQITS, [4]NIMH/MEG Core Facility, [5]NICHD, [6]NINDS/MNB/HMCS, [1]NIH, Bethesda, MD; [7]Univ. of Arizona, Tucson, AZ; [8]Henry Jackson Fndn., Bethesda, MD

**Abstract:** Temporal latencies between constituent parts of any complex dynamical system are an important factor in determining its function and stability. This is the case in neural systems where precise timing of the arrival of neural spikes and signals that propagate along axons is of fundamental importance. While there is a great effort today in reconstructing brain connectivity maps, there is no reliable framework to determine latencies in such networks. To address this, we use a multi-modality approach, combining structural and functional networks, each with their own latency estimates. Using structural information obtained with Mean Apparent Propagator (MAP) MRI data, we estimate the conduction velocity distribution (CVD) from the measured axon diameter distribution (AAD) in white matter. Using functional information derived from transcranial magnetic stimulation (TMS) evoked potentials (EP) via electroencephalography (EEG), as well as time-series analysis of magnetoencephalography (MEG) and electroencephalography (EEG) recordings, we have an independent assessment of the functional brain network connectivity. In deriving the latency from such data it is important to distinguish between temporal delays between neural events and true latencies that we aim to reconstruct. Latencies obtained from time-series analysis can only establish Wiener-Granger causality while incorporating TMS measurements and structural information about axons morphology and white matter path lengths provide additional information that can aid in deducing the true causality. In this abstract, we present preliminary results, with a focus on theoretical aspects and simulations. Our goal here is two-fold: 1) to improve the estimation of latencies from neural recordings using both stationary and non-stationary time-series approaches, and 2) to develop approaches that combine results from different imaging modalities, e.g., diffusion MRI and fMRI. In 1), we compare existing analytical methods that utilize similarity and statistical dependence measures (cross-correlation, transfer entropy, etc.) on time-series, as well as our proposed approach, that is based on non-stationary features of time-series. We test all the approaches using simulations on

four types of models: a) linear delay, b) multivariate autoregressive model, c) coupled non-linear dynamical systems, and d) brain network simulations based on known connectivity. We also apply and compare all the methods considered to MEG recordings (resting state with eyes closed). In 2) we use diffusion MRI-derived latency distributions as priors in a Bayesian framework combining the results from different modalities.

**Disclosures: S. Pajevic:** None. **A.V. Avram:** None. **A. Bernstein:** None. **R. Coppola:** None. **M.J. Curry:** None. **R.D. Fields:** None. **M. Hallett:** None. **T. Holroyd:** None. **G. Leodori:** None. **A.C. Simmons:** None. **N. Thirugnanasambandam:** None. **Z. Ni:** None. **P.J. Basser:** None.

**Poster**

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**Topic:** I.03. Anatomical Methods

**Support:** NIH Grant 1-R24-MH-109068-01

**Title:** Inferring network latencies in the CNS from diffusion MRI data

**Authors: *A. V. AVRAM**[1], A. S. BERNSTEIN[2], R. COPPOLA[8], M. J. CURRY[1], R. D. FIELDS[3], M. HALLETT[9], T. O. HOLROYD[4], G. LEODORI[5], S. PAJEVIC[6], A. C. SIMMONS[2], N. THIRUGNANASAMBANDAM[7], N. ZHEN[5], P. J. BASSER[2]
[1]Natl. Inst. of Biomed. Imaging and Bioengineering, [2]Natl. Inst. of Child Hlth. and Human Develop., [3]NICHD, [4]Natl. Inst. of Mental Hlth., [5]Natl. Inst. of Neurolog. Disorders and Stroke, [7]Human Motor Control Section, [6]NIH, Bethesda, MD; [8]MEG Core Facility, NIMH, Bethesda, MD; [9]Human Motor Control Sec, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

**Abstract:** Descriptors of cytoarchitecture and tissue microstructure such as average axon diameters (AAD) can provide valuable information about the functional organization of normal and pathological white matter. The linear relationship between the diameters of myelinated axons and their action potential propagation velocities is well-established in the literature. Using a recently proposed diffusion MRI method, called Mean Apparent Propagator (MAP)-MRI, we can measure the probability density function of net displacements of water molecules in brain tissues and compute the return-to-axis probability (RTAP) along the orientation of major white matter pathways. In compact white matter regions, myelinated axons can be modeled as collections of parallel impermeable cylinders. In this case, the RTAP allows the estimation of the AAD, from which we can compute the average conduction velocity (ACV) assuming the linear relationship ACV[mm/ms] = 5.5AAD[μm] based upon electrophysiology studies on mammalian myelinated nerves. From the same diffusion MRI data, we can visualize white matter pathways

using whole-brain fiber tractography, and derive estimates of the lengths of white matter pathways connecting different cortical or deep gray matter regions. Finally, by combining ACVs and fiber length measurements we obtain an estimate of the mean latency at a millisecond time-scale between pairs of cortical regions and construct a mean latency matrix (MLM). Whole-brain visualization of AADs is consistent with previous post-mortem studies in monkeys showing larger values in pathways supporting fast sensory integration, e.g. visual/motor functions, and smaller values in association pathways connecting temporal/frontal regions. The MLM shows blocks corresponding to interhemispheric (longer) and intrahemispheric (shorter) latencies. Our results support the potential to non-invasively infer information transfer and functional organization of the human brain at a millisecond time-scale from microstructural measurements using diffusion MRI. The proposed MLMs may provide valuable developmental, neuropathological and functional information complementary to fMRI and standard measures of cognitive performance.

**Disclosures:** **A.V. Avram:** None. **A.S. Bernstein:** None. **R. Coppola:** None. **M.J. Curry:** None. **R.D. Fields:** None. **M. Hallett:** None. **T.O. Holroyd:** None. **G. Leodori:** None. **S. Pajevic:** None. **A.C. Simmons:** None. **N. Thirugnanasambandam:** None. **N. Zhen:** None. **P.J. Basser:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

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**Program#/Poster#:** 623.09/VV65

**Topic:** I.03. Anatomical Methods

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NIH P41-EB015896

NIH S10-RR023034

NIH S10-RR023401

**Title:** The brainstem response to respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) at ultrahigh-field (7T) fMRI and its effect on vagal autonomic outflow

**Authors: R. SCLOCCO**[1], *N. W. KETTNER[2], R. G. GARCIA[1], J. R. POLIMENI[1], K. ISENBURG[1], N. TOSCHI[3], R. BARBIERI[4], V. NAPADOW[1]
[1]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hospital, Harvard Med. Sc, Charlestown, MA; [2]Radiology, Logan Univ., Chesterfield, MO; [3]Univ. of Rome Tor Vergata, Rome, Italy; [4]Dept Anesthes Critical Care, Massachusetts Gen Hosp., Boston, MA

**Abstract:** Transcutaneous electrical stimulation of the auricular branch of the vagus nerve (ABVN) has been used as a neuromodulatory therapy for multiple disorders, and primary brainstem relay occurs in nucleus tractus solitarii (NTS) (Nomura and Mizuno, 1984). We have previously proposed that ABVN stimulation effects are optimized by gating stimulation to the respiratory cycle (Napadow et al. 2012), with increased NTS response shown for exhalation-gated RAVANS (eRAVANS) compared to inhalation-RAVANS (iRAVANS) (Garcia et al. 2017). Here, we exploit the enhanced spatiotemporal resolution afforded by ultrahigh-field functional MRI (7T fMRI) to evaluate brainstem response during RAVANS, concurrently estimating stimulus-evoked instantaneous vagal outflow with a point-process algorithm (Barbieri et al. 2005). Four (4) healthy subjects experienced two 8-minute fMRI runs, with moderately strong (but not painful) eRAVANS or iRAVANS delivered to the left cymba conchae of the ear (450 μs pulse width at 25 Hz, 1 s duration). A third resting state run was used to deliver sham stimulation, and an identical event-related fMRI analysis controlled for respiratory cycle influence on medullary response. Brainstem BOLD fMRI data were collected on a Siemens 7T scanner (1.2mm isotropic voxels, 38 coronal slices, TR=0.99s, TE=23ms, 500 volumes), concurrently with electrocardiogram and respiration monitoring at 500Hz. General Linear Model analyses were performed on preprocessed (RETROICOR, slice-timing, motion and distortion correction, FWHM=2mm spatial smoothing) fMRI brainstem data using both a canonical HRF and a modified HRF having a shorter latency (3s) to peak response. Group maps demonstrated an increased response during eRAVANS compared to iRAVANS and Sham in ipsilateral medulla, consistent with purported NTS. This was not evident when using the canonical HRF during modeling. Vagal outflow increased in response to eRAVANS compared to iRAVANS and Sham, providing promising basis for therapeutic applications of eRAVANS in disorders involving autonomic disruption.

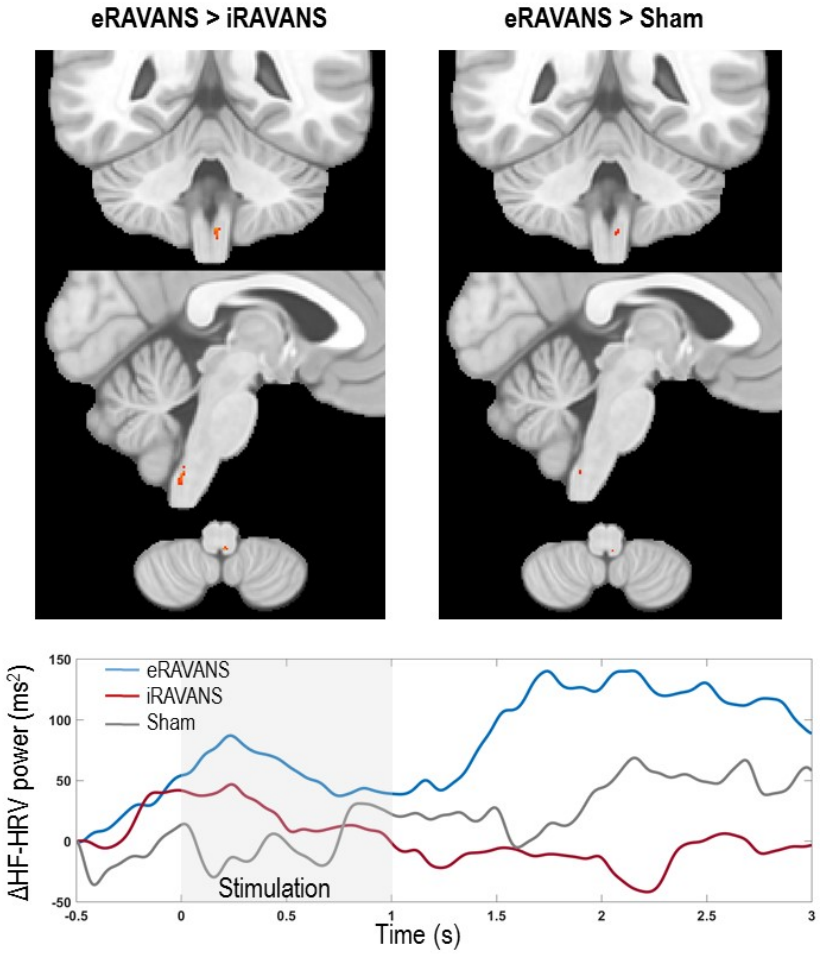


**Figure 1 –** Top panel: group maps (N=4) showing greater NTS activation during eRAVANS compared to iRAVANS and Sham. Bottom panel: peri-stimulus plot of the vagal outflow, as measured by the high-frequency component of the heart rate variability (HF-HRV), showing higher power increase in eRAVANS compared to iRAVANS and Sham.

**Disclosures: R. Sclocco:** None. **N.W. Kettner:** None. **R.G. Garcia:** None. **J.R. Polimeni:** None. **K. Isenburg:** None. **N. Toschi:** None. **R. Barbieri:** None. **V. Napadow:** None.

**Poster**

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**Topic:** I.03. Anatomical Methods

**Support:** NIH Grant MH002798

**Title:** Resting state connectivity of the BNST and CeA in patients with anxiety disorders

**Authors: *S. TORRISI**[1], G. ALVAREZ[2], A. X. GORKA[2], C. GRILLON[2], M. ERNST[2]
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**Abstract: Background:** The bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA), constitute the extended amygdala. They are uniquely implicated in the anxiety response and are suspected to underlie altered threat processing in psychopathological anxiety (Davis et al. 2010). Both the BNST and the CeA are infrequently studied in humans due to their small size and not at all investigated in anxiety patients. Prior work in our group utilized high resolution imaging to map the resting state functional connectivity (rsFC) of the BNST and CeA in healthy subjects at 7 Tesla, confirming and extending structural findings in human and animals (Torrisi et al. 2015; Gorka et al. 2017). In the present follow-up study, BNST and CeA rsFC were contrasted between healthy subjects and anxiety patients. We expected to identify abnormal patterns of rsFC in anxious patients.
**Methods:** 30 patients carrying anxiety diagnoses (generalized anxiety and/or social anxiety) and 30 matched healthy subjects participated in the analysis. The 7 Tesla, 10-minute resting state scan was 1.3 mm isotropic. The T1-weighted structural was 0.7mm isotropic. Publically-available probabilistic BNST and CeA masks were used to extract averaged time series. A two-sample t-test compared rsFC across the brain between healthy subjects and anxiety patients for both structures. Preprocessing and analyses were performed in AFNI.
**Results:** Within-group analyses replicate previous findings showing strong BNST connectivity with many regions including the medial PFC, dorsal amygdala, medial head of caudate and precuneus in both groups. Within-group analyses also replicate previous findings showing CeA connectivity with medial PFC but also temporal and thalamic regions relevant for sensory processing. Between-group analyses revealed that patients displayed hypoconnectivity between the BNST and the left IFG. It was also observed that patients displayed hypoconnectivity between the CeA and the right anterior insula. Patients also displayed significant hyperconnectivity between the CeA and right STS.
**Conclusion:** This is the first resting state study of anxiety patients that probes resting functional connectivity of the BNST and CeA, regions highly implicated in anxiety disorders. Results reinforce the potential for high resolution fMRI to elucidate connectivity with small regions. Findings also suggest that there are distinctions in resting BNST and CeA connectivity in patients with anxiety in prefrontal and temporal regions which may relate to high level cognitive impairments, social processing and the propensity for diagnostic phenotypes such as worry and difficulty concentrating.

**Disclosures: S. Torrisi:** None. **G. Alvarez:** None. **A.X. Gorka:** None. **C. Grillon:** None. **M. Ernst:** None.

**Poster**

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**Program#/Poster#:** 623.11/VV67

**Topic:** I.03. Anatomical Methods

**Support:** NIH NIBIB K01EB019474

NIH NIBIB P41EB015896

**Title:** A probabilistic stereotaxic structural atlas of five mesopontine tegmental nuclei from *In vivo* 7 Tesla MRI

**Authors: *M. BIANCIARDI**[1], C. STRONG[2], N. TOSCHI[1,3], B. L. EDLOW[4], B. FISCHL[1], E. N. BROWN[5], B. R. ROSEN[1], L. L. WALD[1]
[1]Dept. of Radiology, MGH & Harvard Med. Sch., Boston, MA; [2]Dept. of Neurosurgery, Brigham and Women's Hosp. and Harvard Med. Sch., Boston, MA; [3]Med. Physics Section, Dept. of Biomedicine and Prevention, Fac. of Medicine, Univ. of Rome "Tor Vergata", Rome, Italy; [4]Dept. of Neurology, A. A. Martinos Ctr. for Biomed. Imaging, MGH and Harvard Med. Sch., Boston, MA; [5]Dept. of Anesthesia, Critical Care and Pain Medicine, MGH, Boston, MA

**Abstract: Introduction:** Mesopontine tegmental nuclei, such as the cuneiform (CnF), pedunculotegmental (PTg, or "pedunculopontine"), oral pontine reticular (PnO, or "pontis oralis"), paramedian-raphe (PMnR) and caudal-linear-raphe (CLi) nuclei, are crucial for arousal and motor functions. Their localization in conventional MRI of living humans is difficult due to limited MRI sensitivity/contrast. Aim of this work was to develop a tool - a probabilistic atlas of these nuclei - to identify them in *in-vivo* human MRI.
**Methods:** On 12 subjects (6m/6f, age 28 ± 1 - under IRB approval) at 7 T, we acquired 1.1mm-isotropic diffusion tensor MRI (60 directions, b ~ 1000 s/mm$^2$, 7 "b0" images, TE/TR = 60.8/5600 ms, 4 reps), and, after preprocessing, computed fractional-anisotropy (FA) maps, and the average $T_2$-weighted ("b0", $T_2$w) MRI. For each subject, M.B. performed semi-automatic segmentation of FA maps and $T_2$w MRI by k-means clustering. This yielded single-subject labels of the 5 nuclei, which where aligned to MNI space, and averaged across subjects to generate probabilistic atlas labels. This atlas was validated by computing for each subject the Hausdorff distance (mm) between each semi-automatically-segmented label and: (i) each manually segmented (by C.S) label; (ii) the probabilistic label obtained using the other 11 subjects (leave-one-out approach). As a further validation (iii), we compared the segmented-nuclei volumes with *ex-vivo* nuclei volumes [1].
**Results:** The probabilistic labels in MNI space of the 5 nuclei are shown in Figure 1A. Results of the atlas validation are shown in Figure 1B.
**Conclusions:** We created a validated *in vivo* probabilistic atlas of 5 mesopontine tegmental

nuclei by 7 T MRI. This atlas might aid the nuclei localization in conventional (e.g. 3 T) MRI; it might improve the accuracy of interventions (e.g. placement of DBS electrodes), lesion evaluation, and the assessment of arousal/motor connectivity pathways in disorders of consciousness, sleep disorders and neurodegenerative diseases.

**References:** [1] Paxinos et al, Organization of brainstem nuclei, Elsevier, 2012.

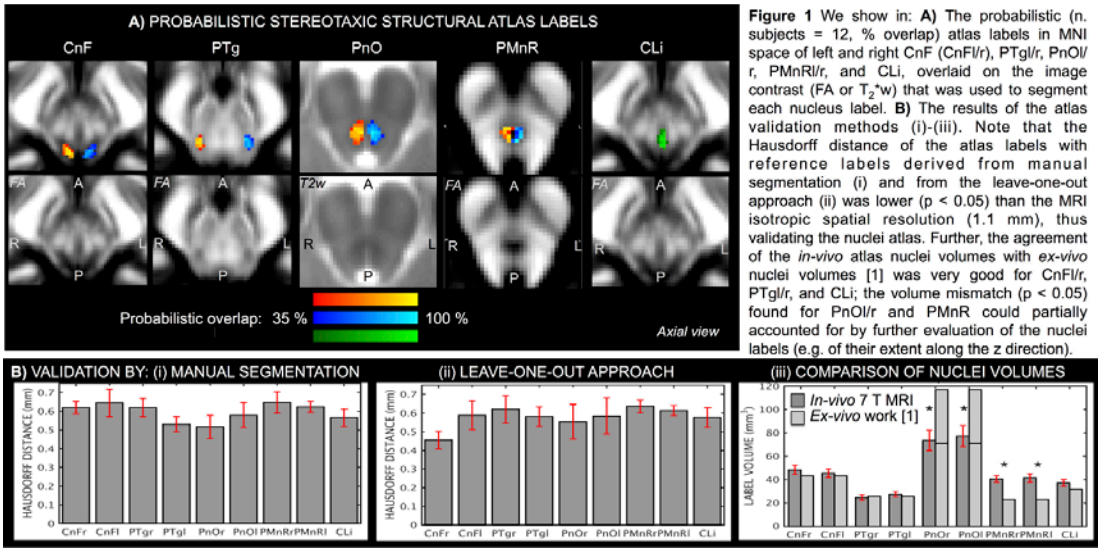


**Figure 1** We show in: **A)** The probabilistic (n. subjects = 12, % overlap) atlas labels in MNI space of left and right CnF (CnFl/r), PTgl/r, PnOl/r, PMnRl/r, and CLi, overlaid on the image contrast (FA or $T_2$*w) that was used to segment each nucleus label. **B)** The results of the atlas validation methods (i)-(iii). Note that the Hausdorff distance of the atlas labels with reference labels derived from manual segmentation (i) and from the leave-one-out approach (ii) was lower ($p < 0.05$) than the MRI isotropic spatial resolution (1.1 mm), thus validating the nuclei atlas. Further, the agreement of the *in-vivo* atlas nuclei volumes with *ex-vivo* nuclei volumes [1] was very good for CnFl/r, PTgl/r, and CLi; the volume mismatch ($p < 0.05$) found for PnOl/r and PMnR could partially accounted for by further evaluation of the nuclei labels (e.g. of their extent along the z direction).

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**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.12/VV68

**Topic:** I.03. Anatomical Methods

**Support:** NIH Big Data to Knowledge (BD2K) Initiative under U54EB020403

SC CTSI NIH/NCRR/NCATS KL2TR000131

NIH 1 L30 CA209248-01

**Title:** Towards creating a probabilistic atlas for contrast-enhanced T1-weighted MR images of the brain: A pilot study

**Authors:** ***M. S. SHIROISHI**[1,2], V. GUPTA[2], J. FASKOWITZ[3], B. BIGJAHAN[1], S. CEN[1], D. HWANG[1], A. LERNER[1], C.-S. J. LIU[1], O. BOYKO[1], P. M. THOMPSON[2], N. JAHANSHAD[2]
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**Abstract:** Introduction: T1-weighted (T1w) MR-based brain morphometry relies on scans that are obtained without gadolinium-based contrast agents (GBCAs). Contrast-enhanced (CE) T1w scans using GBCAs are obtained for many clinical indications like cancer. These represent potentially a wealth of untapped morphometric data that could be used to answer important biomedical questions. However, signal intensity changes from GBCAs confounds image registration, skull stripping, segmentation, etc. In this work, we have created a novel image-processing pipeline to assess the validity of automated brain morphometry derived from CE T1w scans.
Methods: The data set consists of 50 subjects with non contrast-enhanced (nCE) and CE T1w images. For performing any volumetric studies, it is important to construct an atlas for spatial normalization. In most cases spatial normalization is not possible because of the lack of such an atlas. Our current CE atlas is constructed with the help of associated nCE images. The CE images are rigidly registered with NC with 6 DoF using (Figure 1). Steps 2-3 illustrate the NC template construction. Step 4 shows the label transfer from the EVE template to the NC template. The transformation in step 1 and 2 are combined to allow for aligning the CE images. Step 3 and 4 are repeated for CE images. The nCE and the CE templates along with probabilistic ROI definitions are shown on the right. All registrations are computed using ANTS [1].
Conclusions: In this paper, we have proposed an atlas for CE T1w images. The atlas also contains the probabilistic ROI definitions for 175 ROIs in the EVE template [2]. Availability of such an atlas will help us conduct further morphometric analysis with the huge number of CE images across the world.
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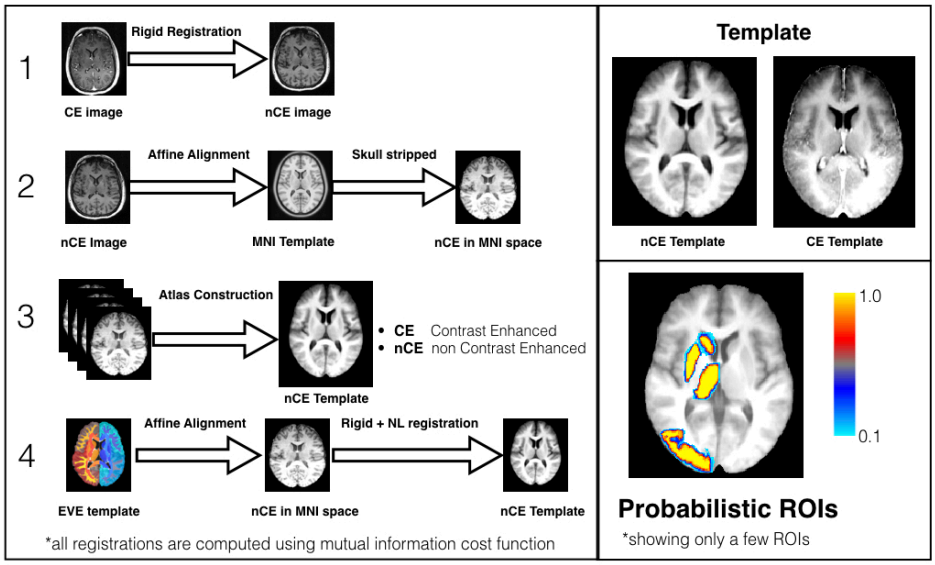

**Disclosures:** **M.S. Shiroishi:** None. **V. Gupta:** None. **J. Faskowitz:** None. **B. Bigjahan:** None. **S. Cen:** None. **D. Hwang:** None. **A. Lerner:** None. **C.J. Liu:** None. **O. Boyko:** None. **P.M. Thompson:** None. **N. Jahanshad:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.13/VV69

**Topic:** I.03. Anatomical Methods

**Support:** Funded by EU H2020 FET Flagship: Human Brain Project (grant no. 720270)

**Title:** Data integration through digital brain atlasing: Human Brain Project infrastructure

**Authors: *K. A. ANDERSSON**[1], M. ØVSTHUS[1], I. E. BJERKE[1], M. A. PUCHADES[1], M. TELEFONT[2], J. MULLER[2], T. DICKSCHEID[3], T. B. LEERGAARD[1], J. G. BJAALIE[1]
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**Abstract:** The Human Brain Project is building an ICT-based scientific research infrastructure that will permit researchers to advance our knowledge in the fields of neuroscience through data exploration, analytics and simulation at multiple levels of brain organization. Experimental neuroscience is connected to the infrastructure through systems for organizing and managing heterogeneous research data. These data systems are initially tested by data producing laboratories in the HBP, and will ultimately be opened for the community. HBP data curation services support users in elevating the level of data consistency and in the migration of data to the open domain. The starting point for research projects that will use HBP resources is the HBP Collaboratory, a rich collaborative workspace which is open to the community. The Collaboratory provides guidance and access to resources, including storage for data and a workbench for entering and organizing metadata. As a central element, the Collaboratory provides high-quality reference atlases of the rodent and human brain, together with appropriate tools and workflows that allow users to register data to the atlases for their study, and to perform initial analysis of data. It also links to important external data repositories and services. Here we present an overview of currently available reference atlases, tools and workflows. We exemplify the use of these resources in a range of neuroscience projects, ranging from brain-wide mapping of molecular level information to identification of precise location of electrophysiology recording sites. With coordinates corresponding to reference atlas space, harvested through the workflow, valuable metadata for future search and analysis of data are captured. Furthermore, with data aligned to reference atlases, analysis of the spatial distribution of events, labeled elements, and regions of interest in image material is strongly supported. Following registration

to reference atlas, subsequent image processing and analysis steps delivers lists of extracted features corresponding to atlas structures, enabling quantitative regional analysis. We exemplify analytical workflows producing automated quantification and spatial analysis of labeling in series of histological section images from whole rodent brain. These and other atlas related workflows will be made available as HBP software services.

**Disclosures: K.A. Andersson:** None. **M. Øvsthus:** None. **I.E. Bjerke:** None. **M.A. Puchades:** None. **M. Telefont:** None. **J. Muller:** None. **T. Dickscheid:** None. **T.B. Leergaard:** None. **J.G. Bjaalie:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.14/VV70

**Topic:** I.03. Anatomical Methods

**Support:** EPSRC HMR00380

**Title:** How gyral geometry affects cortical fibre trajectories; from tract tracing to diffusion MRI tractography

**Authors: *M. COTTAAR**[1], T. E. BEHRENS[1], S. JBABDI[1], S. N. HABER[2]
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**Abstract:** Diffusion MRI tractography streamlines terminate predominantly at the gyral crown, rather than more uniformly across the cortex as seen in histology. This bias to the gyral crown is generally attributed to the limited resolution of diffusion MRI, which is insufficient to resolve the axon curvature at the cortical boundary.
Cortical axons leaving the grey matter to distant regions form a dense, narrow “stalk” (seen as heavily stained, elongated bundles of fibres in individual slices) before splitting up into separate bundles that connect to the striatum, the capsules, and the corpus callosum. This dense stalk implies that at least for the long-distance connections there is a unique relationship between the injection site and the fibre trajectory through the gyral white matter. Our goal is to use tracer injections spread across gyri to delineate the rules governing this relationship and develop new algorithms to guide tractography streamlines more accurately through the gyral white matter to the appropriate cortical termination site.
Ten stalks were charted in six different monkeys with injection sites in the frontal cortex. Six stalks charted in the dorsal prefrontal gyrus show a complicated pattern. In individual slices these stalks can often be seen to have sharp curvature. Furthermore, the in-plane fibre orientations of these stalks were often misaligned and sometimes perpendicular to the long axis. This implies that the elongated shape of the stalks observed in most slices is not due to the projection of a

cylindrical stalk onto the plane, but rather reflects the inherent elongation of the stalks cross-section. In contrast the fibres tended to align with the long axis for stalks in the caudal lateral prefrontal, ventral prefrontal, and premotor gyri, which might reflect the simpler geometry of these gyri. We will present results illustrating how the three-dimensional gyral geometry influences both the shape of the stalk and the fibre orientation. Most importantly for tractography we will present validations of models predicting the path of the stalk based on the injection site and the gyral geometry.

**Disclosures:** **M. Cottaar:** None. **T.E. Behrens:** None. **S. Jbabdi:** None. **S.N. Haber:** None.

**Poster**

**623. Neurophysiology: Humans**

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**Program#/Poster#:** 623.15/VV71

**Topic:** I.03. Anatomical Methods

**Support:** European Union's Horizon 2020 research and innovation programme grant agreement No. 720270

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Flagship Project NanoMAX, and by "Ente Cassa di Risparmio di Firenze"

Proof of Concept Studies for the ESFRI research infrastructure project Euro-BioImaging

RF-2013-02355240 Progetti ordinari di Ricerca Finalizzata

**Title:** Three-dimensional investigation of neuronal layer distribution in human brain cortex

**Authors: *I. COSTANTINI**[1], L. SILVESTRI[2], V. CONTI[3], C. DEL TORTO[1], G. MAZZAMUTO[1], L. SACCONI[2], R. GUERRINI[3], F. S. PAVONE[1,2]
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**Abstract:** Malformations of cortical development (MCD) are heterogeneous disorders frequently associated with epilepsy. The onset of these pathologies is related to alterations of cell proliferation, cell migration, and cortical organization. However, little is known about their pathogenesis. To analyze the architectural abnormalities in the cortical layering of MCD, we exploited the possibility of combining high-resolution 3D imaging with clearing methodologies.

In particular, we successfully integrated the SWITCH immunohistochemistry technique with the TDE clearing method to image pediatric as well as adult human brain tissue. Both light sheet and two-photon fluorescence microscopies were used to reconstruct the three-dimensional structural organization of neurons in human brain cortex. Our data demonstrate that the comparison of neurons distribution in the cortex of healthy and affected individuals allow exploring anomalies in the 3D structural organization of the brain, providing novel insights into the pathogenesis of MCD.In conclusion, this new approach enables to characterize large human brain specimens with high-resolution optical techniques, giving the possibility to expand the histopathological studies to the third dimension.

**Disclosures: I. Costantini:** None. **L. Silvestri:** None. **V. Conti:** None. **C. Del Torto:** None. **G. Mazzamuto:** None. **L. Sacconi:** None. **R. Guerrini:** None. **F.S. Pavone:** None.

**Poster**

**623. Neurophysiology: Humans**

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**Program#/Poster#:** 623.16/VV72

**Topic:** I.03. Anatomical Methods

**Support:** NIH grant R00 NS070821

Icahn School of Medicine a Mount Sinai Medicine Capital Campaign, Translational and Molecular Imaging Institute and Department of Radiology

City College of New York Department of Biomedical Engineering

**Title:** Automated hippocampal subfield segmentation using 7T MRI in patients with major depressive disorder: First results

**Authors: *J. ALPER**[1,3], R. FENG[4], H. DYVORNE[5], H.-M. LIN[6], B. DELMAN[7], P. R. HOF[2], J. W. MURROUGH[8,9], P. BALCHANDANI[1,7]
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**Abstract:** Major depressive disorder (MDD) is a disabling illness of high prevalence [1]. There is a pressing need to elucidate the pathophysiology of MDD to better target treatment. Previous studies have shown an association between hippocampal subfield volumes and MDD, making them potential biomarkers for the disease [2-4]. Using 7 Tesla (7T) MRI to perform volumetric analysis can allow for more accurate measurements enabled by increased contrast and resolution. In this study, we demonstrate the feasibility of automated hippocampal subfield segmentation for

MDD at high resolution 7T MRI. Hippocampal subfield volumes are computed using Automatic Segmentation of Hippocampus Subfields (ASHS) software [5]. We perform a preliminary analysis on differences in subfield volumes between MDD patients and healthy controls. Thirteen MDD patients (ages 29-55) and seven healthy controls (26-55 years) underwent an MRI scan at 7T (Magnetom, Siemens). The imaging protocol consisted of MP2RAGE (TR 6000 ms, TI1 1050 ms, TI2 3000 ms, TE 5.06 ms, voxel 0.8x0.8x0.8 $mm^3$) and $T_2$ TSE (TR 9000 ms, TE 69 ms, voxel 0.45x0.45x2 $mm^3$) acquired at a coronal oblique orientation. We report subfield volumes computed by ASHS for CA1, CA2 3, CA4 DG, and subiculum in the left and right hemispheres. A comparison of the MDD patients to healthy controls was performed for each subfield volume, with age and gender regressed. A significant difference was found between patients and controls in CA1 on the right side ($p=0.0449$), with marginally significant difference in CA1 on the left side ($p=0.1034$). No significant differences were found in the other subfields. We have demonstrated feasibility of automated hippocampal subfield segmentation at 7T for MDD patients and controls. Significant volumetric differences were found in CA1 on the right side, which is concordant with the literature on hippocampal subfield changes associated with stress-related disorders [6, 7]. This volume loss may be due to a reduction in astrocyte density [7]. Future work includes manual tracings of the subfields to validate automated results and increasing the sample size. Hippocampal subfield volumes may serve as imaging biomarkers for MDD, which may help design more targeted treatments for the disease.

We acknowledge Long Xie and Marin Kautz for their help with this study.

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**Disclosures:** **J. Alper:** None. **R. Feng:** None. **H. Dyvorne:** None. **H. Lin:** None. **B. Delman:** None. **P.R. Hof:** None. **J.W. Murrough:** None. **P. Balchandani:** None.

**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.17/VV73

**Topic:** G.07. Other Psychiatric Disorders

**Support:** RO1

**Title:** Similarities between schizophrenia and autism spectrum disorder in functional connectivity of the default mode network

**Authors: L. RABANY**[1], S. BROCKE[1], V. CALHOUN[2], C. J. HYATT[1], S. CORBERA[1,3], B. E. WEXLER[3], B. D. MORRIS[3,4], S. RACHAKONDA[2], K. A. PELPHREY[5], G. D. PEARLSON[3,1], *M. ASSAF[6,3]
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**Abstract: Background:** Schizophrenia (SZ) and autism spectrum disorders (ASD) are severe psychiatric conditions that co-occur at elevated rates. Recent reports indicate phenotypic similarities and shared genetic risk factors, thus stimulating debate about shared underlying neuropathology. The current study examined fMRI resting-state functional connectivity patterns in SZ, ASD and healthy controls (HC), within the default mode network (DMN).
**Methods:** Resting-state fMRI was collected from 100 individuals: 33 SZ, 33 ASD, 34 HC (ages 18-35). Temporally distinct resting state components were determined using the group independent component analysis (ICA) toolbox (GIFT) with a 100 components model. Eight components were identified as DMN nodes, based on the Stanford functional ROI atlas, including 2 frontal, 4 posterior cingulate (PCC), 1 precuneus (PrC) and 1 lateral parietal (LP) nodes. The functional network connectivity (FNC) toolbox was used to compute group differences in DMN components coherence (i.e. connectivity, measured as between-nodes correlations).
**Results:** One way ANOVA demonstrated significant group effect for four pairs of DMN nodes. Post-hoc pair-wise group comparisons indicated that in three of those node-pairs (PCC1-PCC2 $p=0.006$; PCC1-PrC $p=0.024$; PCC2-PrC $p<0.001$) both SZ and ASD had significantly lower correlation than HC (with SZ and ASD not significantly different from each other). For the fourth node-pair (PrC-LP $p=0.025$), SZ showed significantly lower correlation than HC (and although not significant, ASD showed a similar pattern).
**Conclusions:** These preliminary results indicate lower intra-DMN functional connectivity in both SZ and ASD groups, as compared to HC. Interestingly, connectivity of the posterior portions of the DMN (PCC and PrC) was mainly implicated.

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**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.18/VV74

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** NIH Grant NHLBI R01 HL116585-01

Canadian Institute of Health Research Grant MOP-81116

**Title:** Rich club organization of the neonatal functional connectome in newborns with complex congenital heart disease

**Authors: J. D. CRUZ**[1], M. T. DONOFRIO[2], G. VEZINA[2], *C. LIMEROPOULOS[3]
[1]Radiology, Children Natl. Hlth. Syst., Washington, DC; [3]Diagnos. Imaging & Radiology/Developing Brain Res. Lab., [2]Children's Natl. Hlth. Syst., Washington, DC

**Abstract:** Congenital heart disease (CHD) affects brain development over the life span. Recent quantitative MRI studies have demonstrated structural and biochemical disturbances in the brain as early as the fetal/early postnatal period. However, the extent to which functional brain connectivity may be affected is currently unknown. We hypothesize that neonates with complex CHD will have atypical functional brain connectivity likely secondary to *in utero circulatory disturbances*.

We acquired resting state data from 30 neonates (postmenstrual age, PMA: 39.4 weeks, 38.9-39.7 [median, interquartile range]) diagnosed with complex CHD before surgery and compared their rich club organization to 82 healthy, term neonates (PMA: 41.5 weeks, 40.7-42.1). Rich-club organization refers to the tendency of highly connected (higher degree, *k*) brain regions to connect with each other compared to other areas of the brain. Both CHD and control networks demonstrated rich-club organization, however, the range of degrees at which the rich club regime was observed was narrower in CHD infants compared to controls: $k$ = 4-18 versus $k$ = 3-21, respectively. In addition, rich club coefficients were significantly reduced ($p$ = 0.01, paired t-test) in CHD newborns compared to controls. These findings suggest reduced number of connections among rich-club nodes in CHD. The rich club anatomy of the two groups overlapped and included regions such as bilateral hippocampi and insulae, regions identified as rich club nodes in healthy adults and neonates using DTI. The controls' rich club, however, had more members at 24 nodes compared to 13 in CHD. Areas absent in CHD included limbic structures such as the thalamus and amygdala, bilateral Heschl gyri, and parahippocampal cortices, among others.

In summary, we describe for the first time alterations in rich-club organization in neonates with complex CHD before open heart surgery. These finding likely reflect immaturity in brain functional networks possibly due to early life hemodynamic disturbances. Further studies are

needed to elucidate how these alterations impact the long-term neurodevelopment of CHD survivors, which are currently underway.

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**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 623.19/VV75

**Topic:** F.06. Brain Blood Flow, Metabolism, and Homeostasis

**Support:** Grants-in-Aid for Scientific Reserch 17K16410

**Title:** A study of the brain functional MRI in the patients with alcohol dependence

**Authors: *S. FUKUSHIMA**[1,2], S. H. T. T. TAKEFUMI UENO[1]
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**Abstract:** Introduction: The World Health Organization estimates that as of 2010 there were 208 million people with alcoholism worldwide (4.1% of the population over 15 years of age). Alcoholism directly resulted in 139,000 deaths in 2013 up from 112,000 deaths in 1990. A total of 3.3 million deaths (5.9% of all deaths) are believed to be due to alcohol. It often reduces a person's life expectancy by around ten years. Alcohol dependence is complex, multifaceted disorder often characterized by cycles of cutting out alcohol, craving, and relapse. They repeat these cycles despite often-severe negative consequences. This disease results in social problems, health problems, and risky situations.
Objective: Functional magnetic resonance imaging (fMRI) was used to test whether brain activation was detectable in regions previously associated with illegal drugs cue-induced craving. We tried a study to investigate brain activation, using Alcohol images.
Method: 16 patients of Alcohol-dependent and 10 normal controls participated in this study. Blood oxygenation level dependent (BOLD) functional activation was measured with 1.5 T MRI during presentation of visual stimuli containing alternating intervals of orange juice, alcohol-related and mosaic scenes. This study were designed as “block design” and had two blocks. Each block task contained four pictures (orange juice or alcohol drink at random) in 120 sec. The duration of one picture was 15 sec, and the duration of interval between each picture was 15 sec with mosaic scenes. In total, we performed 240 sec (80 scans) on each subject. SPM12 was used to analyze BOLD fMRI data within each subject, as well as a group.
Results: We have a group analysis between 16 patients and 10 healthy people, we detected an interaction effect between brain activation of patients and healthy people with region of interest

(ROI) as right posterior cingulate gyrus (PCC) for analysis. Showing alcohol drinks, the activation of patients is higher than that of healthy people, and showing orange juice, the activation of them is lower with each significant differences (p=0.026, p=0.038). Conclusions: These results suggest that functional MRI may be a useful tool to study the neurobiological basis of cue-induced craving. And, alcohol dependent patients may overreact many kinds of alcohol drinks.

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**Poster**

**623. Neurophysiology: Humans**

**Location:** Halls A-C

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**Program#/Poster#:** 623.20/VV76

**Topic:** I.05. Biomarker and Drug Discovery

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P50MH103222

a State of Maryland contract (M00B6400091)

**Title:** Test-retest reliability of short-interval intracortical inhibition in patients with schizophrenia

**Authors: *X. DU**[1], A. SUMMERFELT[1], J. CHIAPPELLI[1], K. WISNER[1], P. KOCHUNOV[1], F.-S. CHOA[2], L. E. HONG[1]
[1]Maryland Psychiatric Res. Ctr., Catonsville, MD; [2]UMBC, Baltimore, MD

**Abstract:** Schizophrenia is a heterogeneous psychiatric disorder with a broad spectrum of clinical and biological manifestations and inhibitory-excitatory (I-E) imbalance has increasingly been proposed as a fundamental mechanism. One of the robust measurements of clinical I-E abnormality *in vivo* is the short-interval intracortical inhibition (SICI) vs. intracortical facilitation (ICF) elicited by paired-pulse transcranial magnetic stimulation (ppTMS) at 1-6 ms and 9-25 ms, respectively. Reduction of SICI in different stages of schizophrenia has been well demonstrated in the literature. However, the test-retest reliability of SICI in patients with schizophrenia has not been tested which largely limited the application of using SICI in assisting schizophrenia diagnostic. In the present study, the SICI and ICF profiles were obtained using inter-stimulus intervals (ISIs) from 1 to 500 ms, on 2 occasions about 3 weeks apart in 25 patients with schizophrenia (SZ) and 29 age- and gender-matched healthy controls (HC). The TMS coil was placed over left motor cortex for eliciting motor evoked potentials (MEPs) from the right dorsal interosseous. The ppTMS pulses were delivered with a subthreshold stimulus (80% resting motor threshold or RMT) followed by a suprathrehold stimulus (120% RMT) at 1, 3, 6, 9, 12, 15, 18, 21, 30, 40, 80, 120, 200 and 500 ms ISIs. Intraclass correlation coefficient (ICC), a standard method to examine the test-retest reliability, was obtained with a two-way mixed model. Raw MEPs showed moderate-to-good test-retest reliabilities across all ISIs and groups (ICCs for HC: 0.6-0.9; for SZ: 0.7-1). Importantly, moderate test-retest reliability was found for SICI (1 and 3 ms ISIs) in patients (ICCs: 0.6 and 0.7) and controls (ICCs: 0.7 and 0.7). Patients with schizophrenia, but not healthy controls, also showed moderate reliability for ICF (ICC at 9 ms ISI: 0.6 and 15 ms ISI: 0.8). Poor test-retest reliability at other ISIs (e.g., 18 to 500 ms) were observed across groups. To our knowledge, this is the first study demonstrated the acceptable reliability of SICI measurement in schizophrenia. The present data suggests that SICI could be a reliable biomarker for schizophrenia and be used for the development of diagnostics and for improved clinical decision-making.

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**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.01/VV77

**Topic:** I.04. Physiological Methods

**Support:** Dr. Gradinaru is a Heritage Principal Investigator supported by the Heritage Medical Research Institute

Beckman Institute for the Resource Center on CLARITY, Optogenetics, and Vector Engineering for technology development and broad dissemination

**Title:** Embryonic tissue clearing across species by CLARITY with and without perfusion of the fetal vasculature

**Authors: *N. GOEDEN**, N. C. FLYTZANIS, A. GREENBAUM, M. J. JANG, A. M. EZIN, M. L. PIACENTINO, E. J. HUTCHINS, M. E. BRONNER, M. A. YUI, E. V. ROTHENBERG, V. GRADINARU
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**Abstract:** The application of robust clearing techniques to embryonic development across gestational time points and species can benefit many research areas, including neuroscience. Recent advances in tissue clearing have enabled the direct, 3D visualization of whole organs, leading to higher throughput and improved spatial resolution in a variety of tissues. A key step in tissue clearing is transcardial perfusion, which flushes the endogenous blood and uniformly fixes the tissue, especially in hard-to-access areas. However, transcardial perfusion is not directly applicable to mammalian embryos, due to the presence of the placental barrier that separates the maternal and fetal circulations, thus limiting the application of clearing techniques across mid-to-late gestational time points. Furthermore, species lacking distinct maternal-fetal circulations (e.g. chickens, zebrafish, etc.), lack the capacity for utilizing maternal vasculature to flush endogenous blood and deliver fixative to the developing embryo. While some workarounds exist for removing autofluorescence due to the presence of blood, improper fixation degrades the tissue epitopes and endogenous fluorescence signal. Here we describe methodologies to facilitate improved clearing techniques in embryos with and without accessible vasculature. We characterize the effects of fetal perfusion on fluorescent signal preservation in embryonic tissues of mice expressing Bcl11b-mCitrine and Bcl11b-mCherry reporter alleles compared to previously described techniques relying on immersion fixation. Additionally, we present clearing techniques for visualizing the neural crest in early stage chicken embryos by detecting endogenous fluorescence with subsequent immunohistochemistry and HCR on intact embryos. Samples from both studies were imaged with confocal and light-sheet microscopy. Together,

these clearing, labeling, and imaging techniques can aid in the characterization and visualization of neurodevelopmental processes during gestation.

**Disclosures:** **N. Goeden:** None. **N.C. Flytzanis:** None. **A. Greenbaum:** None. **M.J. Jang:** None. **A.M. Ezin:** None. **M.L. Piacentino:** None. **E.J. Hutchins:** None. **M.E. Bronner:** None. **M.A. Yui:** None. **E.V. Rothenberg:** None. **V. Gradinaru:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

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NSF NeuroNex Grant 1707316

A.G. is a Good Ventures Fellow of the Life Sciences Research Foundation

**Title:** Combined soft and osseous tissue clearing for visualization and precise localization of *In situ* implants

**Authors: *A. GREENBAUM**, N. FLYTZANIS, K. Y. CHAN, C. CHALLIS, V. GRADINARU
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**Abstract:** Brain implants such as optical fibers for optogenetics and photometry, and microelectrode arrays for neuronal recording are essential tools for neuroscience research. Given the common practice of removing such implants before tissue examination, it can be difficult to evaluate the implant's exact placement with respect to the brain tissue. In addition to the glial scarring that is caused by implant insertion, implant retraction can result in further tissue damage, widen the implant tract, and distribute blood and necrotic cell debris across a larger volume. This tissue damage approximately marks the implant's tract, however, this approximation lacks precision. Consequently, important experimental details such as the identity of recorded cells or the precise location of the implant relative to region targeted, may be lost. We have therefore developed a technique for preserving the position of the implant and

surrounding tissue during histological processing. This ultimately enables optical investigation of the interface between the implant and the targeted cells. Toward this end, we build upon our previous bone clearing work (Greenbaum, Chan, et al, Science Translational Medicine, 2017), by clearing the brain tissue without removing the skull. This approach ensures that the implant remains firmly anchored and relatively unperturbed during the clearing process – minimizing tissue damage. The clearing process removes minerals with ethylenediaminetetraacetic acid (EDTA) and extracts light scattering lipids, thus allowing for optical investigation of the soft tissue below the skull. This method could be used with a variety of implants such as optical fibers, microelectrode array, EEG sensors and more.

**Disclosures: A. Greenbaum:** None. **N. Flytzanis:** None. **K.Y. Chan:** None. **C. Challis:** None. **V. Gradinaru:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

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**Title:** Integrated brain-wide single cellular mapping

**Authors: *Y.-G. PARK**[1], J. H. CHO[1], G. DRUMMOND[1], D. YUN[1], H. CHOI[1], H.-Y. JUNG[1], T. KU[1], L. RUELAS[1], M. MCCUE[2], K. CHUNG[1]
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**Abstract:** Creating an atlas of all cells in the brain is essential for understanding how complex cellular interactions determine brain function. Extensive efforts have been made to histology-based mapping of the brain. But these methods can map only a few molecules or sites per brain and require averaging over a large number of brains. Although the resulting averages are useful as references, they ignore high inter-individual variabilities. Moreover, the number of molecules and cell-types that these methods can analyze are fundamentally limited by the number of

samples available, creating a particular challenge when studying rare samples such as human embryos. To address this challenge, we have developed a rapid and cost-effective platform for creating an integrated 3D brain atlas with true cellular resolution. To achieve this, we developed eTANGO (electrophoretic Transport of Activity-modulated molecules in a Nanoporous Gel Organ hybrid), a technique that enables uniform staining of SWITCH-processed entire mouse brains in 1-3 days at a fraction of the cost of passive staining methods. To integrate the resulting organ-scale datasets across repeated rounds of labeling at true cellular resolution, we developed a scalable feature-based co-registration algorithm. The combined framework may allow integrated single-cell-resolution mapping of mammalian brains as well as key tissue architectures such as myelinated fiber tracts and vasculatures from a single mouse brain.

**Disclosures: Y. Park:** None. **J.H. Cho:** None. **G. Drummond:** None. **D. Yun:** None. **H. Choi:** None. **H. Jung:** None. **T. Ku:** None. **L. Ruelas:** None. **M. McCue:** None. **K. Chung:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** I.04. Physiological Methods

**Support:** MIT LL ASD R&E

**Title:** Long-range dense axonal fiber tracing using convolutional neural network

**Authors: A. BREWSTER**[1], M. HERNANDEZ[1], M. BULUGIOIU[1], B. TELFER[1], A. MAJUMDAR[1], S. SAMSI[1], T. KU[2], H. CHOI[3], K. CHUNG[4], *L. BRATTAIN[1]
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**Abstract:** Recent advances in intact brain imaging, such as the CLARITY and the MAP (Magnified Analysis of the Proteome) tissue clearing techniques, make it possible to collect large volumetric images of brain tissue at fine subcellular resolutions. The high throughput and high resolution brain imagery, however, poses a challenge for efficient processing and analysis. There is currently no effective tool for automated large-scale connectivity analysis at single fiber resolution. To tackle this unmet need, we developed a semi-automated long-range dense axonal fiber tracing pipeline which consists of four main modules: 1) a convolutional neural network (CNN) detects axon voxels, 2) image processing techniques, such as morphological operations, are applied to extract axon centerlines, 3) tracking logic connects axon segments across low-intensity gaps and unresolved axon crossings, 4) 3D fiber connectivity graphs are computed for network connectivity analysis.
We implemented our algorithms on a CPU cluster, and tested our pipeline on a 250 GB volume

of SMI-312 densely labeled axons, imaged from parts of the hippocampus and cortex of a MAP-processed mouse brain (resolution: 0.325 um in x, y and 1 um in z).
Original tissue size is 5 mm x 5 mm x 170 um. Imaging was performed after 4-fold tissue expansion.
Our automated pipeline traced fibers up to 1 mm long across gray matter and white matter in less than a day, as compared to weeks for one fiber done manually. An interactive front end user interface has also been developed for quick reviews of the raw data and results, allowing for editing of the traces. Accuracy is currently being assessed by experts and will be reported in the poster. Manual review of several 1 mm long fibers found that these were correctly traced.
We are in the process of scaling up our automated axon tracing to analyze terabyte-sized datasets. We plan to expand our algorithms to track other neuron components (e.g. cell bodies and dendrites), for a more complete network connectivity graph. The automated pipeline using CNN is the building block for state-of-art long-range fiber connectivity analysis at resolutions that have not been explored.

**Disclosures:** **A. Brewster:** None. **M. Hernandez:** None. **M. Bulugioiu:** None. **B. Telfer:** None. **A. Majumdar:** None. **S. Samsi:** None. **T. Ku:** None. **H. Choi:** None. **K. Chung:** None. **L. Brattain:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

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Burroughs Wellcome Fund Career Awards at the Scientific Interface

Searle Scholars Program

Packard award in Science and Engineering

JPB Foundation (PIIF and PNDRF)

**Title:** Multi-scale imaging and reconstruction of brain-wide neural circuits using novel chemical approaches

**Authors: *M. G. MCCUE**[1], R. CHEN[2], Y.-G. PARK[2], H. CHOI[3], W. TRIEU[4], K. CHUNG[2,3,5]
[1]Brain and Cognitive Sci. Dept., [2]Picower Inst. for Learning and Memory, [3]Inst. of Med. Engin. and Sci., [4]Biomed. Engin., [5]Dept. of Chem. Engin., MIT, Cambridge, MA

**Abstract:** Genetically encoded fluorescent proteins have greatly facilitated breakthroughs in dissecting neural circuits. Tissue clearing approaches, such as CLARITY, SWITCH, MAP, iDISCO, and CUBIC can render intact brains transparent to visualize 3D structures or expand tissues for nanoscale resolution imaging. However, these approaches often cause loss of fluorescent signals due to the exposure to unphysiological conditions (e.g, high temperature, organic chemicals, detergents) used in these methods. To address this challenge, we have developed a novel strategy, called SHIELD, for protecting fluorescent proteins from denaturation in biological tissue. By combining this technology with our Magnified Analysis of the Proteome (MAP) protocol we can preserve fluorescence in expanded, intact brains. Using SHIELD-MAP, we establish a novel process for rapid, multiscale circuit imaging of a single intact brain sample. This allows us to generate whole brain mesoscale connectome as well as nanoscale synaptic connectivity maps within the same sample. This new method for processing, imaging, and analyzing tissue will unify and expand understanding of whole brain cellular and synaptic connectivity.

**Disclosures: M.G. McCue:** None. **R. Chen:** None. **Y. Park:** None. **H. Choi:** None. **W. Trieu:** None. **K. Chung:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

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**Topic:** I.04. Physiological Methods

**Support:** NIH (1-U01-NS090473-01)

JPB Foundation (PIIF and PNDRF)

**Title:** Simultaneous profiling of nucleotides, proteins, and endogenous fluorescence in transparent tissue enabled by flexible and multifunctional fixatives

**Authors: *R. CHEN**[1], M. MCCUE[1], Y.-G. PARK[1], H. CHOI[1], K. CHUNG[2]
[2]Brain and Cognitive Sci., [1]MIT, Cambridge, MA

**Abstract:** Holistic understanding of complex biological systems, from single biomolecules to cell interactions across whole organs, will require integrated methodologies that can simultaneously probe both nucleotides and proteins in intact tissue. While several tissue preservation strategies, such as CLARITY, SWITCH, and MAP, have been explored to preserve individual molecular features (e.g endogenous fluorescence of protein reporters, mRNA transcripts, or protein antigenicity), a universal tissue clearing method to profile all biomolecule types remains to be developed. Here, we demonstrate how multifunctional cross-linkers with

tightly regulated reaction kinetics can form cohesive tissue gels by binding together biomolecules of interest. We find that these flexible and multifunctional linkers protected proteins and nucleotides from deteriorating in harsh chemical conditions by forming both intra- and intermolecular bonds. This strategy, called SHIELD, enables simultaneous molecular profiling of nucleotides, proteins, and fluorescent reporters in intact tissue, such that correlative studies, from single transcripts to whole organ features, can be performed.

**Disclosures: R. Chen:** None. **M. McCue:** None. **Y. Park:** None. **H. Choi:** None. **K. Chung:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

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Burroughs Wellcome Fund Career Awards at the Scientific Interface

the Searle Scholars Program

Packard award in Science and Engineering

JPB Foundation (PIIF and PNDRF)

**Title:** Advanced Magnified Analysis of Proteome (MAP) for superresolution mapping of biological tissues

**Authors: *T. KU**[1,2], A. ALBANESE[1], K. CHUNG[1,2,3,4,5]
[1]Inst. for Med. Engin. and Sci., [2]Picower Inst. for Learning and Memory, [3]Dept. of Chem. Engin., [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [5]Broad Inst. of Harvard Univ. and MIT, Cambridge, MA

**Abstract:** Intact tissue processing techniques such as CLARITY have facilitated three-dimensional (3D) navigation of biological structures. Magnified analysis of proteome (MAP) further improved the spatial resolution by physically expanding intact tissues and enabling super-resolution imaging of 3D proteome with diffraction limited microscopy. However, the compatibility of MAP with various tissue types and molecular probes remains to be explored. Here, we present an advanced MAP technique, which extends its application to a wide range of tissue types and further enhances antibody compatibility. We confirmed that 36 antibodies targeting diverse synaptic proteins are compatible with MAP tissues, including Western-blot-

compatible antibodies that show no specific signal in PFA-fixed tissues. We were also able to process postmortem human tissues with MAP and achieved 3.5-fold expansion. Using this framework, we mapped diverse cell-types, their projections, and molecular architectures of their synapses. We anticipate that the advanced MAP technique will provide spatial and molecular accessibilities to biological samples beyond conventional approaches.

**Disclosures:** **T. Ku:** None. **A. Albanese:** None. **K. Chung:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LifeCanvas Technologies.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

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**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.08/VV84

**Topic:** I.04. Physiological Methods

**Support:** NIH R01NS068409

**Title:** Developing an *In vivo* optical clearing technique for mouse brain imaging

**Authors: *M. KUME**[1,2], N. KANG[3], A. AKROUH[4], J. DEARBORN[5], D. F. WOZNIAK[8], D. KERSCHENSTEINER[6], T. E. HOLY[7]
[1]Neurosci., Washington Univ. In St Louis, Saint Louis, MO; [2]Biomed. Engin., Washington Univ. in St Louis, Saint Louis, MO; [3]Johns Hopkins Univ., Baltimore, MD; [4]Biol. Sci., Columbia Univ., New York, NY; [5]Intrnl. Med., [6]Dept of Opthalmology and Visual Sci., [7]Anat. & Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO; [8]Psychiatry, Washington Univ. Sch. Med., Saint Louis, MO

**Abstract:** The ability to use light to gain insight into the structure and function of complex biological samples has been invaluable in extending our understanding of biology. These advances have been propelled by improvements in imaging instrumentation and molecular and genetic tools. However, a limitation to optical imaging is imposed by the opacity of biological tissue. Recently, several methods to optically clear tissue have been developed; however, none are compatible with living tissue. To address the challenge of improving imaging resolution and depth in functioning neuronal circuits, we developed a biocompatible clearing agent, iodixanol-ACSF, which is capable of increasing the transparency of living neuronal tissue while still maintaining tissue function and health. Surprisingly, brain-cleared mice were motile and performed well on a variety of behavioral tasks, and extracellular recordings showed that many cellular and circuit phenomena were well-preserved. In live, iodixanol-ACSF cleared mouse brain tissue, both transmission and cellular-resolution fluorescence microscopy indicate improvements of 150-200% in depth penetration with one-third to one-half the laser intensity

when compared to untreated tissue. We successfully imaged calcium activity in behaving animals with iodixanol-cleared brains. Our results show that iodixanol-ACSF clearing can enable deeper imaging and be used to extend our understanding of neuronal circuit function. As this clearing technique is straightforward to implement, this technique can be incorporated into many currently existing setups to further extend our collective understanding of biological processes.

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**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.09/VV85

**Topic:** I.03. Anatomical Methods

**Title:** Large-scale reconstruction of the endoplasmic reticulum and intracellular organelles of neurons using SBEM

**Authors: *M. HABERL**[1,2], E. P. CAMPBELL[3], T. DEERINCK[2], S. PHAN[2], E. BUSHONG[2], B. L. BLOODGOOD[3], M. H. ELLISMAN[2]
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**Abstract:** The endoplasmic reticulum (ER) of neurons extends from the nuclear envelope throughout the axonal and dendritic arbors (Lindsey and Ellisman, 1985). Extensions of the ER reach into dendritic spines (Martone et al., 1993; Spacek and Harris, 1997) forming a distinctive organelle, the spine apparatus, that is commonly found in mature, potentiated spines. Throughout the neuron, the ER comes into extreme proximity to, and even merges with, other organelles, such as mitochondria and the nucleus, as well as the plasma membrane, referred to as membrane contact sites (MCS) (Phillips and Voeltz, 2016). The specialized ER substructures and interfaces with organelle/plasma membranes are important sites for signaling and inter-organelle communication yet their numbers and distributions have not been described. This is largely because reconstructing the ER is a multiscale problem spanning from the nm-scale of MCS to the mm-scale of neuronal projections. Following it in dendrites and axons requires large-scale data acquisition at EM resolution. We have used serial block-face-scanning electron microscopy (SBEM) of the rodent brain and applied learning algorithms for automated reconstructions of these intracellular details as well as other intracellular organelles (mitochondria, Golgi apparatus, nucleus, and nuclear pores). In addition, we performed multi-tilt electron tomography for high-resolution structural reconstruction of the spine apparatus. Our work contributes to deepen the understanding of this fascinating cellular organelle as well as its interactions within the cell.

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**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

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**Program#/Poster#:** 624.10/VV86

**Topic:** I.03. Anatomical Methods

**Support:** NIH U54HD090257

P01 HD083157

**Title:** Large area, high-resolution brain imaging workflow using block-face sem

**Authors:** ***C. A. BRANTNER**[1], L. MATSIYEVSKIY[1], C. CLARKSON-PAREDES[1], C. BRYAN[2], D. MEECHAN[2], T. M. MAYNARD[2], D. S. MENDELOWITZ[2], S. A. MOODY[3], A. S. LAMANTIA[2], A. S. POPRATILOFF[1]
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**Abstract:** Electron microscopy (EM) resolves neuronal ultrastructure; however more “user friendly” optical imaging approaches like confocal microscopy (CM) have recently been used as an alternative to EM analysis to provide limited cellular detail while maintaining neuronal morphology and identity. CM cannot resolve individual synapses, neuronal cytoskeleton or organelles. Thus, we developed an EM method to generate zoomable, Google map-type EM image sets that integrate anatomical localization, neuronal identity, and ultrastructural detail. Our integrated workflow permits acquisition and analysis of complete sections of the murine brainstem for assessment of anterior-posterior position, cranial nuclei, neuronal/glial identity, and organelle morphology in single tiled EM image sets. Brainstem sections (400µm) were fixed in osmium tetroxide, infiltrated with uranyl acetate, dehydrated and flat embedded in resin. Ultrathin sections of the entire brainstem were cut and placed on a silicon wafer for imaging in a FEI Helios FIB-SEM equipped with a concentric backscattering (CBS) detector (accelerating voltage - 2kV; landing current: 200 pAmps; Pixel dwell time: 3 msec, pixel size: 2.095 nm, horizontal field of view: 6.4 µm). Tiled CBS images were acquired using FEI MAPS software, and adjacent images overlapped by 20% for stitching. Tiled CBS images of the entire brainstem were collected at 600X to map regions of interest (ROIs). These ROIs were then imaged at 1000X to assess cellular identity as well as some ultrastuctural detail. Finally we selected a specific 420x240µm area within the ROI to image at 80,000X. Using these image sets we could reliably integrate anatomical, cellular and organelle changes of specific brainstem regions *in silico*. We focused on the hypoglossal nucleus (nXII) in the *LgDel* mouse, a genomically

accurate model of human 22q11 Deletion Syndrome (22q11DS). *LgDel* nXII motor neurons have circuit and excitability changes that likely contribute to perinatal dysphagia, a clinical complication for children with 22q11DS and a robust phenotype in post-natal *LgDel* pups. Our EM analysis of nXII at low, intermediate and high resolution *in silico* demonstrates altered *LgDel* nXII motor neuron and interneuron cellular morphology as well as synapse loss and evidence of oxidative stress. The specificity of this integrated analysis was made possible by our novel approach to EM data collection to guarantee anatomical precision and high quality ultrastructural detail. *Supported by DC Intellectual and Developmental Disabilities* (NIH U54HD090257) and *GW Program for Pediatric Dysphagia* (P01 HD083157).

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**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

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**Topic:** I.03. Anatomical Methods

**Support:** SNF Ambizione grant #161448

**Title:** Localization of endogenous compounds in connectomics-grade volumetric electron microscopy imagery of brain tissue

**Authors: *T. TEMPLIER**[1,2], O. URWYLER[3,2], R. H. HAHNLOSER[4,2]
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**Abstract:** Electron microscopy (EM)-based cellular-resolution Connectomics lacks the ability to visualize endogenous compounds within uncompromised connectomics-grade volumetric EM imagery.
Using postembedding immunohistochemistry after non-destructive ultrathin serial sectioning of connectomics-grade resin-embedded brain tissue we show promising results for the localization of:
1. genetically encoded tags whose antigenicities survive harsh connectomics-grade EM embedding
2. genetically encoded tags that can be labeled
a) in vivo
or b) postmortem without permeabilization in thick tissues by complementary tags whose

antigenicities survive harsh connectomics-grade EM embedding
3. endogenous proteins that are labeled postmortem without permeabilization in thick tissues by tags whose antigenicities survive harsh connectomics-grade EM embedding.
Our approach, if successful, will complement volumetric connectomics-grade EM imagery with multiplexed imagery (fluorescent or cathodoluminescent) showing endogenous compounds (possibly genetically encoded and possibly in a stochastic manner) such as synaptic proteins or tags delineating neuron morphologies.

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**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

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**Program#/Poster#:** 624.12/VV88

**Topic:** I.03. Anatomical Methods

**Title:** Correlative light electron microscopy (CLEM) and quantification of synapses using immunofluorescence

**Authors: I. E. O. REPO**, A. STILLAR, *A. C. WEEKS
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**Abstract:** Quantification of synaptic number in a given brain structure has been traditionally performed using electron microscopy since this approach provides sufficient magnification and resolution for accurate identification. Transmission electron microscopy (TEM) is, however, very time consuming due to need for physical sectioning of tissue and stereological reconstruction from slices to volumes. This issue makes TEM only practical for analyzing small synaptic populations in small tissue volumes and not entire brain regions. While confocal laser scanning microscopy is able to sample large tissue volumes due to the optical sectioning, it has lower resolution than TEM and does not allow for direct visualization of functional synaptic contacts. The present study examined the capabilities of confocal microscopy to study synaptic populations by visualizing the co-localization of pre and post synaptic proteins (SV2A and PSD-95 respectively) and determining the efficacy and accuracy of this method at detecting specific synapse. Here, correlative light electron microscopy (CLEM) was employed to reveal the underlying ultrastructure of confocal images. Results demonstrated that a confocal approach can be used to accurately estimate the total number of synaptic contact in a given brain area.

**Disclosures: I.E.O. Repo:** None. **A. Stillar:** None. **A.C. Weeks:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.13/VV89

**Topic:** I.03. Anatomical Methods

**Title:** X-ray scattering: A new tool to probe myelin content and fiber direction. Application in mouse brain and comparison with MRI, histology and CLARITY

**Authors: *M. GEORGIADIS**[1,2,3], Z. GAO[1], M. LIEBI[4,5], C. LEUZE[6,7], V. ZERBI[8], D. ZINGARIELLO[1], S. SOMMER[1], M. AUGATH[1], J. MCNAB[6], O. BUNK[4], M. GUIZAR-SICAIROS[4], A. SCHROETER[1,2], M. RUDIN[1,2]
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**Abstract:** Disintegration of axonal myelin sheaths leads to impaired impulse transduction and is associated with severe neurological/neurodegenerative diseases. Yet, spatially resolved assessment of myelin content in the brain remains a challenge. Currently, magnetic resonance imaging (MRI) methods such as magnetization transfer (MT) assessing the amount of macromolecules including myelin, and diffusion MRI (dMRI) inferring on axonal direction by probing the directionality of water diffusion are methods of choice, though they are not directly reporting on myelin levels. The recently developed *small-angle X-ray scattering tensor tomography* (SAXSTT)[1,2] might constitute a more immediate approach for probing myelin content and fiber direction given its sensitivity to a structural feature of the myelin sheath, its ~17.5nm periodicity[3].
We applied SAXSTT to mouse brain, and compared it to readouts obtained from MT and dMRI. Specifically, brains from 5-month-old C57BL/6 mice were studied *in vivo* and *ex vivo* using MRI and *ex vivo* with SAXSTT. In general, we found a high correlation between myelin content measures from MT and SAXSTT, though the quality of the correlation varied across brain regions with deviations found in frontal and lateral temporal cortex as well as olfactory tracts. Given the molecular signature of the SAXSTT signal (17.5nm periodicity) we attribute these differences to confounding contributions from other macromolecules to the MT signal. Comparisons of dMRI- and SAXSTT-derived fiber directions displayed a high level of similarity; yet region-specific differences were observed as well, e.g. for cingulum and brainstem. This discrepancy might arise from fundamental differences in properties measured: while SAXSTT probes the orientation of structural elements of myelin sheaths, dMRI assesses

the direction of intra-axonal water diffusion. Imaging results on myelin content and fiber directions were validated by histological analysis using Luxol Fast Blue and whole brain analysis using CLARITY[4] and anti-neurofilament staining.
In summary, the sensitivity to structural features renders SAXSTT an attractive technique for probing the local concentration and orientation of specific molecular entities in tissue *ex vivo* at high spatial resolution. SAXSTT might complement and validate experimental and clinical (MRI) readouts of brain disorders affecting neural connectivity, for which myelin content and integrity of white matter tracts play an important role.

***References***

1. Liebi M, et al., Nature. 2015;527:349.
2. Schaff F, et al., Nature. 2015;527:353.
3. Jensen TH, et al., NeuroImage. 2011;57:124.
4. Chung K, et al., Nat Meth. 2013;10:508.

**Disclosures:** **M. Georgiadis:** None. **Z. Gao:** None. **M. Liebi:** None. **C. Leuze:** None. **V. Zerbi:** None. **D. Zingariello:** None. **S. Sommer:** None. **M. Augath:** None. **J. McNab:** None. **O. Bunk:** None. **M. Guizar-Sicairos:** None. **A. Schroeter:** None. **M. Rudin:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.14/VV90

**Topic:** I.03. Anatomical Methods

**Support:** MH106245

GM115042

NSF1659427

NSF1137725.

**Title:** Higher resolution and precision correlative and integrated volume electron microscopy: Solving sample surface charging and electron beam damage

**Authors: *E. ROSA-MOLINAR**[1], I. I. TORRES-VASQUEZ[1], N. MARTINEZ-RIVERA[1], C. M. SANTIAGO-ROBLES[1], H. SHINOGLE-DECKER[1], P. S. THAPA-CHETRI[1], J. P. KILCREASE[2], V. JOSHI[3], R. D. POWELL[3]
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**Abstract:** Here we describe results of our efforts to develop conductive reagents and sample preparation workflows that together overcome the challenges of “charging” and “electron beam damage” posed by volume electron microscopy. Our efforts began with a conductive epoxy resin developed and optimized for infiltration, immunocytochemistry, and serial-sectioning. Although the resin did not infiltrate evenly throughout the tissue sample and did not serial-section well, it provided tissue section stability as well as eliminated tissue section charging, beam damage, and shrinkage. These results led to the development of an electron conductive *en bloc* stain and sample preparation workflow. Prior to embedding the tissue sample in a non-conductive epoxy resin, the tissue sample was made electron-conductive using an *en bloc* stain. This *en bloc* stain coupled with the sample preparation workflow dramatically increased tissue contrast and electrical conductivity to overcome challenges posed by charging, radiation, ion beam- and electron-induced surface contamination/artifacts. However, again, serial-sectioning was an issue. These results then led to a third development, an *en bloc* electron-conductive ionic liquid stain and sample preparation workflow. The workflow dramatically increased tissue contrast in addition to electrical conductivity and overcame challenges posed by charging, radiation, ion beam- and electron-induced surface contamination/artifacts. It also serial-sectioned well and allowed for the segmentation / volume rendering of spinal motor neurons as well as their dendritic arbors and spines. On-going developments in volume electron microscopy specimen preparation, such as those described here will allow us to overcome challenges and permit artifact free neural circuit reconstruction.

**Disclosures: E. Rosa-Molinar:** None. **I.I. Torres-Vasquez:** None. **N. Martinez-Rivera:** None. **C.M. Santiago-Robles:** None. **H. Shinogle-Decker:** None. **P.S. Thapa-Chetri:** None. **J.P. Kilcrease:** None. **V. Joshi:** None. **R.D. Powell:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.15/VV91

**Topic:** I.04. Physiological Methods

**Support:** NIH Grant 1R01MH107238-01

NSF Grant 007289-00001

Moore Foundation Grant GBMF3396

**Title:** Light sheet and light field microscopy platform for structural and functional neuroimaging

**Authors: *T. V. TRUONG**[1], A. ANDREEV[2], S. MADAAN[2], D. B. HOLLAND[1], M. JONES[1], S. E. FRASER[1]
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**Abstract:** We present our development of an integrated imaging platform based on light sheet and light field microscopy strategies, to allow structural and functional neural imaging of whole small animals such as the zebrafish larvae. Light sheet microscopy, also known as Selective Plane Illumination Microscopy (SPIM), enables high resolution, high speed, low-photodamage imaging, ideal for following changes to brain structure and activity as a function of behavioral states. Light field microscopy, with the tradeoff of reduced resolution, enables synchronous volumetric imaging, where a single snapshot captures information over an entire extended 3D volume. This enables observation of neural activity at speeds even higher than possible with SPIM, and allowing for the sample to move in space during acquisition. We recently introduced Selective Volume Illumination to light field microscopy (SVIM), illuminating only the volume of interest of the sample, hence significantly reduce the background and enhance the contrast. We apply SPIM and SVIM to a variety of neural imaging applications, from observing sleep/wake behavior, to monitoring structural and functional changes due to learning. We envision the integration of SPIM and SVIM, spanning the full performance space of high-resolution to high-speed to whole-animal coverage, as the ideal platform for experimental paradigms that aim to record and understand neuronal structures and functions across scales, from the cellular to whole-brain, and correlating them with the animals' behavior.

**Disclosures: T.V. Truong:** None. **A. Andreev:** None. **S. Madaan:** None. **D.B. Holland:** None. **M. Jones:** None. **S.E. Fraser:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.16/VV92

**Topic:** I.03. Anatomical Methods

**Support:** Pilot Grant from IDDRC at Children's National

George Washington University

**Title:** Correlative workflow from fluorescence to 3D ultrastructural reconstruction of intracellular organelles via FIB-SEM imaging

**Authors: P. PARLANTI**[1], A. W. OAKS[2], C. BRANTNER[2], A. S. POPRATILOFF[2], *M. MANZINI[3]

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**Abstract:** Correlative methods are a powerful tool to obtain multiple types of information on biological samples with increasing resolution. The samples can be imaged with microtomography, light microscopy (LM) and electron microscopy (EM) to achieve a complete overview, both in 2- and in 3-dimensions. We developed a workflow to apply correlative imaging between fluorescent LM and focused ion beam-scanning EM (FIB-SEM) to visualize the organization of aberrant endolysosomal structures in neurons within the dendrite and under synaptic spines.
Proof of concept experiments were performed by overexpressing CC2D1A-GFP in HEK cells. CC2D1A (coiled-coil and C2 containing 1A) is a key regulator of intracellular signaling via endosomal trafficking, and is mutated in intellectual disability and autism spectrum disorder. Previous studies have shown that gain or loss of function of CC2D1A in different cell types disrupts endolysosomal trafficking and signaling, generating enlarged vesicular structures. Transfected HEK cells were cultured on gridded glass coverslips, fixed, and imaged by both fluorescent and transmitted LM over the entire coverslip on a FEI CorrSight microscope. CC2D1A-GFP expressing cells of interest were then imaged again to build a 3D stack of fluorescent features. Cells were then embedded in resin for EM analysis and imaged again using transmitted light. All fluorescent and LM data were loaded on FEI MAPS software, which allowed the following alignment between the LM and EM images. The following EM analysis has been performed with the FEI Helios NanoLab 660 dual beam FIB-SEM. The fluorescent cells were identified in the MAPS software, and the same cells were localized with the FIB-SEM, using as reference the coordinates acquired during the LM. The FIB-SEM instrument allowed to build a trench in the resin in the region of interest (ROI), 20-50nm thickness serial sections were removed with the gallium ion beam and exposed block faces were imaged using SEM to obtain serial images with ultrastructural resolution. The images from up to a 15µm area were then aligned, and the volume of endosomal vesicles segmented with Arivis software. The entire process was performed at the Nanofabrication and Imaging Center at GWU.
This workflow allows to identify specific cells or subcellular ROIs via fluorescent LM and to reconstruct the fluorescent features at the ultrastructural level in 3D. We confirmed that CC2D1A-GFP-positive regions within the cells colocalize with enlarged and abnormal vesicular structures, and we are now extending these studies to correlative imaging analysis of endosomal vesicles under the dendritic spines of primary neurons.

**Disclosures: P. Parlanti:** None. **A.W. Oaks:** None. **C. Brantner:** None. **A.S. Popratiloff:** None. **M. Manzini:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.17/WW1

**Topic:** I.03. Anatomical Methods

**Support:** NIH BRAIN Initiative Grant (NEI and NIMH 1-U01-MH106027-01)

NIH Single Cell Grant 1 R01 EY023173

**Title:** Batch processing of ultrathin sections for large-scale, serial section electron microscopy

**Authors: *T. LEE**[1], D. J. BUMBARGER[2], R. REID[3], C. FOREST[4]
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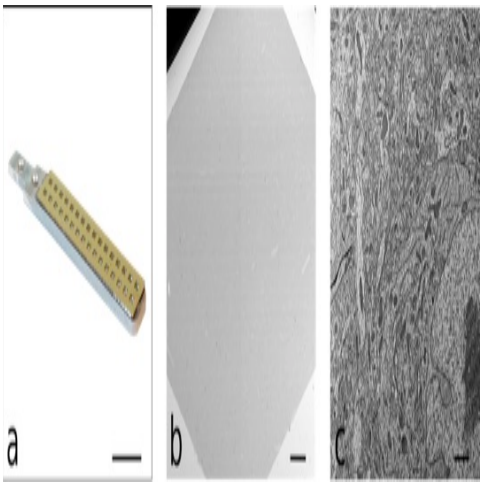
**Abstract:** Over the past decade, the field of connectomics has emerged as one of the most promising approaches to exploring the nature of neural circuits. A microscale (~1 $mm^3$) connectome—a neuron-to-neuron wiring diagram of a neural circuit—would potentially contain a vast trove of information regarding information processing and memory. The field is held back, however, by the great difficulty in consistently collecting anatomical datasets with serial section electron microscopy. In the cerebral cortex, for instance, a local circuit is contained in a cubic millimeter, but single sections—obtained by cutting plastic-embedded brain samples with a diamond knife—must be 40 nanometers or thinner, thus requiring 25,000 consecutive sections to be processed. Here, we present novel tools for high-throughput, high-reliability, batch serial section processing that will enable rapid, millimeter-scale neural circuit reconstruction. (1) Using modern microfabrication technologies, we have developed high packing density substrates that are compatible with either serial section transmission or scanning electron microscopy (see Figure 1a, scale bar: 10 mm). Our substrates, with silicon nitride windows, are electron transparent, do not exhibit charging in the electron microscope, and are patterned with hydrophilic domains to eliminate misplacement of sections. Heating substrates during the section placement process allows for rapid evaporation of water droplets and facilitates wrinkle-free dry-down of ultrathin sections (see Figure 1b, scale bar: 100 um). (2) With off-the-shelf, high precision linear actuators, high-resolution cameras, and custom image processing, we have developed a semi-automated, robotic method for picking up and placing individual serial sections onto our heated substrates. High-magnification images of resulting ultrathin sections exhibit sufficient contrast for segmentation (see Figure 1c, scale bar: 1 um). The integrated toolset promises high-throughput, high-reliability ultrathin section processing for serial section electron microscopy.




**Disclosures:** **T. Lee:** None. **D.J. Bumbarger:** None. **R. Reid:** None. **C. Forest:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.18/WW2

**Topic:** I.03. Anatomical Methods

**Support:** Australian Research Council (ARC) Centre of Excellence Scheme

**Title:** Novel probes for background-free cellular tracing

**Authors: *L. M. PARKER**[1], M. DAS[1], N. M. CORDINA[1], P. REINECK[3], X. XU[2], Y. LU[1], B. GIBSON[3], N. H. PACKER[1]
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**Abstract:** Tracer compounds such as cholera toxin B (CTB), wheat germ agglutinin (WGA) and tomato lectin have greatly increased our understanding of central nervous system (CNS) cell function, location and connectivity. Organic dye derived fluorophores are currently the most popular tracing conjugates even though they are highly susceptible to photobleaching, compete heavily with brain and spinal cord autofluorescence, and cannot be used for drug delivery. CNS tissue autofluorescence is high across a range of fluorescent excitation wavelengths from 300-650nm, particularly due to flavoprotein and reduced pyridine nucleotide (NADH) levels that correlate with neuronal excitability and metabolic activity *in vivo*, as well as from common neurotransmitter precursor amino acids such as tryptophan amongst others. Nanoparticles can offer the same advantages of fluorescent dyes for tracing studies but have superior properties that

give them photostability and clear signal discrimination over tissue/cellular autofluorescence. We have developed fluorescent nanoparticle conjugated tracers with near-infrared or infrared excitation, which are beyond the excitation wavelengths that produce almost all CNS autofluorescence. Fluorescent nitrogen vacancy centre nanodiamonds and lanthanide based upconversion nanoparticles were conjugated to CTB, WGA or tomato lectin via PEG using EDC/NHS based chemistry. These tracers were added to neurons, microglia and astrocyte cells. Uptake capacity and specificity were characterised at time points ranging from 1 minute to 4 hours by widefield and confocal microscopy. Our novel conjugated tracers were readily endocytosed by CNS cells over time, their presence and spectra were confirmed by single particle fluorescence spectroscopy and they demonstrated excellent photostability. The long and tuneable fluorescent lifetimes of these nanoparticles are ideal for time-resolved imaging, allowing for the detection of eight or more populations simultaneously, which is not possible using conventional dyes. Thus, the novel tracers developed here are ideal for future use in long term *in vivo* or tissue slice neuroimaging in a background fluorescence-free environment whilst also offering great multiplexing capabilities.

**Disclosures:** **L.M. Parker:** None. **M. Das:** None. **N.M. Cordina:** None. **P. Reineck:** None. **X. Xu:** None. **Y. Lu:** None. **B. Gibson:** None. **N.H. Packer:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.19/WW3

**Topic:** I.03. Anatomical Methods

**Support:** MEXT (A)(25250005)

MEXT (B)(25290012)

MEXT (15K14324)

MEXT (26112006)

MEXT (15H01456)

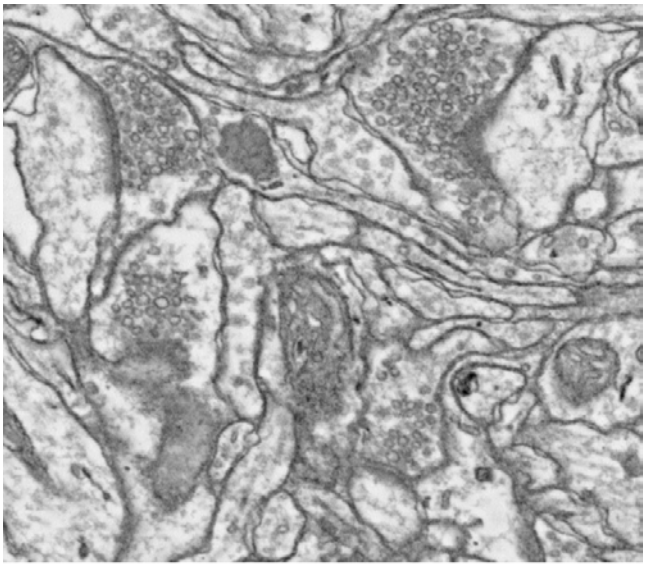
The NOVARTIS Foundation (Japan) for the Promotion of Science

the Okazaki ORION project

**Title:** A conductive novel tape material and a new staining protocol for volume electron microscopy applications

**Authors: *Y. KUBOTA**[1], J. SOHN[1], S. HATADA[1], M. SCHURR[2], J. STRAEHLE[3], A. G. GOUR[4], R. NEUJAHR[5], S. MIKULA[6], Y. KAWAGUCHI[1]
[1]Natl. Inst. Physiol Sci. (NIPS), Okazaki, Japan; [2]Connectomics, MPI For Brain Res., Frankfurt, Germany; [4]Dept. of Connectomics, [3]Max Planck Inst. For Brain Res., Frankfurt, Germany; [5]ZEISS Group, Carl Zeiss Microscopy GmbH, Oberkochen, Germany; [6]Electrons - Photons - Neurons, Max-Planck Inst. For Neurobio., Martinsried, Germany

**Abstract:** Electron microscopy (EM) analysis using 3D reconstruction from serial ultrathin sections has attracted considerable attention, even after the introduction of super resolution microscopy, because EM is the most reliable method to analyze dense nanoscale details of biological structures, e.g. synaptic connections. The volume EM datasets for analysis were obtained using a several kinds of new EM system including automated tape-collecting ultramicrotomy (ATUM), in addition to conventional serial-section EM using an ultramicrotome in conjunction with transmission electron microscopy (TEM).
The ATUM method automatically collects large numbers of ultrathin sections quickly, consistently and continuously and is capable of repeated observations of the ultrathin sections on tape. We found a novel, optimal tape for ATUM, plasma-hydrophilized, carbon nanotube (CNT)-coated polyethylene terephthalate (PET) tape with an extremely high surface conductance. The CNT-PET tape was superior in many features in comparison to the currently used carbon-coated Kapton tape (polyimide film, DuPont, Wilmington, USA), which is not completely optimal due to its imperfect conductivity, hydrophobic surface nature and/or frequent surface scratches. SEM of brain tissue sections on CNT-PET tape stained using a modified en bloc staining protocol revealed detailed synaptic structures and vesicles. The images are comparable to those obtained with the tissue section on the carbon coated Kapton tape.
In conclusion, CNT-PET tape is a useful addition to automated serial sectioning workflows and is expected to facilitate serial-section SEM reconstructions of brain circuits, which we expect will lead to substantial advances in brain microcircuit analysis.



Electronmicrograph captured with BSE detector showing synaptic contacts of rat frontal cortex stained with the modified block staining protocol.

**Disclosures:** **Y. Kubota:** None. **J. Sohn:** None. **S. Hatada:** None. **M. Schurr:** None. **J. Straehle:** None. **A.G. Gour:** None. **R. Neujahr:** None. **S. Mikula:** None. **Y. Kawaguchi:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.20/WW4

**Topic:** I.03. Anatomical Methods

**Support:** Dutch Science Foundation (NWO) VIDI Grant (#14637)

ERC Starting Grant (MULTICONNECT, #639938)

**Title:** An optical clearing and labelling platform for 3D cytoarchitectural characterization of large adult human brain samples

**Authors: *S. HILDEBRAND**, A. SCHÜTH, A. ROEBROECK
Maastricht Univ., Maastricht, Netherlands

**Abstract:** Optical clearing of brain tissue rapidly became a standard for investigating the rodent brain [1], [2]. However clearing and staining of adult human brain tissue proves to be far more difficult and currently only imaging of thin human specimen has been performed [3], [4]. This is partly due to the clearing capacity of the protocols, but even more to the limited penetration of common labels. Here, by combining an optimized clearing approach with small molecule dyes, we demonstrate 3D cytoarchitecture in thick adult human brain samples.
Brain tissue of 3 body donors, giving informed and written consent regulated by Dutch law, was obtained. Tissue was first perfusion fixed with 10 % formalin, post-fixed in 4 % PFA in PBS and sectioned into slices of approx. 15 x 20 x 5 mm.
Several dyes were tested for fluorescent Nissl or nuclei labeling by immersing slices in 6 ml solution for 2 - 7 d at $4^0$C.
Clearing was performed via a modification of the iDISCO+ method [1] with a new refractive index matching solution (RIMS) of the same RI as dibenzyl ether (DBE).
Light sheet fluorescent microscopy was performed with an Ultramicroscope II (La Vision Biotech, Germany; 2x objective, 4-10 mm working distance (WD)), two-photon laser scanning microscopy on a Leica TCS SP5 MP (Leica Mikrosysteme Vertrieb GmbH, Germany; HXC APO L 20x, 2 mm WD water immersion objective).
The iDISCO+ protocol [1] in combination with our new RIMS renders samples of human brain highly transparent (Fig. 1), while being less toxic, corrosive and photobleaching than DBE.
With the tested labels it is possible to stain thick samples reliably and fast (5 mm deep labelling possible in 5d). These inexpensive dyes provide a broad basis for cytoarchitectonic labeling and

counterstaining for cleared specimen, with various wavelengths to match research questions and microscope setups.
This opens up the possibility of high-throughput processing and imaging of human brain tissue for the examination of human neocortical architecture at the mesoscale.

**References:**

1. Renier, N., et al. (2016)
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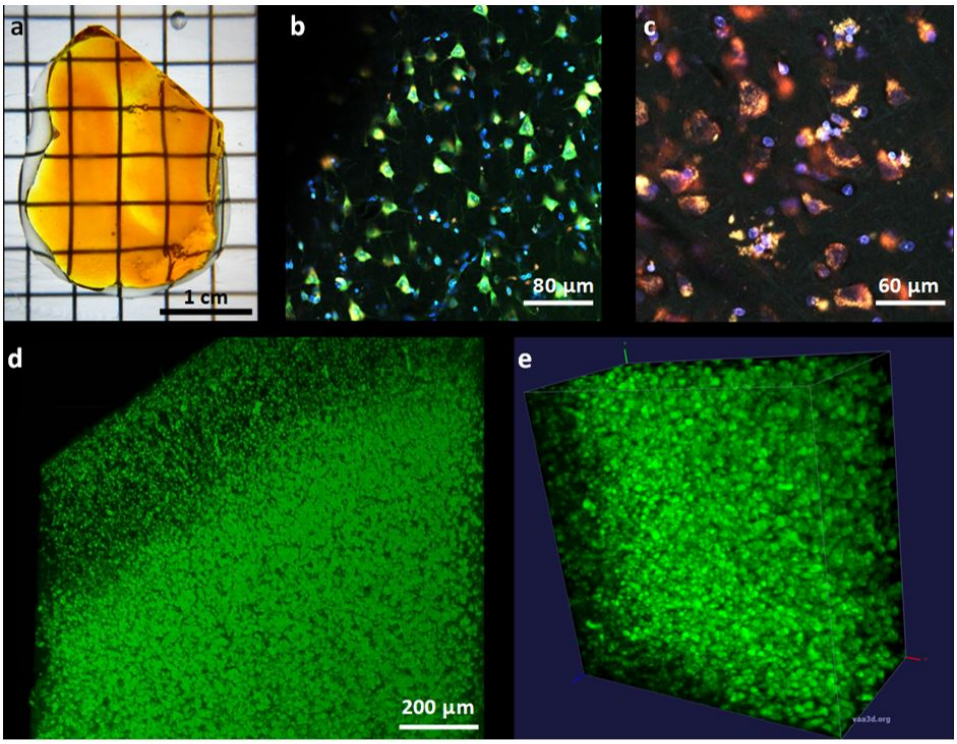


**Figure 1: Cleared adult human brain (5 mm thickness, a).** b and **c** show TPLSM images of a green and a red fluorescent Nissl stain and DAPI stained nuclei in blue. Cell bodies are also clearly distinguishable in the projection of a LSFM stack (**d**) and the 3D rendering of the same sample (**e**).

**Disclosures: S. Hildebrand:** None. **A. Schüth:** None. **A. Roebroeck:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.21/WW5

**Topic:** I.03. Anatomical Methods

**Title:** *In vitro* biocompatibility evaluation of nanostructured polymers

**Authors: *J.-M. MAYAUDON**[1], A. QUESNEL-HELLMANN[2], L. ROUSSEAU[4], B. YVERT[3], G. PIRET[2]
[1]U1205 BrainTech Lab., INSERM, Grenoble Cedex 9, France; [2]BrainTech lab U1205, INSERM, Grenoble, France; [3]BrainTech Lab., INSERM, Grenoble Cedex 9, France; [4]Esiee-Paris, Noisy le grand, France

**Abstract:** Multi-Electrode cortical arrays are key neuronal interfacing systems in neurophysiological and clinical research to better understand healthy and pathological brain dynamics. One of the major challenge is to propose a biocompatible device that remains stable over a long period of time and therefore that minimizes undesirable brain tissue reactions. Several studies indicate that surface structuration can promote neuron adhesion to the implant surface while it could limit glial cell proliferation. As the major implant surface is composed of the insulating material, we investigated different plasma etching procedures to get reproducible nanostructure topologies of SU-8, polyimide and parylene polymers. We have shown that, using a simple and rapid etching technique we were able to get nanostructures with controllable size. In a second step, the biocompatibility of these different nanostructured polymers was studied on rat primary cortical cells cultures.

**Disclosures: J. Mayaudon:** None. **A. Quesnel-Hellmann:** None. **L. rousseau:** None. **B. Yvert:** None. **G. Piret:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.22/WW6

**Topic:** I.03. Anatomical Methods

**Support:** McKnight Foundation

NIDDK

**Title:** The enteric nervous system 'connectome' with and without the microbiome

**Authors: *V. SAMPATHKUMAR**[1], V. DE ANDRADE[3], R. VESCOVI[1], H. LI[1], K. FEZZAA[3], M. DU[4], V. LEONE[2], E. CHANG[2], N. B. KASTHURI[1]
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**Abstract:** Despite increasing evidence that the gut microbiome influences neurological functioning, little is known about how microbiome changes the cellular and synaptic

organization of neurons. Part of the problem is that many studies focus on the effects of microbiome on the large and complicated central nervous system (CNS), where finding the putative site(s) of influence remains difficult. The enteric nervous system (ENS), often termed the 'second brain', has a potentially simpler structure than the CNS, functions in a semi-autonomous manner, and is far more proximate to the microbiome and its potential influences. It can thus serve as an ideal model system to study neuronal connectivity changes influenced by the microbiome. We have developed a multi-scale imaging pipeline that maps the cellular composition of large samples of the ENS (~1 $cm^3$, ~ 1μm resolution) using synchrotron source x-ray microscopy (μXCT) and nanometer reconstructions of neuronal connectivity in the same sample using automated large volume serial electron microscopy (EM). We are applying this pipeline to create unbiased maps of the cellular and synaptic differences in the ENS of wild type (WT) and germ free (GF) mice . Preliminary data already reveals potential cellular, vascular and neuronal changes in the absence of the microbiome. This data will reveal, for the first time, the influence of intestinal microbiota on the normal development of ENS and serve as a model for similar studies of CNS.

**Disclosures: V. Sampathkumar:** None. **V. De Andrade:** None. **R. Vescovi:** None. **H. Li:** None. **K. Fezzaa:** None. **M. Du:** None. **V. Leone:** None. **E. Chang:** None. **N.B. Kasthuri:** None.

**Poster**

**624. Histological Approaches: Brain Clearing, Expansion, and Reconstruction**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 624.23/WW7

**Topic:** I.03. Anatomical Methods

**Support:** Conacyt CB 169861

**Title:** Effect of incubation with streptolisynie o, in the pore formation in boar spermatozoa

**Authors: *M. BARRIENTOS**[1], E. JACOME-SOSA[1], B. DOMINGUEZ-MANCERA[1], P. CERVANTES[1], A. HERNANDEZ BELTRAN[1], M. JUAREZ-MOSQUEDA[2]
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**Abstract:** The aim of the present study was to determinate the formation of pores in the plasma membrane of the boar spermatozoa, after incubation with streptolysin O (SLO) and determining the effect on the viability post-thawing. The study was conducted at the reproduction biology lab of the diagnostic unit "Augusto R. Mancisidor Ahuja" at the "Torreón Del Molino" ranch. Fifteen ejaculates of four boars were obtained by the gloved hand technique. The inclusion criteria of the sample was ≥ 4 for mass motility and ≥ 70% for individual motility. The Scanning

electron microscopy was used to observe the presence of transmembrane pores. The Eosin-Nigrosin test and Coomassie Brilliant Blue in combination with Hypo-Osmotic Swelling Testing (HOST) were used for assessment of sperm viability and acrosome damage. Cells were incubed with SLO (0.6 UI/ml) and trehalose (200 μM) at three periods: T1: 5 minutes, T2: 15 minutes and T3: 30 minutes, all treatments at 37 °C for the membrane permeabilization. In the presence of trehalose (200 uM) and a control group (TC: diluted semen). After this, they were cryopreserved by the protocol described by Westendorf (1975); for sealing pores fetal bovine serum (FBS) was used at 5%. The data obtained were analyzed through Kruscal Wallis H test program STATISTICA V7.01. Was concluded that SLO is capable of forming pores in the plasma membrane of swine sperm cell. The motility do not have difference ($p > 0.05$) between T1 and T2, but there is with the T3 ($p < 0.05$). The TC had the highest percentage of living cells and functional membrane ($p < 0.05$) compared to other treatments, same as were similar between them ($p > 0.05$). The percentage of sperm with intact acrosome was similar between the TC, T1 and T2 ($p > 0.05$) treatments, as well as between T2 and T3 ($p > 0.05$). It can be concluded that in this study the SLO was able to form pores in the plasma membrane of swine sperm cell; however, incubation in SLO and trehalose was not a determining factor in the increase in viability after thawing.

**Disclosures:** **M. Barrientos:** None. **E. Jacome-Sosa:** None. **B. Dominguez-Mancera:** None. **P. Cervantes:** None. **A. Hernandez Beltran:** None. **M. Juarez-Mosqueda:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.01/WW8

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NINDS NS091260

The Blaustein Pain Research Fund

**Title:** Development of an immortalized huamn DRG neuronal cell line to model diabetic and chemotherapy idncued peripheral neuropathies

**Authors:** ***W. CHEN**[1], A. HOKE[2]
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**Abstract:** We have created a human neuronal cell line, HC-1, by immortalizing partially differentiated hESC cells with Sv-40 large T, MycT58A and hTert genes. Upon differentiation with the presence of human Activin A, GRO/MGSA (growth regulated protein/melanoma

growth stimulatory activity), NGF, GDNF and cAMP, these cells extended neurites and displayed human DRG characterizations. Specifically, they expressed high levels of neuronal markers such as TPRA, P2x3, TrkA, TrkB, TrkC, Trpm8, P75, GDNF and Pirt. Immunostaining and ELISA confirmed they were also positive for beta-3 tublin, neurofilament, calcium binding protein S100B, GFAP, isolectin B4 and CGRP proteins. In addition, they exhibited functional properties of human nociceptive afferent sensory neurons in electrophysiological studies. About half cells (51/116) were able to fire spikes upon the current pulse injection. Voltage clamp traces showed that HC-1 cells also expressed $Na^+$ currents, which could be blocked by pretreatment of 1 *u*M TTX. Complex outward current and voltage-gated potassium and sodium currents could also be evoked by depolarizing pulse protocol in these cells. Importantly, over 50% of the HC-1 cells (23/41) were responsive to capsaicin (a TRPV1 agonist). Calcium imaging confirmed that 15.38% (20/130) of cells responded to capsaicin and 23.07% (30/130) responded to menthol. We have tested HC-1 cells' suscepility to anticancer drugs cisplatin, *bortezomib, and taxol by measuring ATP production after treatment for 48 hours. About 40% of toxicity can be observed at 2 uM, 20nM and 10 uM, respectively, and higher concentrations of the drugs resulted in more toxicity.* These results indicated that these cells might be used in high throughput systems for the discovery and development of new drugs for the treatment of peripheral neuropathy in chemotherapy patients. Further more, we have conditioned HC-1 cells in a culture system in which glutamine, other than glucose, was used as the major source of nutri-energy. These adapted cells were susceptible to high glucose stress at physiologcal pH, and 35-45% toxicity (loss in ATP level) was recorded after treatment for 24 hours with 25mM glucose. This indicated yet another application of the cells in exploring the leads to the treatment for diabatic neuropathy.

**Disclosures: W. Chen:** None. **A. Hoke:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.02/WW9

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Development of an *In vitro* myelination assay using mouse oligodendrocytes and engineered nanofibers

**Authors: *Y. YANG**[1], B. BAI[2], S. LUNN[2], J. JOHNSON[3], N. KLEINHENZ[3], V. SHENOY[2], B. D. TRAPP[4]
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**Abstract:** Oligodendrocytes (OLs) are the only cells capable of generating myelin in vertebrate central nervous system (CNS). Myelinated axons are crucial for efficient and rapid conduction of action potential throughout the CNS. In multiple sclerosis (MS), pathological insults have been shown to cause a loss of OLs, leading in turn to demyelination, neuronal axon degeneration and ultimately irreversible neurological disability. Remyelinating therapies are keys for changing the long-term course of the MS progression. The development of myelination/remyelination therapies requires a reliable and sensitive in vitro myelination system. Although co-culture of neuron-oligodendrocyte or slice culture has been used to study myelination and remyelination, however, these in vitro myelination assays have limited throughput and sensitivity for drug efficacy studies. We present here the development of a myelination assay using purified mouse oligodendrocyte progenitor cells (OPCs) and custom-designed nanofibers that mimic white matter as suitable scaffolds for myelin wrapping. We found that immunopurified mouse OPCs are capable of differentiating into mature OLs and forming concentric wraps along the nanofibers, as revealed by electro-ultrastructure analysis. When OPCs were treated with thyroid hormone T3, a known activator of OPC differentiation and myelination, we found significant increases in the number of immunostained PLP+ and MBP+ cells in the nanofiber culture system. Moreover, these cells show longer myelin length and extended myelin wrapping along the fibers compared with controls. The efficacy of other repurposed drugs that have been shown to promote OL differentiation and myelination/remyelination has also been tested in the system. Our data demonstrate that mouse OPCs can differentiate into OLs and form multiple-layers of myelin wrapping on the nanofibers without axonal signaling. This system can be used effectively to evaluate the potential of remyelinating therapies. In addition, the use of mouse OLs in this culture system could provide a new avenue to investigate the mechanism of action of therapeutics by taking advantage of the mouse genetic system.

**Disclosures:** **Y. Yang:** A. Employment/Salary (full or part-time):; employee, Renovoneural Inc. **B. Bai:** A. Employment/Salary (full or part-time):; employee, Renovoneural Inc. **S. Lunn:** A. Employment/Salary (full or part-time):; employee, Renovoneural Inc. **J. Johnson:** A. Employment/Salary (full or part-time):; employee, Nanofiber Solution, Inc. **N. Kleinhenz:** A. Employment/Salary (full or part-time):; employee, Nanofiber Solution, Inc. **V. Shenoy:** A. Employment/Salary (full or part-time):; employee, Renovoneural Inc. **B.D. Trapp:** F. Consulting Fees (e.g., advisory boards); Renovoneural, Novatis, Biogen.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.03/WW10

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** CAPES-PROEX

FAPESP

**Title:** Glutamate transporter activator Parawixin-10: Neuroprotective effects in brain and retinal ischemia models in Wistar rats

**Authors: *J. L. LIBERATO**[1,2], M. V. A. AGUIAR[1], M. V. B. CELANI[1], T. BRONHARA[1], J. MARIN-PRIDA[3], N. P. LOPES[4], W. F. SANTOS[1,2]
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**Abstract:** Both stroke and retinal ischemia (RI) acute neuronal injury involve L-Glu-mediated excitotoxicity. Compounds that enhance L-Glu clearance represent promising tools for providing an efficient protection against ischemic injury, however only a few compounds promptly activate L-Glu transporters. In this context, we have investigated molecules isolated from spider venoms such as Parawixin10 (Pwx10), which presents a potent activity in increasing L-Glu uptake and did not present behavioral changes in neuroethological assessment. To evaluate the neuroprotective effects of Pwx10, we submitted Wistar rats to translational models of stroke and RI that reproduce ischemia and reperfusion. Thereby, in RI model, intraocular pressure was increased to 120 mmHg for 60 min in ischemia (ISC), which could be followed by reperfusion (IR). Pwx10 (2µg/µL), Riluzole (23,45 µg/µL) or Saline (0.9%, 1µL) injections were administrated intravitreal and neuronal protection and neurodegeneration were evaluated by quantification of Hematoxylin-Eosin (HE) and Fluoro-Jade C positive cells (FJC), respectively. The Pw10 protected the retina ganglion cell layer (GCL) more effectively than treatment with Riluzole both in HE ($n=6$; $p<0.0001$) and FJC ($n=6$; $p<0.0001$) assessment. In stroke model, rats were implanted with a steel guide cannula into the piriform cortex to access Middle Cerebral Artery (MCA) and in the right ventricle to deliver drugs. Occlusion of the right MCA was induced in awake rats by endothelin-1 (ET-1; 400ρmol in 4µL) injection. SHAM animals were injected with saline (in 4µL). Neurologic deficit (inclusion parameters) were evaluated 10 minutes after ischemia in Open field test (OFT) and Tail Suspension Test (TST). Treatment was conducted 30 min from behavioral analyses, with Pwx10 (2µg/µL; 1µL) or Saline 0.9% (1µL) injections. Neurologic deficits were assessed by OFT, TS additionally with Adhesive Test (AT), after 3 days post stroke. Neuronal death in cortical and striatal infarcted areas was evaluated with FJC and triphenyltetrazolium chloride (TTC) method. Treatment with Pwx10 improved neurological function in AT test ($n=6$, $p<0.001$), and in OFT ($n=6$, $p<0.0001$) and TST tests ($n=6$, $p<0.001$). More remarkably, Pwx10 was efficient in reducing infarct volume area ($n=6$, $p<0.0025$) in TTC method and decreasing FJC-positive cells in the cortex ($n=7$, $p<0.0001$) and striatum ($n=7$, $p<0.0001$). Our findings suggest that Pwx10 presents neuroprotective effect and L-Glu transporter activators can be considered a safe and effective approach to treating conditions involving excessive L-Glu such as ischemic injury.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.04/WW11

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Development of a quantitative targeted mass spectrometry platform for Parkinson's disease biomarkers

**Authors: *M. ROTUNNO**[1], P. WOLF[1], M. LANE[1], P. OLIVOVA[1], L. SHIHABUDDIN[1], K. ZHANG[1], P. SARDI[2]
[2]CNS Rare Dis. Research, Neurosci. Therapeut. Area, [1]Sanofi, Inc., Framingham, MA

**Abstract:** Parkinson's disease (PD) is the second most frequent neurodegenerative disease. There are no cures or disease-modifying therapies for PD, and this may be due in part to our inability to monitor biological markers of disease. Currently, PD patients are diagnosed, cared for, and assigned to clinical trials based on relatively arbitrary clinical scales. In order to develop disease-modifying therapies, dependable and robust biomarkers are required for monitoring disease progression and facilitating patient stratification. In this study, we employed parallel reaction monitoring (PRM) mass spectrometry to develop a targeted quantitative method for elucidating potential biomarkers in cerebrospinal fluid (CSF). By using both in-house data dependent mass spectrometry analyses and literature mining, we produced a list of over 125 quantifiable peptides representing 90 protein targets, including neurocan (*NCAN*) and vitamin D-binding protein (*GC*). Quality control analyses of these protein targets show high reproducibility with a CV < 15% for the majority of peptides assessed with the PRM method. Preliminary results from CSF analyses from 21 control and 19 PD patients indicate altered protein levels for a subset of these proteins. Among several disease relevant findings, altered vitamin D-binding protein was observed in patient compared to control CSF, and vitamin D insufficiency has been associated with PD. Currently, all 90 protein targets are being quantified with PRM in a validation cohort of 160 CSF samples from control and PD patients to assess their potential for biomarker application. In summary, we have developed a reliable quantitative targeted mass spectrometry platform to explore multiple putative biomarkers in PD that can be applied to other disorders.

**Disclosures:** **M. Rotunno:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **P. Wolf:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **M. Lane:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **P. Olivova:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **L. Shihabuddin:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **K. Zhang:** A. Employment/Salary (full or part-time):; Sanofi, Inc. **P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi, Inc..

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.05/WW12

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) (No. CRC-15-04-KIST)

**Title:** Alleviation of memory impairment in mouse model for alzheimer's disease by a novel inhibitor of aberrant gaba synthesis

**Authors: *J.-H. PARK**, J. CHOI, B. JANG, Y. JU, M. PARK, A. PAE, J. CHO, C. J. LEE, K. PARK
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**Abstract:** Alzheimer's disease (AD) is a complex and multifactorial disease for which various attempt to rescue memory impairment are still not produced actual results. We found that reactive astrocytes aberrantly and abundantly produce the inhibitory transmitter GABA by over-expressed monoamine oxidase-B (MAO-B) in AD. Based on this novel target, we have developed a promising lead compound that shows excellent selectivity and efficacy against hMAO-B and fully restores the cognitive impairment of APP/PS1 mice. It also displays excellent drug-like properties in ADME/Tox test. We proposed novel disease-modifying therapy in AD which inhibit aberrant GABA synthesis in reactive astrocytes.

**Disclosures: J. Park:** None. **J. Choi:** None. **B. Jang:** None. **Y. Ju:** None. **M. Park:** None. **A. Pae:** None. **J. Cho:** None. **C.J. Lee:** None. **K. Park:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.06/WW13

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Intramural Research Program of NIH/NIDA

**Title:** Molecular mechanisms underlying the oligomerization and activity of sigma 1 receptor

**Authors: M. XU**, H. YANO, *L. SHI
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**Abstract:** The sigma 1 receptor (σ1r) is a unique endoplasmic reticulum membrane protein, which shares no sequence homology with other mammalian proteins. It is involved in numerous cellular processes by interacting with various client proteins as a chaperon or modulator. Such extensive interaction network implicates its vital role in several human diseases such as drug addiction, depression and neurodegenerative disorders. Moreover, some σ1r ligands have been reported to alter the oligomeric state and the cellular behavior of σ1r. The categorization of these compounds, however, were based on different assay models and often not entirely consistent. Thus, it is essential for us to investigate the structure-function relationship for σ1r, in order to clarify the ambiguity of the classification and predict the pharmacological profile for new ligands. The recent crystal structures solved by Schmidt *et al. 2016* lay the foundation for this mission. The structures were solved as trimers and each subunit is composed of a single transmembrane helix and a barrel-like ligand-binding domain. Starting from the crystal structures, we investigate the conformational change of σ1r under different oligomeric states and ligand-bound conditions, using all-atom molecular dynamics (MD) simulations. Specifically, we characterized the different binding modes for four σ1r ligands, (+)-pentazocine (PNT), PRE084, PD-144418 (PD) and haloperidol (HDL) in both the trimeric and monomeric σ1r models. To this end, by the network analysis of the MD data, we identified the gate and anchor regions in the ligand-binding domain that have distinct dynamics under different conditions. The gate region, featured by the GXXXG motif, can adopt two states, open and closed. We demonstrate that all the trimeric conditions stabilize the gate in the closed conformation, which is further stabilized by binding with PD or HDL. The anchor region is where the transmembrane helix is attached. The mutation E102Q in this region was found to affect the mobility and activity of σ1r in the apo or PNT-bound conditions by Wong *et al. 2016*, which may play a role in amyotrophic lateral sclerosis. The analysis of our MD data showed different interaction networks within the anchor region, which are more well-connected in the PNT-bound and E102Q mutant conditions. In summary, our MD simulations are in line with the results from the previous studies and our BRET assay, indicating that the gate region is critical for the oligomerization of σ1r, whereas the anchor region contributes significantly to the activity of σ1r. The uncovered conformational changes also shed light on the pharmacological profiling for the σ1r ligands.

**Disclosures: M. Xu:** None. **H. Yano:** None. **L. Shi:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

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**Program#/Poster#:** 625.07/WW14

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Supported by the Intramural Research Program of the National Institutes of Health, National Institute on Drug Abuse.

**Title:** Pharmacological profiling of the sigma 1 receptor ligands by novel *In vitro* and in silico approaches

**Authors: *H. YANO**[1], M. XU[1], A. BONIFAZI[1], A. FANT[1], W. C. HONG[2], A. H. NEWMAN[1], L. SHI[1]
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**Abstract:** The sigma 1 receptor (σ1R) is a structurally unique transmembrane protein not sharing sequence homology to any other known protein family. Mainly functioning as a molecular chaperone in the endoplasmic reticulum (ER), its ligands have shown therapeutic potential in cancer, neuropathic pain, and psychostimulant abuse. Despite physiological and pharmacological significance, mechanistic underpinnings of structure-function relationships for σ1R are poorly understood. Particularly, the distinction between agonist and antagonist is not clearly defined, due to the lack of congruence among results from different assay models, which can be influenced by specific cellular environment and events leading up to specific assay readouts. Here we have established an assay system away from the downstream readout models. By incorporating a molecular proximity assay, bioluminescence resonance energy transfer (BRET), we categorized σ1R ligands independent of factors preceding the readout events. By focusing on the ligand induced conformational changes associated to σ1R-σ1R homomeric interaction, we have parsed out the ligands into those that promote the interaction and those that do not. In comparison, we demonstrated that the non-promoting ligands can reduce the BRET signals elicited by the promoting ligands. Further these results were substantiated with other cellular functional readouts for immediate downstream effects such as ER and intracellular calcium activities. In light of the seminal work of recent crystal structures by Schmidt *et al.*, we carried out molecular dynamics (MD) simulations in parallel in the trimer as well as monomer states. A consistent correlation regarding the differential characteristics of the ligand binding modes induced by interaction promoting and non-promoting ligands have been observed. Together with the results observed from MD simulations, we propose a novel BRET assay herein as a reliable method to functionally categorize σ1R ligands based on the conformational states of the receptor that a particular ligand would prefer or induce. The method could serve as a useful pharmacological profiling tool in its relevance within neuropharmacology.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.08/WW15

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** FONACIT 2013001606

**Title:** Pharmacological inhibition of the neuron restrictive silencer factor under hypoxia situations

**Authors: *Y. A. RODRIGUEZ**[1,2], J. C. MARTINEZ[1], M. L. SERRANO[3], C. CASTILLO[1], J. CARBALLO[1]
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**Abstract:** It is known that the neuron-restrictive silencer factor (NRSF) or Repressor Element 1-Silencing Transcription factor (REST), is part of a repressor complex that acts in the promoter region of more than 2000 neuronal genes. Patients suffering from a stroke have an increase in NRSF/REST expression that increases post-ischemic neuronal death in the affected regions. Therefore, the aim of this study was to evaluate if the use of a drug that prevents the transcriptional repression carried out by NRSF/REST could reduce neuronal death in hypoxia situations. The lysine-specific histone demethylase 1 (LSD1) participates in the repression complex of NRSF and was selected as a target protein to direct the search for drugs, because it was available in its three-dimensional protein structure and had been co-crystallized with several ligands. Then, it was used to screen compound libraries to look for drugs that were validated by molecular docking tools (AutoDock software, Vega ZZ) followed by *in vitro* assays. We chose the four best potential inhibitors based on their binding energy (C1, C2, C3, C4). When evaluating these compounds using *in vitro* assays in rats neuroblastoma Neuro 2A cells, none was found to have cytotoxic effect. In addition, we investigated the neuroprotective action of the compounds against oxygen-glucose deprivation (OGD)-induced injury. Cell viability was investigated using the 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) reduction assay. When the compound C3 was present, we observed a significant increase in cell viability compared to control treatments. When we examined the expression of the *HIF-1α* gene, a REST target gene, using RT-PCR, we found that the C3 compound favored the expression of the HIF-1α gene. This gene has been reported to exert an anti-apoptotic effect, which suggests the usefulness of such drug to promote cell survival. The compound C3 could be an epigenetic drug for treatment of strokes or could be a scaffold for future epigenetic drug discovery.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

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**Program#/Poster#:** 625.09/WW16

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NIH R01CA161056

P30 MH075673-S1

National Multiple Sclerosis Society Grant

**Title:** Enhanced CNS uptake of prodrugs of the GCPII inhibitor 2-PMPA following intranasal administration

**Authors: A. J. GADIANO**[1], S. C. ZIMMERMANN[2], R. P. DASH[2], C. GARRETT[3], J. ALT[1], C. ROJAS[3], A. G. THOMAS[1], Y. WU[1], P. MAJER[4], R. RAIS[2], *B. S. SLUSHER[1]
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**Abstract:** 2-(Phosphonomethyl)pentanedioic acid (2-PMPA) is a potent and selective inhibitor of glutamate carboxypeptidase-II (GCPII), a membrane-bound zinc metalloprotease that plays a key role in regulating extracellular glutamate availability in the brain. As disrupted glutamate homeostasis is implicated in several neurological and psychiatric conditions, GCPII is a promising drug target. Several independent laboratories have shown robust neuroprotective efficacy with 2-PMPA in dozens of preclinical neurological and psychiatric disease models (e.g. e.g. neuropathic pain, peripheral neuropathy, stroke, schizophrenia, addiction, multiple sclerosis, traumatic brain injury), when given at very high systemic doses ($\geq$100 mg/kg) or when directly injected into the brain. However, the clinical development of 2-PMPA has been hampered by its low brain penetration, presumably due to its multiple acidic functionalities. We recently reported an improvement in the brain-to-plasma ratio of 2-PMPA after intranasal (IN) dosing in both rodents and primates. We also recently employed a 2-PMPA prodrug strategy to decrease its polarity and showed that we could improve its oral availability by >20-fold in both rodents and dogs We therefore hypothesized that combining a IN delivery with a prodrug approach could further enhance permeability and increase drug exposures in the brain. Herein, we have undertaken the synthesis of several 2-PMPA prodrugs with masked γ-carboxylates and performed pharmacokinetic evaluations of these compounds in Wistar rats and rhesus macaques.

Our results show that the prodrugs investigated have enhanced lipophilicity and further improve brain as well as systemic delivery of 2-PMPA after IN administration. When compared to IN 2-PMPA in rats at 1 hour post-administration, the prodrug γ-(4-acetoxybenzyl)-2-PMPA (JHU 144)**,** resulted in substantially higher 2-PMPA delivery to both plasma (4.1-fold) and brain (11-fold). Subsequent time-course evaluation of JHU 144 also showed high brain and plasma 2-PMPA exposures with the brain/plasma ratios of 1.45, 0.23, and 0.20 for olfactory bulb, cortex, and cerebellum, respectively. Further, in primates IN administration of JHU 144 more than doubled 2-PMPA concentrations in the CSF relative to previously reported levels and provided a CSF to plasma ratio of 1**.** In conclusion, the results of these experiments provide a promising strategy of enhancing delivery of drugs that demonstrate therapeutic potential for neurological and psychiatric disorders.

**Disclosures: A.J. Gadiano:** None. **S.C. Zimmermann:** None. **R.P. Dash:** None. **C. Garrett:** None. **J. Alt:** None. **C. Rojas:** None. **A.G. Thomas:** None. **Y. Wu:** None. **P. Majer:** None. **R. Rais:** None. **B.S. Slusher:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.10/WW17

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Grand Valley State University Office of Undergraduate Research and Scholarship's Student Summer Scholar Grant

**Title:** microRNA 34b/c and alpha synuclein gene expression in SH-SY5Y cells for Parkinson's disease study

**Authors: *E. HAHS**, S. KHOO
Cell and Mol. Biol., Grand Valley State Univ., Grand Rapids, MI

**Abstract:** Parkinson’s disease (PD) is the second most common neurodegenerative disorder, affecting 1-2% of adults over the age of 60. The pathological hallmark of PD is alpha synuclein (aSyn) protein inclusions, known as Lewy bodies and Lewy neurites, in the brain. Accumulation of this misfolded protein, especially in the dopaminergic neurons, has been implicated to cause PD. Thus, developing new drug therapies that block aSyn aggregation could potentially slow or stop the disease progression. MicroRNAs (miRNAs) are small RNAs that bind to messenger RNA (mRNA) and regulate their gene expression. miRNA-34b and 34c are predicted targets for aSyn and are shown to be down-regulated in PD brains. Here, we aim to establish a cell model to study the effects of miRNAs on aSyn aggregation. SH-SY5Y cells were incubated in DMEM/F12 and 10% FBS. Retinoic acid and brain-derived neurotrophic factor were then added

for cell differentiation. Trypan blue was used for cell viability assay. Rotenone, a pesticide that can induced Parkinson-like phenotypes in cell, was added in day 8 and cells (in triplicate) were collected every day at day 9-16 of differentiation. RNA that contains miRNAs was extracted from cells using Qiagen miRNeasy kit. Quality and quantity of mRNA and miRNA were measured with a Qubit fluorometer. Using quantitative real-time PCR, we found miR-34b/c expression down-regulated in rotenone treated SH-SY5Y cells that mimic PD when compared with non-treated control (miRNA-34b p-value = 0.0073; miRNA-34c p-value = 0.0006). However, aSyn gene expression was not up-regulated in rotenone treated cells when using Taqman primers that cover short aSyn transcript (exon 1-3; Hs01103383_m1). Our next step is to use primers that cover aSyn full-length transcript (exon 1-5; Hs00240907_m1) and anticipate up-regulation of aSyn in rotenone-treated cells. This study will allow testing of miRNA mimics or inhibitors to investigate their effects on aSyn aggregation to provide valuable information on future miRNA-based therapies for PD.

**Disclosures: E. Hahs:** None. **S. Khoo:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.11/WW18

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** AbbVie

**Title:** Reduced lifespan, but absence of Alzheimer's-like pathology and cognitive impairment in commercially-sourced SAMP8 mice

**Authors: *J. W. BROWN**, V. A. RODERWALD, H. M. ROBB, C. Z. ZHU, E. G. MOHLER
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**Abstract:** In-vivo discovery efforts to screen and validate novel therapeutics for Alzheimer's Disease (AD) predominantly use transgenic mice harboring human mutations associated with early-onset familial AD (APP, PS1, and PS2). While Tg AD mice do have utility in certain applications, particularly when evaluating therapeutics whose mechanism of action is directly linked to such proteins and pathways, they model only a tiny fraction (1-5%) of the AD patient population. SAMP8 mice have been considered as a model of senile dementia and non-familial sporadic AD and therefore may be a more representative model of the predominant AD population of late-onset. SAMP8 mice, in addition to an accelerated aging phenotype, are reported to feature many AD-like characteristics (e.g., increased Aβ and pTau, neuroinflammation, neuron loss, synaptic dysfunction, and cognitive dysfunction), some of which are often absent in Tg AD mouse models. To confirm the reported phenotype of SAMP8

mice, we evaluated 6, 9, and 12 month old male and female mice in a cross-sectional design using commercially-sourced animals from a purpose-bred colony at EnVigo (Indianapolis, USA) using a variety of cognition, biochemical, and immunohistochemical (IHC) assays. While both male and female SAMP8 mice had significantly reduced lifespan compared to sex-matched SAMR1 controls, consistent with the established accelerated aging phenotype for these mice, no robust cognitive impairments or AD-like pathological changes were observed relative to SAMR1 controls. There were no strain differences in Barnes Maze, contextual fear conditioning, or spontaneous alternation in cross-maze across all ages. Only in the inhibitory avoidance task were SAMP8 mice impaired relative to SAMR1 mice, but there was no age-related decline in performance observed. Biochemical analysis of whole brain homogenates by Meso Scale Discovery immunoassay revealed no increases in $A\beta_{40}$, $A\beta_{42}$, total tau, pTau, or neuroinflammatory cytokines in SAMP8 compared SAMR1 across all ages. Furthermore, IHC analysis of the CA3 region of hippocampus demonstrated no strain differences in pTau (AT8) or microglial activation (Iba-1) across all ages as well. The lack of strain differences across multiple measures of cognition and absence of AD-like pathological features reported here suggests the possibility of phenotypic drift in SAMP8 mice sourced from EnVigo, at least in the colony of mice produced for our purposes.

**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: J.W. Brown:** A. Employment/Salary (full or part-time):; AbbVie. **V.A. Roderwald:** A. Employment/Salary (full or part-time):; AbbVie. **H.M. Robb:** A. Employment/Salary (full or part-time):; Abbvie. **C.Z. Zhu:** A. Employment/Salary (full or part-time):; AbbVie. **E.G. Mohler:** A. Employment/Salary (full or part-time):; AbbVie.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.12/WW19

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Biomarker for KMO inhibitor, CHDI-00340246: Pharmacokinetic and pharmcodynamic effect in CSF of conscious non-human primate

**Authors: *M. VAN GAALEN**[1], R. CACHOPE[2], J. SUTCLIFFE[1], A. RASSOULPOUR[4], M. S. HEINS[5], C. SCHLUMBOHM[1], K. HOFFMANN[1], J. H. A. FOLGERING[5], T. I. F. H. CREMERS[5], V. KHETARPAL[2], C. DOMINGUEZ[2], I. MUNOZ SANJUAN[2], L. MRZLJAK[3]
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**Abstract:** Information about the concentration of drug candidates and their pharmacodynamic (PD) effect in the brain and cerebrospinal fluid (CSF) is vital to decrease the attrition rate in drug discovery/development; poor pharmacokinetics has been recognized as one of the leading causes of failure in the clinic. Therefore, the ability to measure concentrations of a drug candidate and its PD effect in the brain, CSF and plasma as biomarkers are of particular interest. Metabolites of kynurenine pathway (KP) are implicated in the pathophysiology of neurodegenerative disorders including Huntington's disease (HD). Kynurenine 3- monooxygenase (KMO) inhibitors may have potential to treat HD, given that KMO inhibition should shift the metabolism of kynurenine to increase the formation of the neuroprotective metabolite kynurenic acid and reduce the neurotoxic metabolites 3-hydoxykynurenine and quinolinic acid in the brain. This has been confirmed in rodent and non-human primate microdialysis studies using the potent KMO inhibitor CHDI-00340246. In the present study, CHDI-00340246 or vehicle was dosed in a Latin square design up to 10 mg/kg p.o. in awake, cynomolgus macaques. To enable serial CSF collection, a catheter was placed in the cisterna-magna and connected to a subcutaneous port under anaesthesia. CSF and plasma was sampled at pre-defined time-points across a 60 hour period following a minimum of a 1 week recovery period after surgery. The maximum concentration of CHDI-00340246 in the CSF was found at 2 hours post-dose, correspondingly in the plasma the maximum concentration was found at 1 hour after drug administration at 10 mg/kg p.o. Furthermore, concentrations in CSF were significantly lower compared to plasma. The maximal concentration of kynurenic acid, indicating KMO inhibition, was dose-dependently increased in the CSF, whereas in the plasma a maximal increase was found at 3 mg/kg. Comparison to results from a previous microdialysis experiment in the cynomolgus macaque revealed that the concentration of CHDI-00340246 in the CSF is 1.3 - 2.7 fold lower than that measured in the brain directly via microdialysis. Finally, CHDI-00340246 increased kynurenic acid concentrations in the brain, CSF and plasma, however, kynurenic acid concentrations rose more rapidly in plasma compared to the brain and CSF. These findings demonstrate that PK and PD parameters in the CSF can be helpful as a biomarker for CHDI-00340246 reflecting brain concentration and on target effects.

**Disclosures:** **M. Van Gaalen:** A. Employment/Salary (full or part-time):; Encepharm. 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.; Encepharm, Brains On-Line. **R. Cachope:** A. Employment/Salary (full or part-time):; CHDI. **J. Sutcliffe:** A. Employment/Salary (full or part-time):; Encepharm. **A. Rassoulpour:** A. Employment/Salary (full or part-time):; Brains On-Line. **M.S. Heins:** A. Employment/Salary (full or part-time):; Brains On-Line. **C. Schlumbohm:** A. Employment/Salary (full or part-time):; Encepharm. **K. Hoffmann:** A. Employment/Salary (full or part-time):; Encepharm. **J.H.A. Folgering:** A. Employment/Salary (full or part-time):; Brains On-Line. **T.I.F.H. Cremers:** A. Employment/Salary (full or part-time):; Brains On-Line. **V. Khetarpal:** A. Employment/Salary (full or part-time):; CHDI. **C. Dominguez:** A. Employment/Salary (full or part-time):; CHDI. **I. Munoz SanJuan:** A.

Employment/Salary (full or part-time):; CHDI. **L. Mrzljak:** A. Employment/Salary (full or part-time):; CHDI.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.13/WW20

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** The American Parkinson Disease Association (APDA)

**Title:** Analysis of neuron- and oligodendroglia-derived blood exosomes for diagnostics of synucleinopathies

**Authors: *S. DUTTA**[1], I. D. ROSARIO[2], K. PAUL[2], J. BRONSTEIN[1], B. RITZ[2], G. BITAN[1]
[1]David Geffen Sch. of Med. At UCLA, Los Angeles, CA; [2]Dept. of Epidemiology, Fielding Sch. of Publ. Hlth., Univ. of California Los Angeles, Los Angeles, CA

**Abstract:** Synucleinopathies, including Parkinson's disease (PD), Lewy body dementia (LBD) and multiple system atrophy (MSA) are all characterized by aggregation and deposition of α-synuclein in the brain. Though the normal cellular function of α-synuclein is unclear, the protein is highly enriched at presynaptic terminals. Multiple observations suggest that misfolding and self-association of α-synuclein into oligomers and aggregates may cause neural dysfunction and neurodegeneration in these diseases. Nonetheless, diagnosis of synucleinopathies is challenging due to overlapping symptoms among the synucleinopathies themselves and with other atypical parkinsonian syndromes. Thus, reliable biomarkers for these diseases are highly sought after. Recently, exosomes and other extracellular vesicles (EVs) isolated from blood have been shown to provide a useful source of biomarkers for Alzheimer's and Parkinson's diseases. Following similar strategy, here, we used antibody-coated magnetic beads to immunochemically isolate EVs released by neurons or oligodendrocytes from serum of healthy individuals and patients with PD and other synucleinopathies and measured biomarker concentration in them by a highly sensitive electrochemiluminescent ELISA. Our data show that although the α-synuclein concentrations are indistinguishable between healthy and patient samples of whole serum or serum exosomes, a significant difference is found in α-synuclein concentration in neuron- and oligodendroglia-derived blood exosomes. Supporting previous reports, the α-synuclein concentrations observed were substantially lower in the exosomes isolated from healthy individuals compared to patients with PD. Our data support the use of brain-derived blood exosomes for diagnosis of synucleinopathies, and suggest that the method can be expanded for other neurodegenerative diseases.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.14/WW21

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Current and future directions of the ninds parkinson's disease biomarkers program

**Authors: *C. R. SWANSON**[1], K. ARCE[2], D. J. BABCOCK[1], K. K. DAVID[1], L. FRAME[2], J. LIU[2], C. LUNGU[1], B.-A. SIEBER[1], M. SUTHERLAND[1]
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**Abstract:** The NINDS Parkinson's Disease Biomarkers Program (PDBP) promotes biomarker discovery to improve clinical trial design for PD and related disorders. Over 1400 participants, to date, have contributed data and biosamples to the PDBP. The program has four pillars: 1) Standardized longitudinal clinical data and biosample collection from PD subjects and healthy controls, harmonized with complementary PPMI and BioFIND biomarker cohorts. The NINDS PDBP recently expanded to include subjects with Atypical Parkinsonisms and other movement disorders (e.g. Multiple System Atrophy, Corticobasal Degeneration, Progressive Supranuclear Palsy, Essential Tremor), as well as four studies on Lewy Body Dementias (LBD) that will enroll 590 participants. 2) Laboratory-based biomarker discovery and replication studies utilize platforms including whole genome sequencing (WGS), transcriptomics, digital immunoassays, and exosomal characterization. The studies include longitudinal follow-up and independent replication of candidate biomarkers. 3) PDBP biospecimens banked and distributed to the research community through the NINDS Biorepository (BioSEND). New efforts in 2017 include collection and banking of PBMCs at the NINDS Human Cell and Data Repository (NHCDR). 4) The PDBP Data Management Resource (DMR) infrastructure, provides broad sharing and access to clinical data and associated biospecimens for research. The PDBP engages and collaborates with stakeholders, including the FDA, industry and nonprofit organizations, to reach common goals. Using a multifaceted approach, the NINDS PDBP is advancing biomarker discovery for PD prognosis and progression via the expansion of standardized clinical data and biosample collection in PD and related disorders, banking and distribution of samples, coordination of broad data access and new technological approaches. Coordinated and collaborative NINDS PDBP activities align with a multimodal approach to biomarker discovery required to facilitate effective drug development for the treatment of PD and related disorders

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.15/WW22

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** ITS/163/16

CUHK-4903747

CUHK-7105306

**Title:** An easily integratable behavioral system for high-throughput drug screening in zebrafish

**Authors:** ***S. L. WALKER**, D. C. CHAN, H. LIU, W. YUNG, Y. KE
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**Abstract:** A major bottleneck which limits the discovery of new neuro-active compounds for treating disorders such as Parkinson's disease or epilepsy is the duration it takes to screen said drugs which are relevant to the nervous system. While cell culture can detail toxicity and basic cellular responses of a potential drug, the model is not sophisticated enough to define the drug effects within a complex system like the brain. Individual drugs need to be tested in regards to behavior for hidden side effects and drug efficacy in order to identify active neural compounds which could treat the disorder efficiently without complicating one's health. To test the drug effects on behavior and in a high-throughput manner, we developed a behavioral system around zebrafish larvae. Zebrafishes are easy to maintain and produce large numbers of embryos, lending them particularly suitable for high-throughput drug screens. Although many drugs screens have been performed on zebrafish prior, these screens focused primarily on toxicity and basic cellular responses. To identify neural active drugs, we have developed a behavioral box which can actively test multiple plates at once and is easily integrated into a robotic system. The system utilizes an identical setup utilized for behavioral tests under the multi-photon microscope, allowing for easy and quick comparisons between a macro-view of behavior and a neural imaging view of behavior.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.16/WW23

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Brown University Upjohn Professorship

**Title:** Characterizing the role of ceramide in the dual cytotoxic and metabolic stimulative effects of sigma-2 receptors in SK-N-SH neuroblastoma cells

**Authors: *C. Z. LIU**, W. D. BOWEN
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**Abstract:** Sigma-2 receptors mediate cell survival. Sigma-2 agonists cause cell death via a variety of pathways and thus have been of interest as potential cancer therapeutics, while antagonists may have value as neuroprotective agents. Recently, a new class of sigma-2 agonists has been discovered, e.g. CM764, that do not induce cell death but that affect cellular metabolism, as shown by increased MTT reduction, increased ATP level, and HIF1α stabilization. Sigma-2 agonists modulate the sphingolipid pathway, increasing ceramide and decreasing sphingomyelin levels. Since the ceramide pathway plays both pro-survival and pro-apoptotic roles, we investigated its role in the divergent sigma-2 receptor mediated effects in human SK-N-SH neuroblastoma cells. Fumonisin B1 (10 μM), a ceramide synthase inhibitor, significantly decreased CM764-induced MTT reduction but did not affect CM572-induced cytotoxicity. This indicates that de novo ceramide synthesis may play a role in the metabolic stimulative effect. NVP 231 (1 μM), a ceramide kinase inhibitor, had no effect on either CM572-induced cell death or CM764-induced MTT reduction, indicating that ceramide-1-phosphate may not be involved. Exogenously added C6-ceramide induced dose-dependent cell death in SK-N-SH cells. However, these cells were markedly less sensitive to the pro-apoptotic effects of C6-ceramide compared to effects in other cancer cell types. C6-Ceramide induces mitochondrial depolarization. Treatment with CM572 (24 h) caused dose dependent mitochondrial depolarization, while CM764 caused less depolarization. C6-Ceramide-induced mitochondrial depolarization was only partial and more similar to that caused by CM764 as opposed to CM572, having a significant effect only at the highest concentration. This may be consistent with ceramide involvement in the metabolic stimulative effect and the relative resistance of SK-N-SH cells to the cytotoxic effects of exogenous C6-ceramide. In light of the observation that combination of C6-ceramide with cytotoxic chemotherapy agents causes additive or synergistic cancer cell death in other cancer cell types, we examined the effect of combining C6-ceramide with sigma-2 agonists. Treatment of cells with C6-ceramide (20 μM, producing ~25% cell death alone) caused an additive decrease in cell viability caused by CM572, SV119, and siramesine.

There was a synergistic increase in cytotoxicity with low dose treatments of CM572 and siramesine (3 μM) in combination with C6 ceramide. Taken together, the results suggest that ceramide may play a dual role in sigma-2 signaling, mediating metabolic effects at low concentrations and apoptosis at higher concentrations.

**Disclosures: C.Z. Liu:** None. **W.D. Bowen:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.17/WW24

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Zhejiang Provincial Scientific Project of Medicine and Health 2017KY705

Zhejiang Provincial Scientific Project of Medicine and Health 2016KYA186

Taizhou Science and Technology Plan 162yw01

Taizhou Science and Technology Plan 15yw01

**Title:** Serum endocan levels are associated with large-artery atherosclerotic stroke

**Authors: *X.-P. JIN**, X.-W. HE, S.-F. KE, W.-J. HONG, Y.-Y. BAO, Y.-G. SHEN, F. ZHU, E. WANG
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**Abstract:** Background: Accumulating evidence has suggested that endocan may play important roles in cardiovascular disease. However, no previous study has focused on its circulating levels in patients with large-artery atherosclerotic (LAA) stroke.
Methods: Serum levels of endocan in 114 patients with LAA stroke and 114 age- and gender-matched controls were measured by ELISA. Serum samples from patients were available on days 1 and 6 and in the 4th week after IS. Stroke severity was determined based on the National Institutes of Health Stroke Scale and the stroke volume. An unfavourable outcome was defined as a modified Rankin Scale score > 2 on day 90 after IS.
Results: The serum levels of endocan were significantly higher in patients with LAA stroke compared with the controls ($p = 0.001$), and after adjustment for other factors (AOR 1.553, 95 % CI 1.184-2.038, $p = 0.001$). In addition, higher endocan levels were independently associated with unfavourable outcomes on both day 1 and day 6 after IS (AOR 1.903, 95 % CI 1.115-3.248, $p = 0.018$ and AOR 2.031, 95 % CI 1.179-3.500, $p = 0.011$, respectively).
Conclusions: This study is the first to show that endocan levels are higher in patients with LAA stroke and can help in predicting the short-term unfavourable outcome.

**Disclosures:** **X. Jin:** None. **X. He:** None. **S. Ke:** None. **W. Hong:** None. **Y. Bao:** None. **Y. Shen:** None. **F. Zhu:** None. **E. Wang:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.18/WW25

**Topic:** I.05. Biomarker and Drug Discovery

**Title:** Characterization of fast-spiking interneurons differentiated from human induced pluripotent stem cells

**Authors: *S. SUYAMA**[1], K. TAKASU[2], Y. ISHIDA[1], K. TAKAHASHI[1], N. SUZUKI[1], M. HASEGAWA[2], K. OGAWA[2]
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**Abstract:** Gamma oscillations are high-frequency rhythmic activities in the brain. Gamma rhythms are observed with higher cognitive functions and are disrupted in many disorders such as Alzheimer's disease (AD), schizophrenia, autism and epilepsy. Therefore, elucidating mechanisms of gamma oscillations will lead to development of new medication for cognitive disorders. However, there are few methods to study gamma oscillations in human *in vitro*. Here we developed a new *in vitro* tool for examining gamma oscillation using human induced pluripotent stem cell (hiPSC).
Gamma oscillations are driven by fast-spiking parvalbumin positive GABAergic interneurons in mammals from rodent to human. Deficiencies of fast-spiking GABAergic interneurons are also observed in a variety of disease model mice including AD model mice. In the previous study, we reported that fast-spiking interneurons in AD model mice (PSAPP transgenic mice) were dysfunctional and characterized *in vitro* phenotypes of fast-spiking GABAergic interneurons in hippocampus slices from normal and PSAPP AD model mice. GABAergic interneurons in these slices showed kainate dependent fast-spiking activities. In AD model mice, they showed lower action potential (AP) frequency and amplitude and were reversed by a histone deacetylase inhibitor (SAHA).
Here, we generated forebrain GABAergic interneurons from hiPSCs which possessed similar phenotypes to mice fast-spiking interneurons. HiPSC-derived GABAergic interneurons showed kainate dependent fast-spiking activities which were inhibited by CNQX, a kainate receptor antagonist. These kainate receptor mediated fast-spiking activities were disrupted by Aβ42 peptides, and the disruptions induced by Aβ42 peptides were ameliorated by SAHA. These data indicated that hiPSC-derived fast-spiking GABAergic interneurons would be useful *in vitro* tools for examining pathophysiology of gamma oscillation and drug discovery.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

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**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NIH Grant AG017586

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Arking Family Foundation

**Title:** Multimodal MRI-based classification of the primary progressive aphasias using eigenanatomy

**Authors:** ***C. A. OLM**[1], L. H. ZHAO[2], P. A. COOK[1], C. T. MCMILLAN[3], J. C. GEE[1], M. GROSSMAN[3]
[1]Dept. of Radiology, [2]Dept. of Statistics, [3]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Introduction: Primary progressive aphasia (PPA) is a class of neurological disorders characterized by progressive speech and language deficits, and are associated with different pathologies. As treatment trials are developed, finding reliable methods of distinguishing pathologies is becoming increasingly important. Machine learning methods help reduce the massive dimensionality of structural MRI data and have been shown to capture meaningful variance related to cognitive decline in neurodegenerative disease. We hypothesize that data driven regions of interest (ddROIs) of both gray matter (GM) and white matter (WM) are informative for classification of PPA variants.
Methods: 67 individuals with a PPA diagnosis (N=23, logopenic variant (lvPPA); N=20, nonfluect/agrammatic (naPPA); N=24, semantic (svPPA)) underwent T1-weigthed and diffusion-weighted MRI, which were used to generate cortical thickness (CT) images and fractional anisotropy (FA) images, respectively. To reduce data dimensionality, eigenanatomy

was used to find ddROIS consisting of regions of homogeneous variance, somewhat like principle component analysis, however with sparsity, non-negativity, and spatial constraints imposed. Eigenanatomy was performed on CT images to determine GM regions and FA images for WM regions. All GM and WM ddROIs were entered as predictors in a cross-validated multinomial logistic regression, wherein LASSO was used to identify the subset of ddROIs that contributed maximally to classifying participants. Each PPA phenotype was classified relative to the other two groups combined.
Results: Two ddROIs include anterior, medial, and inferior temporal cortex, with the left ddROI also extending to anterior insula, and these are reduced in svPPA. Also, a WM region consisting of portions of bilateral superior longitudinal fasciculus (SLF) is reduced in both naPPA and lvPPA relative to svPPA. naPPA displayed reduced GM relative to the other PPAs in a region encompassing much of the right frontal cortex, including the inferior frontal regions. FA in left parietal areas extending to include bilateral WM adjacent to fusiform cortex is reduced in lvPPA. Using only these 5 ddROIs, overall accuracy of 79.1% was achieved in 3-way classification.
Conclusions: ddROIs map well onto distributions of disease associated with each the PPAs: anterior-medial temporal cortex in svPPA, inferior frontal cortex in naPPA, parietal WM in lvPPA, and SLF with naPPA and lvPPA. Eigenanotomy generates ddROIs that reduce data dimensionality, are representative of different disease phenotypes, and can be used to achieve reasonable classification accuracy.

**Disclosures: C.A. Olm:** None. **L.H. Zhao:** None. **P.A. Cook:** None. **C.T. McMillan:** None. **J.C. Gee:** None. **M. Grossman:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.20/WW27

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** JSPS KAKENHI JP16H03288

JSPS KAKENHI JP25350974

JSPS KAKENHI JP16K14704

JSPS KAKENHI JP16K01909

**Title:** Screening of amyloid-β aggregation inhibitors from aromatic low molecular compounds by a microliter-scale high-throughput screening system with quantam-dot nanoprobes

**Authors: *Y. ANDO**[1], K. OTA[2], I. ITO[3], H. KIKUCHI[3], Y. OSHIMA[3], Y. ENDO[2], K. UWAI[1], K. TOKURAKU[1]

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**Abstract:** Alzheimer's disease (AD), a severe neurodegenerative disorder, accounts more than half of dementia cases. Currently, the amyloid cascade hypothesis, in which the accumulation of Aβ in the brain is the primary influence driving AD pathogenesis, is widely accepted as the molecular pathology of AD. On the basis of the hypothesis, inhibitor for Aβ aggregation may become key compounds for AD prevention and treatment. Recently, we established a microliter-scale high-throughput screening (MSHTS) system for Aβ aggregation inhibitors using quantum dot (QD) nanoprobes (Ishigaki *et al*., PLOS ONE 8, e72992, 2013). This novel system can be estimated the inhibitory activity as a half-maximal effective concentration ($EC_{50}$) at a small scale (5 μL) and high-throughput (1536-well). In this study, we screened 137 aromatic low-molecular compounds using this MSHTS system, and revealed that 15 compounds showed inhibitory activity lower than 200 μM $EC_{50}$. Interestingly, some compounds containing tropolone structure showed particularly high activity among them. The top three samples with the highest activity, TR-003 ($EC_{50}$: 6.6 ± 5.5 μM), TR-007 ($EC_{50}$: 14.9 ± 6.0 μM), and MO-009 ($EC_{50}$: 19.1 ± 5.6 μM), were higher than that of rosmarinic acid ($EC_{50}$: 20~25 μM) that is well known as an Aβ aggregation inhibitor. The results of evaluation also showed that the difference of the substituent groups strongly influence the inhibitory activities. The inhibitory activities of the active compounds containg tropolone strucutre were also confirmed by ThT assay although there are some differences between the $EC_{50}$ values determined by the MSHTS system and ThT assay. These results demonstrated that the MSHTS system is a powerful tool for screening a large compound library.

**Disclosures: Y. Ando:** None. **K. Ota:** None. **I. Ito:** None. **H. Kikuchi:** None. **Y. Oshima:** None. **Y. Endo:** None. **K. Uwai:** None. **K. Tokuraku:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.21/WW28

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** AG042178

AG47812

**Title:** Identification of novel circulatory microRNA signatures linked to patients with stroke

**Authors: *M. VIJAYAN**, S. KUMAR, P. REDDY
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**Abstract:** The purpose of our study was to determine circulatory microRNAs (miRNAs) as early detectable peripheral biomarkers in patients with stroke. To achieve our objective, we measured expression levels of miRNAs in stroke serum samples (n=34; age: 43-86 years) and healthy controls (n=11; 51-80 years). We used Illumina deep sequencing (Illumina GAIIx, ACGT101-miR v4.2, LC Sciences) analysis. Expression of selected potential candidate and unique miRNAs were further validated by SYBR green based quantitative real-time PCR assay (qRT-PCR). Target prediction of selected miRNAs was done using Targetscan and the potential targets of miRNAs were collected for GO/Pathway analysis using the DAVID functional annotation database. A total of 484,651,777 raw RNA reads were obtained in our deep sequencing analysis. Seventy percent (341,678,616) of reads were mapped for miRNAs. A total of 4,656 differentially expressed miRNAs were found in serum samples from stroke patients relative to controls. 272 miRNAs were found to be significantly differentially expressed (173 upregulated and 76 down regulated) in stroke patients. 17 most significantly deregulated miRNAs were selected for further validation. Interestingly, several previously unidentified miRNA candidates were found to be significantly differentially expressed in stroke patients relative to healthy controls. Moreover, target genes prediction of the co-expressed miRNAs and further Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses revealed that some candidate miRNAs were involved in the regulation of the stroke event. MiRNAs identified in the present study could potentially be used for the development of novel therapeutic approaches for stroke. Further studies are necessary to better understand miRNAs-regulated stroke event.

**Disclosures: M. Vijayan:** None. **S. Kumar:** None. **P. Reddy:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.22/WW29

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Ohio Third Frontier

**Title:** MRI detects the effects of Cuprizone/Rapamycin-mediated chronic demyelination and remyelination in rodent brain

**Authors: *H. BATTAPADY**[1,2], S. JOHNSON[1], L. LOOSE[1], S. LUNN[1], V. SHENOY[1], J. CHEN[2], B. TRAPP[2]
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**Abstract:** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). MRI is extensively used for non-invasive diagnosis and monitoring progression of MS pathology in the CNS. The cuprizone-rapamycin (CR) mouse model of MS

demonstrates extensive demyelination in both white (WM) and gray matter (GM) with axonal injury; however studies looking at regional effects of chronic demyelination and remyelination in the in-vivo mouse brain are limited. In this longitudinal MRI study, using CR mouse model of reversible demyelination, we demonstrate that MRI can detect the loss and restoration of myelin in WM: corpus callosum (CC), GM: cortex, hippocampus, and deep GM structures: caudate putamen and thalamus.

Eight-week-old C57Bl/6J mice were divided into 2 groups: Controls (AM-WT) and chronically demyelinated mice treated with 12 weeks (w) of CR. 3D T2-weighted structural MRI and 3D MT-weighted MRI were acquired on a 7T Bruker system, at 12+0w, 12+3w and 12+6w of spontaneous remyelination to segment desired regions of interest and quantify myelin loss sensitive Magnetization Transfer Ratio (MTR) respectively. Immunohistochemistry was performed to quantify the density of myelin (PLP), myelinated axons (PPD), activated astrocytes (GFAP) and axonal ovoids (SMI32). All image processing was performed in-house using automated pipelines developed using AFNI, FSL and ImageJ tools.

In the WM, CC MTR significantly decreased after 12w of chronic demyelination compared to controls ($p<0.001$); after 3w and 6w of spontaneous remyelination, CC MTR significantly increased compared to the 12+0 CR mice ($p<0.01$), but was not completely restored to control levels ($p<0.001$). This data was corroborated by decreased density of myelinated axons and increased density of activated astrocytes and axonal ovoids after chronic demyelination suggesting myelin loss and axonal injury; after 6w of spontaneous remyelination, these parameters restored towards control levels. MTR in GM and deep GM regions also showed similar statistically significant results as WM suggesting that these metrics and regions may be used to study disability and cognitive impairment in this model. These myelin loss sensitive MTR-histology results are consistent with demyelination and remyelination pattern that is typically observed in these mice.

Our results show that MRI is sensitive enough to detect the regional effects of CR-mediated chronic demyelination and remyelination in the mouse brain. Moreover, these data suggest that MRI metrics can be used in preclinical studies with therapeutic interventions that aim to aid neuroprotection and remyelination in MS patients.

**Disclosures:** **H. Battapady:** A. Employment/Salary (full or part-time):; Employee of Renovo Neural Inc., Renovo Neural Inc. **S. Johnson:** A. Employment/Salary (full or part-time):; Employee of Renovo Neural Inc., Renovo Neural Inc. **L. Loose:** A. Employment/Salary (full or part-time):; Employee of Renovo Neural Inc., Renovo Neural Inc. **S. Lunn:** A. Employment/Salary (full or part-time):; Employee of Renovo Neural Inc., Renovo Neural Inc. **V. Shenoy:** A. Employment/Salary (full or part-time):; Employee of Renovo Neural Inc., Renovo Neural Inc.. **J. Chen:** None. **B. Trapp:** A. Employment/Salary (full or part-time):; Cleveland Clinic. F. Consulting Fees (e.g., advisory boards); Renovo, Biogen, Novartis.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.23/WW30

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** NIH R01 NS644912-1A1

RC2 NS69476-01

NIH NRSAF31NS058224

**Title:** Utilizing skin derived astrocytes to screen for effective therapeutic treatments for ALS patients with C9ORF72 mutations

**Authors: *C. N. DENNYS**[1], L. FERRAIUOLO[3], *C. N. DENNYS[4], D. MOTTI[2], S. LIKHITE[2], C. MIRANDA[2], K. MEYER[2], B. KASPAR[2]
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**Abstract:** Amyotrophic Lateral Sclerosis (ALS) is a severe adult onset neurodegenerative disorder leading to progressive paralysis and death within 2-5 years. Patients diagnosed with ALS either have a genetic mutation (familial, fALS) or the disease develops due to an unknown origin (sporadic, sALS). While the most prominent feature of the disease is death of motor neurons, oligodendrocytes and astrocytes were shown to play a crucial role in disease progression in mouse models. However, tissue from the central nervous system of human patients can only be collected after death, complicating the study of these cell types in disease progression. The new and fast reprogramming method we developed allows generating both cell types from skin biopsies of patients. We have previously shown that induced astrocytes (iAs) and oligodendrocytes from ALS patients are toxic to motor neurons. Knockdown of SOD1 prevented motor neuron toxicity in most familial and sporadic lines, with C9ORF72 mutations being the exception. Following this observation we have begun screening potential therapeutics for patients with mutations in C9ORF72. iAs were treated with multiple compounds and their effects on motor neuron survival was quantified. We are currently working with a molecule that shows high promise for this specific type of mutation. In other familial and sporadic patient iAs, the effectiveness of the same molecule appeared to be patient specific. The specificity of responses revealed patient subpopulations that may benefit from a more tailored therapeutic approach. Importantly, treatments that worked on a subset of ALS patient cells, were ineffective in others. These observations may explain why translation of therapies to clinical trial has a high failure rate in ALS. Our model system thus proves useful as a clinical screening tool to subclassify

patient populations as responders and nonresponders to a certain therapeutic approach. This underlines the power of the new method for screening of new therapeutics as well as in comparing large patient groups to identify and select adequate subpopulations for future clinical trials.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.24/WW31

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** VA Merit

**Title:** Validation of hnRNP A1 as a novel biomarker in an early stage of Experimental Autoimmune Encephalomyelitis

**Authors: *S. LEE**[1,2], Y. SHIN[1,2], M. C. LEVIN[3,2]
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**Abstract:** The heterogeneous nuclear ribonucleoprotein (hnRNP) A1, which is expressed predominantly in the central nervous system, is involved in RNA metabolism. Dysregulation of hnRNP A1 is implicated in various neurodegenerative diseases, including multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) is a well-known mouse model for human MS disease. The EAE model enabled to discover the disease mechanism and drug discovery for MS. Also, EAE mice can mimic the chronic and relapsing-remitting form of human MS.
Proteases and their inhibitors are involved in the development and progression of MS. Proteolysis plays a direct role in immune cell vasodilation, myelinolysis and damage to oligodendrocytes (OLG) and axons. Limited proteolytic events also play an important role in the regulation of the activity and expression of cytokines, chemokines, and their receptors. Therefore, proteolytic events are essential initial mediators in the development and progression of MS and EAE. In the EAE model, protease activation leads to angiogenesis that is followed by T cell infiltration event. Damaged vascular cells release exosomes containing cleaved hnRNP A1 to biological fluids such as plasma, and thus the cleaved hnRNP A1 can be used as a new biomarker in the early stage of EAE.
In this study, we report here for the first time, the stage specific (pre-onset, onset, peak and degradation) cleaved hnRNP A1 levels and patterns in plasma exosomes of the EAE mouse

model. We found that cleaved hnRNP A1 from the plasma exosome of EAE mice was found much earlier (day 5 post immunization) than disease onset (day 12). We also evaluated the drug response of FTY720 to hnRNP A1 cleavage. Identification of disease stage-specific cleaved hnRNP A1 in plasma exosomes can distinguish between prognosis and clinical subtypes, and helps to determine treatment and its effects.

**Disclosures: S. Lee:** None. **Y. Shin:** None. **M.C. Levin:** None.

**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.25/WW32

**Topic:** I.05. Biomarker and Drug Discovery

**Support:** Swedish Medical Research Council

Erling-Persson Family Foundation

Swedish Parkinson Foundation

Swedish Parkinson's Disease Association

**Title:** Alpha synuclein-related microRNAs as biomarkers for Parkinson's disease

**Authors: *S. KHOO**[1], R. WRIGHT[4], B. HISKES[5], R. MITCHELL[2], B. ARMISTEAD[2], E. HAHS[2], L. FORSGREN[6], S. OTIENO[3], D. PETILLO[4]
[2]Cell & Mol. Biol., [1]Grand Valley State Univ., Grand Rapids, MI; [3]Statistics, Grand Valley State Univ., Allendale, MI; [4]Mol. Diagnostics, Ferris State Univ., Grand Rapids, MI; [5]Carleton Col., Northfield, MN; [6]Pharmacol. and Clin. Neurosci., Umea Univ., Umea, Sweden

**Abstract:** Parkinson's disease (PD) is a complex and heterogeneous neurodegenerative disorder. It is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra and the intraneural accumulation of alpha synuclein protein. The current diagnosis of PD relies primarily on the presence of its motor symptoms. Consequently, by the time PD is diagnosed, 50-80% of the patient's dopaminergic neurons have already been lost or damaged. Thus, there is a need to develop measurable and unbiased biomarkers that can be used for early detection of PD. MicroRNAs (miRNAs) are small RNAs that regulate gene expression post-transcriptionally by binding to 3′- or 5′-untranslated regions (UTRs) of specific messenger RNAs (mRNAs). miRNAs are tissue-specific, stable, quantifiable, and easily isolated, making them ideal candidates for biomarker development. MiRNA miR-34b and miR-34c have been shown to bind at the 3′-UTR of alpha synuclein mRNA and repress alpha synuclein protein expression. They were also shown to be down-regulated in brains of PD patients. miR-7 and miR-153 also bind to

3′-UTR of alpha synuclein and down-regulate its mRNA and protein expression. Here, we evaluate miR-34b/c, miR-7, and miR-153 as potential diagnostic biomarkers for PD. 30 Patients with PD (10 newly-diagnosed and 20 advanced PD) and 8 healthy controls were recruited from the Department of Pharmacology and Clinical Neuroscience at Umeå University in Sweden. Total RNA, including miRNAs, of EDTA-treated plasma supernatant was isolated using a Qiagen miRNeasy Serum/Plasma Kit. miRNA expression of biomarker candidates was evaluated using the Taqman miRNA-specific assay. qRT-PCR was then performed in an Agilent MX3000P QPCR system. miR-7 and miR-153 did not show statistical significance in miRNA expression between PD and healthy controls. Expression of miR-34b/c was significantly lower in advanced PD when compared with newly diagnosed PD or healthy controls. Thus, miR-34b/c may be used as potential biomarkers to diagnose and track PD progression.

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**Poster**

**625. Novel Approaches in Neurodegeneration and Stroke**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 625.26/WW33

**Topic:** C.06. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** DARPA/BTO/HAPTIX N66001-15-C-4018

**Title:** Three dimensional device-capture histological assessment of hd-time nerve-machine interface

**Authors: *S. W. CURRLIN**[1], A. KUNDU[2], F. DELGADO[3], E. PATRICK[2], N. MAGHARI[3], R. BASHIRULLAH[3], K. J. OTTO[4]
[1]Neurosci., [2]Electrical Engin., [4]Biomed. Engin., [3]Univ. of Florida, Gainesville, FL

**Abstract:** The Implantable Multimodal Peripheral Recording and Stimulation System (IMPRESS) project aims to develop a high-density stimulating and recording microelectrode system for peripheral nerve implantation. Each year nearly 200,000 amputations occur in the United States caused mainly from vascular disease, trauma, or cancer. The loss of an upper extremity has profound psychological and physiological effects upon daily life. Prosthetic devices, such as robotic limbs, can interface with humans via electrodes implanted within remaining muscle or nerves, and in rare cases the brain to improve quality of life.
Sensory feedback enables finer control of robotic neuroprosthetic limbs. Stimulation of peripheral nervous tissue in amputees enables recapitulation of somatic perceptions such as intensity, texture, and velocity to improve cognitive control of prosthetics. Consolidating signal acquisition and stimulation within an implantable device is critical to achieve closed-loop,

naturalized control of robotic limbs. IMPRESS incorporates a next generation *hd*-TIME, an active CMOS probe embedded within a flexible polyimide substrate, for insertion into human nerve fascicles. The *high-density* transverse intrafascicular multichannel electrode (*hd*-TIME) aims to improve spatial selectivity within implanted peripheral nerves.
Implantation of peripheral microelectrodes generates an immunological foreign body response (FBR). Within the central nervous system, a chronic FBR to implanted electrodes leads to reduced or lost signal acquisition. Within the peripheral nervous system (PNS), a chronic FBR to the hd-TIME also threatens to degrade device functionality. New technologies and techniques have greatly improved our ability to perform device capture histology thus preserving the critical data at the nerve-machine interface (NMI). The ability to associate electrode functionality with specific tissue-interface sites is a critical step towards understanding and combating the FBR. Following chronic (4-week) implantation, we excised the rat sciatic nerve with mock hd-TIME to preserve the NMI between electrode sites and nerve. CLARITY techniques produced cleared nerves while maintaining device position. Light sheet fluorescence microscopy (LSFM) captured three-dimensional device-capture histological data for markers of axon fiber types, immune cells, Schwann cells, and nodes of Ranvier. These markers were quantified for each hd-TIME electrode site and for the entire implanted portion of the probe.

**Disclosures: S.W. Currlin:** None. **A. Kundu:** None. **F. Delgado:** None. **E. Patrick:** None. **N. Maghari:** None. **R. Bashirullah:** None. **K.J. Otto:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.01/WW34

**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH Grant R21NS093727

**Title:** Automatic surface electromyogram decomposition based on progressive fastica peel-off

**Authors: M. CHEN**[1,2], X. ZHANG[1], *P. ZHOU[3,2]
[1]Univ. of Sci. and Technol. of China, Hefei, China; [2]Guangdong Work Injury Rehabil. Ctr., GuangZhou, China; [3]PM&R, Univ. of Texas Hlth. Sci. Ctr. At Houst, Houston, TX

**Abstract:** Electromyogram (EMG) decomposition is the process of breaking down the multiunit EMG signal into the contributions of the underlying motor unit action potential trains (MUAPTs), which provides a powerful tool for investigation of neuromuscular control. This study presents automatic decomposition of high density surface EMG signals through a progressive FastICA peel-off (PFP) framework. By incorporating FastICA, constrained FastICA and a peel-off strategy, the PFP can progressively expand the set of motor unit spike trains

contributing to the EMG signal. A series of signal processing techniques were applied and integrated in this study to automatically implement the two tasks that often require human operator interaction during application of the PFP framework, including extraction of motor unit spike trains from FastICA outputs and reliability judgement of the extracted motor units. Based on these advances, an automatic PFP (APFP) framework was consequently developed. The decomposition performance of APFP was validated using simulated high density surface EMG signals with different motor unit numbers (30, 70, 91) and signal to noise ratios (SNRs) (20, 10, 0 dB). The results demonstrated relatively large numbers of extracted motor units and high accuracies (high F1-scores). The APFP was also evaluated with experimental surface EMG signals, and the decomposition results were comparable to those achieved from the PFP with human operator interaction. Among 111 trials of experimental surface EMG signals, the average extracted number of APFP and PFP is 13.6±5.4 and 14.1±5.0, respectively. Wherein, there are 9.8±4.7 common motor units were identified by both methods with a highly average matching rate as 97.57 ± 2.42%. The results show that the decomposition performance of APFP and PFP are highly consistent.

**Disclosures:** **M. Chen:** None. **X. Zhang:** None. **P. Zhou:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.02/WW35

**Topic:** I.07. Data Analysis and Statistics

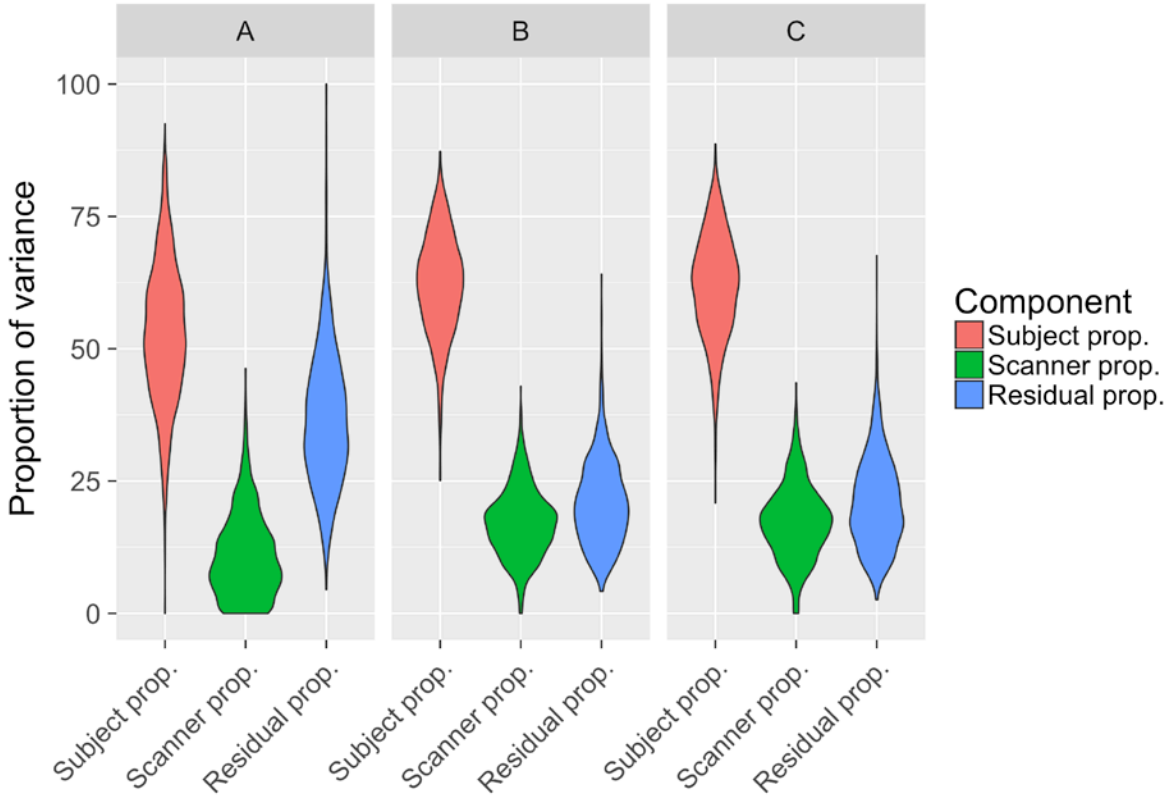
**Support:** NIMH Intramural Research Program

**Title:** Towards a framework for comparing functional magnetic resonance imaging data across scanners, vendors, and models

**Authors: *P. J. MOLFESE**[1], J. A. LEE[2], S. T. MARRETT[3], A. G. THOMAS[2], V. ROOPCHANSINGH[3], D. M. NIELSON[2], J. VARADA[3], A. DERBYSHIRE[3], P. A. BANDETTINI[1]
[1]Section on Functional Imaging Methods, [2]Data Sci. and Sharing Team, [3]Functional MRI Facility, NIMH/NIH, Bethesda, MD

**Abstract:** Neuroimaging studies are increasingly targeting diverse populations represented by different genetic, social, phenotypic, and geographical profiles (e.g. Rueckl et al., 2015), which are made more feasible through data collection at multiple sites using a mixture of equipment. These studies often involve both cognitive tasks (Gee et al., 2015; Brown et al., 2010, Yendiki et al., 2010) and/or resting state (Turner et al., 2013). Some recommendations from multi-site studies exist (FBIRN; Zou et al., 2005), and some portion of these methods have been

implemented in the recent Human Connectome Project (HCP; Van Essen et al., 2013). In this study, we collected fMRI data consisting of a rotating checkerboard stimulus on eleven participants, each imaged using four different fMRI machines with different scanner platforms (GE, Siemens), and corresponding multi-channel phased array whole-head “coils”. Data were collected using similar imaging parameters (e.g. slice number, FOV, flip angle) across all scanners. Data were analyzed in AFNI using a standard preprocessing pipeline. Comparisons of this standard pipeline to others with current “best practices” including distortion correction with blip-up/blip-down data, regression of physiological signals, smoothing to a set level of smoothing, and regressing events using ARMA were also performed (Figure 1, A). Group level analysis was conducted using a either a more traditional ANOVA (Figure 1, B), or a Linear Mixed Effects model (3dLME; Chen et al., 2013) with voxel-wise SFNR covariate (Figure 1, C). Results of these preprocessing and group level analyses are expressed in variance accounted for by the subject, scanner, and unexplained variance. The addition of each of these preprocessing and group level steps increased variance accounted for by subject, while decreasing the variance accounted for by scanner.




**Disclosures:** **P.J. Molfese:** None. **J.A. Lee:** None. **S.T. Marrett:** None. **A.G. Thomas:** None. **V. Roopchansingh:** None. **D.M. Nielson:** None. **J. Varada:** None. **A. Derbyshire:** None. **P.A. Bandettini:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.03/WW36

**Topic:** I.07. Data Analysis and Statistics

**Title:** Reevaluation of the form of nonlinear age-related changes in brain volume

**Authors: *D. NICHOLS**
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**Abstract:** It has previously been established that some aspects of the brain change volume at a fairly constant rate across the life span, i.e. linearly with respect to age, whereas other aspects of the brain change volume at different rates within different age ranges, i.e. nonlinear age-related changes. Theoretically relevant distinctions can be made between nonlinear age-related changes that switch direction, e.g. decades of size reduction followed by decades of growth, versus those that change amplitude, e.g. accelerated size reduction beginning later in life. Simulated data with known trajectories were used to compare locally-linear models of nonlinear age-related changes based on a small set of theoretically derived critical ages to smoothing spline estimations. Three particular categories of trajectories were explored - quadratic, with a change in direction across the life span; acceleration, with changes in amplitude but not direction; sigmoidal, with a period of change preceded and followed by no change in volume. Smoothing splines, which have previously been used to estimate the rate of change of individual aspects of the brain along the life span, were shown to lack the precision required to differentiate the onset of nonlinear changes less than one to two decades apart and exhibited consistent inaccuracies for non-polynomial trajectories. To demonstrate the utility of the locally-linear modeling approach, which also determines confidence intervals on the rates of change within regions of the life span, a reevaluation of published data [Fjell et al. (2013) Critical ages in the life course of the adult brain: Nonlinear subcortical aging. Neurobiol Aging 34:2239-47] was carried out for 17 aspects of MRI scans from 1100 subjects. Establishing the qualitative form of the trajectories and bounded quantitative estimates along the life span allowed for categorization of the nature of the nonlinear age-related changes for each aspect of the brain. This approach can be useful for determining distinctions between brain areas in the timing of developmental or degenerative events that influence their volume as well as making statistical comparisons between brain areas regarding their rates of change.

**Disclosures: D. Nichols:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.04/WW37

**Topic:** I.07. Data Analysis and Statistics

**Title:** Effects of dopamine depletion on signal variability and functional connectivity of resting state brain networks

**Authors: *G. SHAFIEI**, Y. ZEIGHAMI, A. DAGHER, B. MISIC
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**Abstract:** Dopaminergic projections are hypothesized to stabilize neural signaling and neural representations (Kroener et al., 2009, *PLoS One*), but their role in shaping local information processing and large-scale network interactions remains unclear. Here we investigate the effects of lowered dopamine levels on functional connectivity and signal variability of the healthy brain at rest. Acute phenylalanine and tyrosine depletion (APTD) technique was used to induce dopamine depletion in 51 healthy participants who underwent high-resolution resting-state functional MRI (fMRI). Functional data were parceled into 83 cortical and subcortical areas using the Desikan-Killiany atlas (Desikan et al., 2006, *Neuroimage*), and then further subdivided into 1015 approximately equally sized parcels (Cammoun et al., 2012, *J Neurosci Meth*). Functional connectivity was estimated as a Pearson correlation coefficient between regional time series. Sample entropy (SE) analysis was used to estimate regional signal variability by quantifying the similarity of any two sequences of data points in the fMRI time series (Richman and Moorman, 2000, *Am J Physiol Heart Circ Physiol* ). Multivariate partial least squares (PLS) analysis was used to statistically assess changes in signal variability before and after dopamine depletion (McIntosh and Lobaugh, 2004, *NeuroImage*). PLS results in a set of latent variables (LV), that are weighted combinations of experiment design (i.e. a contrast) and signal variability patterns that optimally covary with each other. We found one significant latent variable (permuted $p \approx 0$, accounting for almost 100% of covariance), capturing a pattern of increased signal variability following dopamine depletion. The pattern was spatially diffuse, but most pronounced in areas associated with the somatomotor and salience resting-state networks. Finally, changes in signal variability were concomitant with changes in functional connectivity, such that nodes with the greatest increase in signal variability following dopamine depletion also experienced the greatest decrease in functional connectivity. Our data suggest that dopamine may act to stabilize neural signaling, particularly in areas related to somatomotor function and orienting attention towards behaviorally-relevant stimuli. Moreover, dopamine-dependent signal variability is critically associated with the functional embedding of individual areas in large-scale networks.

**Disclosures:** **G. Shafiei:** None. **Y. Zeighami:** None. **A. Dagher:** None. **B. Misic:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.05/WW38

**Topic:** I.07. Data Analysis and Statistics

**Support:** from the Casa Colina Foundation

J. Yang & Family Foundation

**Title:** Prediction of functional outcome in tbi patients by machine learning strategy

**Authors: *H. GHASEMI DAMAVANDI**[1], M. KACHUEE[2], M. SUN[1], J. CHAMBERS[1], S. ROSENBERG[3], J. C. LEITER[1], M. SARRAFZADEH[2], E. R. ROSARIO[3], D. C. LU[1]
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**Abstract: Abstract:** The ability to accurately predict functional recovery upon hospital discharge and hospital length of stay (LOS) in patients with neurological injury such as traumatic brain injury (TBI), spinal cord injury (SCI) and stroke is important as it provides information that may guide clinical management, resource allocation, and patient guidance. This study presents a novel approach to predict functional improvement and LOS for patients admitted with TBI. A retrospective analysis of a database containing 409 patients with a history of TBI was provided by Casa Colina Hospital and Centers for Healthcare. We used Functional Independence Measure (FIM) scores as an index of each patients ability to complete daily activities. An advanced machine learning technique, Random Forests (RF), which uses an ensemble of learning algorithms to optimize data classification, was applied to the longitudinal inpatient data, which included features such as age, gender, rehabilitation impairment category, actual LOS and 19 subcategories of FIM scores. An RF classifier was trained to predict the LOS and the discharge FIM scores where the predicted LOS along with the features listed above were used to develop an RF regression model to predict the discharge FIM scores. The performance of the method was optimized to minimize the regression error between the predicted and actual values for both LOS and discharge FIM scores. Based on this training dataset, the RF was capable of predicting LOS with an error of less than a week and the discharge FIM scores of motor and cognitive tasks with a mean absolute error rate of 0.96 and 0.87, respectively. The regression model developed may be applied prospectively to TBI patients for further validation.
**Support:** This research was made possible by generous support from the Casa Colina Foundation and J. Yang & Family Foundation. D.C.L. is a 1999 Paul & Daisy Soros New

American Fellow.
**Keywords:** FIM Score, Length of Stay, Machine Learning

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Program#/Poster#:** 626.06/WW39

**Topic:** I.07. Data Analysis and Statistics

**Title:** Unresponsive states with and without report of conscious experience show distinct patterns of EEG-based effective brain connectivity in humans

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**Abstract:** Background: Quantifying effective connectivity using the Directed Transfer Function (DTF) on 1-second segments of raw clinical EEG has shown promising performance as a method for classifying conscious and unconscious states in patients undergoing anesthesia. Here, we test whether the DTF-based classification algorithm can be used as a general objective measure of consciousness, and compare its performance with a leading electrophysiological marker of consciousness - the perturbational complexity index (PCI).
Method: We reanalyzed data from an experiment in which healthy volunteers were randomly assigned to one of three forms of general anesthesia: propofol, xenon or ketamine. Spontaneous EEG was recorded from each participant before and during anesthesia, and DTF was calculated from each one-second segment of the EEG data to quantify the effective connectivity between channel pairs. From this, the state of the person was classified as either conscious or unconscious, using DTF from 1-second segments as input. The resulting classifications was compared with the subjects’ own report of experience and previously calculated PCI values from the same experiments.

Results: The DTF analysis yielded two distinct connectivity patterns, both at group and individual levels. This difference was sufficient to accurately classify the state as conscious or unconscious with 93% accuracy, compared to the participants' report of subjective experience. Specifically, the algorithm was more likely to conclude with consciousness in the awake state than during propofol and xenon anesthesia ($p<0.05$), but not during ketamine anesthesia ($p>0.05$). Finally, the DTF-based classification confidence correlates with PCI ($r^2=0.48$, $p<0.01$). Conclusion: The DTF based method seemed to be able to distinguish reliably between conscious and unconscious states in accordance with the individual's own report, and correlated well with PCI. These results provide further evidence that effective connectivity, here quantified by DTF, can be used to separate conscious from unconscious states based on spontaneous EEG activity.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH – 1R01EB022858-01 FAIN – R01EB022858

NIH – 1R01LM012087

NIH BD2K – 5U54HG008540-02 FAIN – U54HG008540

**Title:** Diagnosis of autism spectrum disorder by causal connectivity strength from resting state functional magnetic resonance imaging data

**Authors: *B. HUANG**, K. ZHANG, R. S. ROMERO, J. RAMSEY, M. GLYMOUR, C. GLYMOUR
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**Abstract:** Diagnosing Autism Spectrum Disorder (ASD) with brain signals has received much attention in neuroscience and clinic diagnosis. It has been postulated that ASD is underpinned by atypical brain connectivity. Most analyses for neuroimaging data use correlation coefficients as a measure of brain connectivity strength to distinguish between ASD and typical control (TC). However, correlation does not directly reveal causal influences among brain regions. To identify atypical causal connections in ASD, we propose a diagnostic approach that first recovers the causal structure among brain regions and then uses the causal connectivity strength for

classification. The proposed approach is able to recover the whole causal structure including feedbacks among brain regions. We demonstrate the efficacy of the proposed methods with resting state functional magnetic resonance imaging (R-fMRI) data from the Autism Brain Imaging Data Exchange. Particularly, the R-fMRI signal is extracted from thousands of voxels clustered into regions of interest by the Automated Anatomical Labeling atlas. We choose datasets which have relatively long time period and remove unqualified data, resulting in 30 ASD subjects and 48 TCs. We use the estimated causal strength of connections as features for diagnosis, and achieve 81% out-of-sample prediction accuracy with support vector classification (SVC), which significantly improves on previously reported results. To avoid overfitting, we do feature ranking to remove uninformative features before running SVC. In addition to the causal strength, we find that the time-varying property of certain causal connection strengths may further help distinguish between ASD and TC. More specifically, the causal coefficients of certain causal relations in ASD appear to change faster than those in TC. Figure 1 shows identified causal connections which are effective for diagnosis.

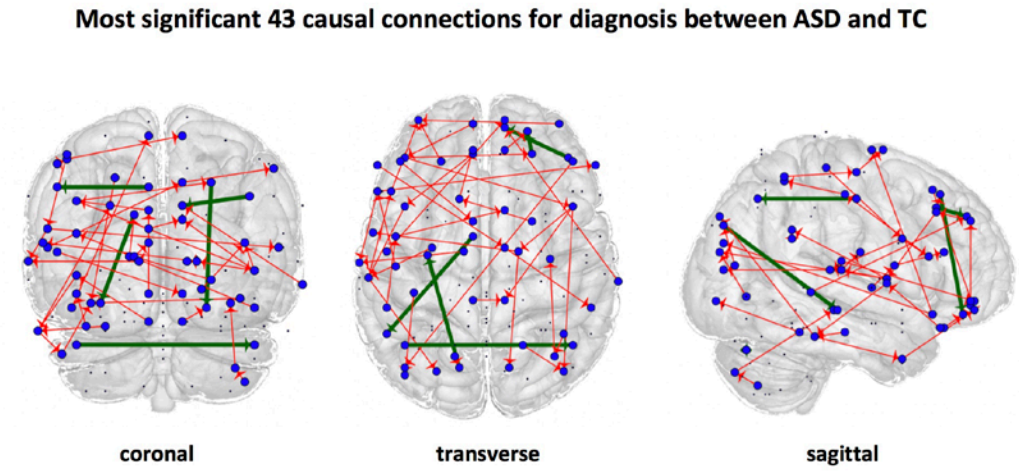


Figure 1. Coronal, transverse and sagittal views of the 43 causal connections that maximize out-of-sample prediction accuracy. Nodes represent centroids of 116 ROIs in AAL atlas. Radiological orientation. The 5 most relevant connections are in green: right midcingulate -> right angular gyrus; left superior frontal gyrus -> left superior orbital frontal gyrus; right crus 1 of cerebellum -> left crus 1 of cerebellum; left middle frontal gyrus -> left medial frontal superior gyrus; right cuneus -> right hippocampus.

**Disclosures:** **B. Huang:** A. Employment/Salary (full or part-time):; Carnegie Mellon University. **K. Zhang:** A. Employment/Salary (full or part-time):; Carnegie Mellon University. **R.S. Romero:** A. Employment/Salary (full or part-time):; Carnegie Mellon University. **J. Ramsey:** A. Employment/Salary (full or part-time):; Carnegie Mellon University. **M. Glymour:** A. Employment/Salary (full or part-time):; Carnegie Mellon University. **C. Glymour:** A. Employment/Salary (full or part-time):; Carnegie Mellon University.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

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**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH Grant 1R21NS096590-01A1

**Title:** Multi-band coherence motifs for assessing network dynamical abnormalities associated with focal injury

**Authors: *S. KHANMOHAMMADI**[1], O. LAURIDO-SOTO[3], L. N. EISENMAN[3], T. T. KUMMER[3], S. CHING[2]
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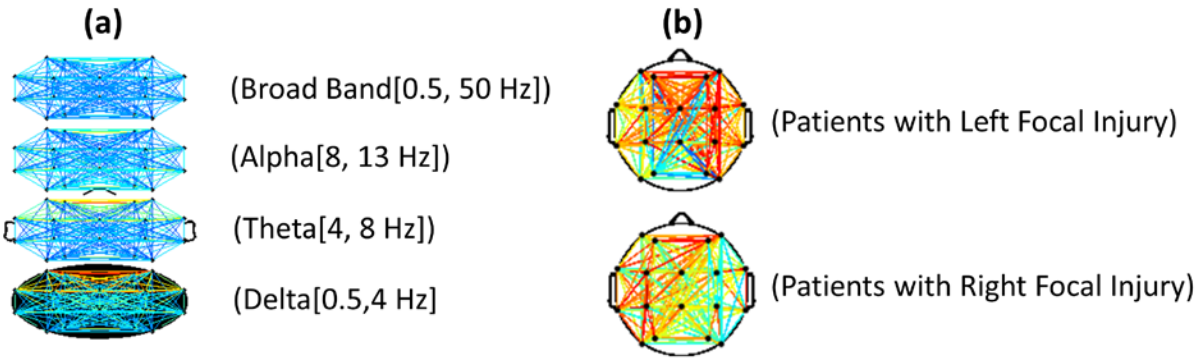
**Abstract:** Several studies suggest that a disruption of functional connectivity, i.e., statistical relationships between brain region activity, is associated with focal brain injury. However, most of these characterizations rely on a single metric, nominally the zero-lag correlation, upon which to quantify connectivity. Frequency-domain metrics such as the coherence similarly are usually limited to a particular frequency band of interest. Less emphasis has been placed on assessments of network temporal dynamics that may span multiple time-scales, thus obscuring potentially more complex network relationships. In this study, we examined time (cross-correlation) and frequency (multilayer coherence) domain dynamical abnormalities of EEG signals recorded from patients who suffered severe cerebral injury leading to coma, as well as from an uninjured control population. By aggregating both correlation and coherence matrices from several frequency bands into a single construct, we extracted multi-band motifs that characterize both the spatial location of their injury and the severity of their behavioral deficits as measured in terms of the clinical Glasgow Coma Score. We observed that the most severe behavioral deficits are associated with a pronounced decrease in slow frontal connectivity. Furthermore, our analysis reveals a lateralized, broadband increase in connectivity that is, interestingly, dominant in the hemisphere contralateral to the injury. These characterizations illustrate that focal brain injuries give rise to widespread, but systematic effects on brain network dynamics that may correlate with cognitive deficit and, in turn, enable new strategies for neuromonitoring and clinical assessment.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

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**Program#/Poster#:** 626.09/WW42

**Topic:** I.07. Data Analysis and Statistics

**Support:** Sloan Foundation

**Title:** Assessing approaches for estimating the electrophysiological 1/f background spectrum

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**Abstract:** Electrophysiological neural recordings have a characteristic 1/f shape in frequency space, in which the power exponentially decreases for higher frequency activity. In log-log space, this relationship is approximately linear, and can be measured by calculating the slope of the power spectral density (PSD). Measurements of this slope value, as a measure of the 1/f background, have been shown to vary. This variance can be cross-sectional—such as in aging—or within subjects across different states such as sleep wake cycles, and anesthetic conditions or even varying on a trial-by-trial basis with cognitive or perceptual state. This 1/f noise may also index disease states, including epilepsy and schizophrenia. Despite the burgeoning interest and potential applicability of examining the 1/f background, there is no clear consensus regarding the best approach for doing so. In particular, a key challenge in the characterizing 1/f dynamics is the co-existence of oscillatory 'bumps' of activity in the PSD. This oscillatory activity is not 1/f distributed, but does also vary with demographics, disease states, and behavior. It is therefore critical that methodological approaches are specific to measuring 1/f changes, and are not confounded by oscillatory changes. Here we evaluate different methodological approaches to estimating 1/f activity, using synthetic data with statistical properties mimicking neural power

spectra, in which ground truth is known. We evaluate a number of existing metrics in the literature, and also test novel approaches. Findings support that robust linear fitting procedures in log-log space, ignoring oscillatory regions, are accurate and reliable, however these approaches work best when using data-driven exclusion regions (instead of fixed ranges). Depending on the specific algorithm, and characteristics of the data, fixed-band exclusions can sometimes perform similarly, or slightly worse than, no exclusion band. Strengths and weaknesses of the various fitting procedures are discussed. We also apply these methods to existing electroencephalography (EEG) datasets of data collected from different age groups, replicating observations that 1/f as measured from the PSD of resting state data systematically varies with age. Applying best practice methods, as defined by the initial work with the synthetic data, is shown to more robustly demonstrate this relationship, using metrics that are also characterized as to their sensitivity to oscillatory confounds.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Program#/Poster#:** 626.10/WW43

**Topic:** I.07. Data Analysis and Statistics

**Title:** Eeg based computation of group-level dynamic causal cortical connectivity in humans performing motor and decision making tasks

**Authors: *H. COURELLIS**[1], J. R. IVERSEN[2], D. A. PETERSON[3], G. CAUWENBERGHS[1]
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**Abstract:** Dynamic causal intracortical interactions offer a wealth of information that can be used to develop an understanding of brain functionality and quantify interaction among brain regions during the performance of cognitive tasks. Traditional analysis and evaluation of cortical electrophysiology focuses on individual brain regions or simple interactions among them but does not address spectro-temporally dynamic causal interactions between such regions. Dynamic connectivity present between different regions of the cortex can be readily interrogated in humans using a non-invasive neuroimaging modality, the electroencephalogram (EEG). We quantified spectro-temporal causal cortical connectivity dynamics at the millisecond time scale by developing a new computational methodology for processing multichannel EEG recordings. Our methodology involved the use of a group-level source localization and connectivity analysis approach. Cortical regions of interest (ROIs) were identified by clustering Independent Components across subjects into a common brain volume using both spatial and functional-activity metrics. Current source density estimation was then conducted in the identified ROIs

using a cortical boundary element model, and employing Cortically Constrained Low Resolution Electromagnetic Tomography (cLORETA), assuming cortical dipole orientations normal to the surface of the cortex. A representative current signal from each ROI was then extracted using a maximum-power selection heuristic, and causal spectrotemporal connectivity between ROIs was quantified by fitting multivariate autoregressive models to the ROI signals and computing directed transfer function-based causality measures. We applied the developed computational methodology to elucidate the cortical dynamics associated with reaching and saccading to spatial targets (RSST) (using a cognitive task performed by young adults) and to characterize the process of reward-based decision making (RBDM) through the cortical projection of sub-cortical dopaminergic basal nuclei activity (using a cognitive task performed by older adults). Analysis of connectivity dynamics in the RSST group revealed task relevant fronto-parietal communication occurring over varying frequency bands during both planning and execution of reaches and saccades. Analysis of connectivity dynamics in the RBDM group revealed that regions of the frontal cortex receiving direct input from dopaminergic subcortex, namely the Anterior Cingulate, drive activity in motor regions during decision making associated with steady-state image valuation, that is, after reward based learning has occurred.

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**Poster**

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**Topic:** I.07. Data Analysis and Statistics

**Support:** Moore Foundation

Sloan Foundation

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Netherlands Organization for Scientific Research: 016_VENI_178_048

**Title:** BIDS-iEEG: A data structure for intracranial electrophysiology that facilitates open data and integration with other human imaging methods

**Authors: *C. HOLDGRAF**[1], *C. HOLDGRAF[1], K. E. BOUCHARD[2], O. DEVINSKY[3], A. FLINKER[4], D. M. GROPPE[5], A. GUNDUZ[6], L. S. HAMILTON[7], C. J. HONEY[9], K. J. GORGOLEWSKI[10], R. T. KNIGHT[14], B. L. FOSTER[15], K. J. MILLER[16], M. PERRY[11], N. PETRIDOU[17], N. F. RAMSEY[18], G. SCHAEFER[11], N. C. SWANN[8], B. VOYTEK[19], B. A. WANDELL[12], J. WINAWER[4], D. HERMES[13]

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**Abstract:** Neuroscience is experiencing an exponential growth in the ability to collect larger, more complex datasets from the human brain. Increasingly data from a single brain includes measurements made with multiple modalities, spanning functional and structural MRI data as well as electrophysiology measurements. To support reproducibility, many of these datasets are being openly shared with collaborators and the broader scientific community. These rapid developments come with new requirements for effectively managing data workflows, as well as a new set of best-practices in curating and storing data. For MRI data, a community driven effort resulted in the Brain Imaging Data Structure (BIDS), a framework for organizing data and metadata to satisfy these needs. This has now been adopted by many labs around the globe. The BIDS framework invites community involvement in shaping the specifications such that they are broadly useful and applicable. Intracranial electroencephalography (iEEG) data always have a multi-modal aspect, as data are integrated with at least a structural MRI to estimate electrode positions. While there are currently several data formats that store one iEEG dataset (including event or task information), there is no common data organization structure that allows for multi-modal integration following best-practices in data-heavy workflows. Here we propose an extension of the BIDS format to iEEG data. Our proposal is a starting point intended to spark a public discussion to adopt community standards in storing data recorded from iEEG patients, with a focus on being easy to understand and incorporate into many workflows. The BIDS format allows for the integration of iEEG with functional and structural MRI measurements in 3D or surface space and, as such, provides a standard operating procedure for storing complex multi-modal data from human subjects. BIDS also provides a common ground for data transfer within labs, between labs, and in open-data repositories. Adopting a standard will enable more complex, reproducible analyses that cut across datasets from different experiments, sites, and analysis workflows, allowing scientists to cooperate and check one another's work as they study the human brain. A link for public comment on the BIDS-iEEG specification can be found at `bit.ly/bids-ieeg-draft`.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.12/WW45

**Topic:** I.07. Data Analysis and Statistics

**Support:** NSF Grant EFRI-MC3: # 1137237

**Title:** "Off-course" and emotional: The role of cognitive and limbic circuits during movements in humans

**Authors: *M. S. BREAULT**[1], P. SACRÉ[2], M. S. KERR[3], M. D. JOHNSON[4], J. BULACIO[5], J. GONZALEZ-MARTINEZ[5], J. T. GALE[6], S. V. SARMA[1]
[1]Biomed. Engin., [2]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [3]Dept. of Engin., Univ. of Cambridge, Cambridge, United Kingdom; [4]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN; [5]Epilepsy Ctr., Cleveland Clin., Cleveland, OH; [6]Dept. of Neurosurg., Emory Univ., Atlanta, GA

**Abstract:** Nonmotor brain regions in humans are rarely studied during motor control due to their secondary, nontrivial, or even nonexistent role. Furthermore, capturing neural data from these regions can be cumbersome during movement, especially if motor regions are being probed simultaneously. However, it is important to understand what nonmotor regions encode for during movements to not only treat nonmotor neural diseases that impact movement but also to design brain-computer interfaces (BCIs) that rely solely on recordings from some of these areas. Such BCIs may provide the opportunity for patients with damaged motor cortex to regain motor control. We exploited a rare opportunity to record local field potential (LFP) activity from 680 contacts covering over 70 nonmotor structures in 9 human subjects as they executed a goal-directed reaching task using a robotic manipulandum. These subjects are medically refractory epileptic patients implanted with multiple depth electrodes for clinical purposes using the stereoelectroencephalography (SEEG) technique, which provides both high spatial and temporal resolution data. The question we set out to answer is whether or not nonmotor regions encode any path related information. To explore our relatively large data set, we developed a method that both quickly scans over all contacts, trials, and subjects and accurately extracts meaningful neural correlates of behavior. First, a behavioral signal was derived for each trial that captures modulating properties of path trajectory. That is, this signal increases or decreases when the subject's path deviates or is "off-course" from the target. Neural data was then transformed into spectrograms (time by frequency matrices) that were summarized as one-dimensional signals using singular value decomposition. These neural signals captured time periods where the spectrogram significantly modulated during trials for any frequency. Finally, the behavioral and neural signals during movement execution were cross-correlated for every trial and contact. The

top contacts with a significant number of trials with high correlations between brain and behavior signals were extracted. Preliminary results show that limbic and visual cortical areas were most highly correlated with path modulation. This suggests that when subjects move “off-course”, they see the deviation and then may emotionally react to it. Our findings provide early evidence of the important role that nonmotor regions have during motor control, suggesting that these regions should no longer be overlooked during sensorimotor control.

**Disclosures:** **M.S. Breault:** None. **P. Sacré:** None. **M.S. Kerr:** None. **M.D. Johnson:** None. **J. Bulacio:** None. **J. Gonzalez-Martinez:** None. **J.T. Gale:** None. **S.V. Sarma:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Topic:** I.07. Data Analysis and Statistics

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Center for Brain Activty Mapping

Qualcomm Institue Calit2 Strategic Research Oppurtunities

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ONR N00014-13-1-0672

**Title:** Spatial filtering of high density human electrocorticography (ECoG)

**Authors:** ***J. HERMIZ**[1], N. ROGERS[1], E. KAESTNER[1], M. GANJI[1], D. R. CLEARY[1], B. CARTER[1], S. S. CASH[2], D. BARBA[1], S. DAYEH[1], E. HALGREN[1], V. GILJA[1]
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**Abstract:** The advancement of electrophysiological neural interfaces and data acquisition systems has enabled increases in the number of electrodes. Human electrocorticography (ECoG) has the potential to see an exponential increase in sensors from 100s to 10,000s because of scalable manufacturing and integration of electronics on the neural interface substrate. With increasing sampling across space, techniques for leveraging this spatial information becomes more relevant. One important class of techniques utilized is spatial filtering. Time domain filtering is frequently used in ECoG to isolate important signals of interest such as high frequency (70 - 170 Hz) band (HFB) or remove noise (eg. DC and 60 Hz). Spatial filters have been used extensively in other domains such as array and image processing to for example

isolate sources coming from certain directions or extract relevant features from a scene. This highlights the utility of spatial filters, which may turn out to be important tools for the next generation of high density neural interfaces.
In this work, we explore a common set of spatial filters used in image processing and applied them to μECoG recordings from 2 subjects, SD007 and SD008 collected intraoperatively. The implanted surface probe has 7x8 electrodes spaced 400μm apart with a 50um diameter. Each electrode is coated with PEDOT:PSS to reduce the impedance magnitude. Classification of two stimuli types presented to the subjects were performed using HFB features and Elastic Net Logistic Regression.
We explored how spatial filters altered the classification performance. Common averaging reference (CAR), which we interpret here to be a spatial "DC" blocking filter was performed prior to the following filters: Laplacian (high pass), Laplacian of Gaussian or LoG (band pass) and Gaussian (low pass). Also, just CAR and no filtering was performed. In both, subjects, Gaussian filtering yielded maximum single channel median classification accuracy (ACC) with 70% and 73% for SD007 and SD008, respectively (note chance is 50%). For SD007, Gaussian filtering significantly outperformed the 4 other techniques, while for SD008, it outperformed all but CAR ($P < 0.01$, $n_{SD007} = 468$, $n_{SD008} = 552$, Kruskal-Wallis Test with Tukey's HSD criterion). Laplacian filtering resulted in among the lowest median ACC with 50% and 55% for SD007 and SD008. Finally, LoG produced intermediate results. This suggests that for these 2 subjects, low pass spatial filtering improves signal-to-noise ratio of HFB, while high pass filtering does the converse. Further spectral analysis of HFB and other signals of interest may shed new insights on electrophysiology measured from the cortical surface.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

**Time:** Tuesday, November 14, 2017, 1:00 PM - 5:00 PM

**Program#/Poster#:** 626.14/WW47

**Topic:** I.07. Data Analysis and Statistics

**Support:** NJCSCR Grant CSCR15FEL002

**Title:** Calibrating task evoked hemodynamic response of functional near infrared spectroscopy using resting state fluctuations

**Authors:** ***K. KARUNAKARAN**[1], S. GOHEL[2], A. AZEEZ[1], T. L. ALVAREZ[1], B. B. BISWAL[1]

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**Abstract: Background**: Functional MRI studies have typically used breath hold response as a measure of vascular reactivity. However, recent studies use resting state signals to calibrate task activation signal changes. The goal of this study was to utilize resting state fluctuations to calibrate task evoked response from fNIRS. **Methods**: The data were collected using TechEn fNIRS system using a wavelength of 690 and 830 nm with 10 channels over the sensorimotor cortex hand area in each hemisphere. We scanned 10 male participants (25.57±3.81) with a single session. Each session consisted of a resting scan, two trials of imaginary bilateral finger tapping (IBFT), two trials of bilateral finger tapping (BFT) and a breath holding task. The task duration was 3 min each. Preprocessing includes detrending, normalization and band-pass filtering (0.01-0.5 Hz). Average activation of a channel during task was computed using a wavelet coherence transform between the fNIRS signal and the corresponding stimulus design. Resting state fluctuation of amplitude (RSFA) was computed as the standard deviation of the rest data for 0.01-0.08Hz. Task activation measures were scaled by RSFA for each channel. A repeated measure ANOVA was performed to compare the two tasks during the two trials. **Results**: BFT task during trial #1 showed an average of 28.6 % (±7.4) greater activation compared to the IBFT task with a false discovery rate (FDR) corr-$p<0.05$ in 17 out of 20 channels. During trial #2 BFT had an average response of 9.4 % (±10.2) greater than IBFT task at $p>0.05$. No difference was observed at FDR corr-$p<0.05$ between the two trials in both tasks. Further, channel near task relevant region (post-central gyrus) greater activation in right hemisphere at FDR corr-$p<0.05$. Specifically, IBFT trial #1, IBFT trial #2 and BFT trial #2 showed 48.8% (±36.5) ,54.39% (±45.39) and 41.03% (±27.2) greater activation in the right hemisphere as compared to dominant (left) hemisphere. Lastly, correlation between RSFA and breath hold activation using bootstrapping with 1000 samples showed 18 out of 20 channels with significant negative correlation. **Conclusions**: This preliminary study using fNIRS demonstrates differences in task activation between BFT and IBFT. It also supports published results from fMRI studies applying RSFA to measure vascular reactivity. Further, the inter hemispheric differences observed after RSFA scaling suggests that resting state fluctuations may be more sensitive to underlying vasculature than breath hold response, as the latter is influenced by large vessels and subject compliance. Further investigation is required to study the effect of resting state on task activation in fNIRS.

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**Poster**

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**Title:** Temporal-autoencoding neural network extracts task-relevant spatiotemporal features utilizing dynamic information of functional MRI time-series

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**Abstract:** We proposed a temporal-autoencoding neural network (TANN) for the functional magnetic resonance imaging (fMRI) data analysis. Compared to conventional data-driven approaches based on a single-layer neural network such as independent component analysis (ICA), the TANN is an artificial neural network model that has a hidden layer in between the input and output layers. In each node in the hidden layer, an input value of the hidden node is transformed into an output value via a non-linear function such as a hyperbolic tangent function. Thus, the input pattern in the input layer is non-linearly transformed into the output layer via this hidden layer. Compared to a conventional autoencoder to reconstruct the input pattern in the input layer to the output pattern in the output layer via the hidden layer pattern, the TANN is well suited to the analysis of the time-series data such as fMRI since the two-consecutive fMRI volumes are modeled as the input and output patterns of the TANN. The learning rule of the weights of the TANN was derived from a minimization of mean-squared error between actual output pattern and predicted output pattern as well as the L1-norm and L2-norm regularization of the weights to prevent an overfitting to the training data. The utility of the TANN was evaluated using the fMRI data from the Human Connectome Project (HCP). Each of the fMRI volume series from the HCP motor and language fMRI runs from 50 subjects were used to train the TANN. The spatial features of the TANN (i.e., weights) and the temporal features (i.e., hidden node output) were calculated and these features were compared to those from a group ICA (GICA), a popular data-driven model for the fMRI data analysis. From the results, the correlation coefficients between the temporal features and task-related hemodynamic response functions were greater from the TANN than GICA. Thus, the TANN appeared to estimate the increased number of task-relevant spatial and temporal features than the GICA. Also, the two sets of spatial features from each of the motor and language fMRI runs seemed to be distinct from the TANN compared to the GICA models. The TANN is a novel promising data-driven approach to estimate the task-relevant spatial and temporal features of the task-fMRI and potentially useful to extract intrinsic functional networks from a resting-state fMRI as an alternative to conventional data-driven approaches for fMRI data analysis.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Support:** This research was supported by the Intelligence Advanced Research Projects Activity (IARPA) via contract # 2014-13121700007.

**Title:** Strengthening human adaptive reasoning and problem-solving (SHARP) data repository

**Authors: R. SINGH**, A. SHARP JOINT ANALYSIS COMMITTEE, *M. A. HALKO
Neurol., Harvard Med. Sch. / Beth Israel Deaconess Med., Boston, MA

**Abstract:** Adaptive reasoning and problem solving represent crucial skills in increasingly information-rich working environments. The SHARP research project is a multi-institutional competitive and collaborative effort that seeks to understand the neurobiological substrates of fluid intelligence and its malleability in response to a wide variety of interventions. This research effort, across all involved institutions collected the largest dataset on the training of fluid intelligence and its possible neural correlates. To ultimately share this resources to all researchers, a repository has been created to hold this data (https://sharp.bidmc.harvard.edu). We have hosted this data using the XNAT data repository infrastructure. This infrastructure has been extended to fit the data elements of interest, including EEG, quantitative phenotypical information, cognitive assessment and MR imaging data. At present data includes over 500 research participants, and over 800 imaging sessions. API data access is available for researcher direct access to data elements. Sample API analysis scripts are available through the repository. This repository offers a valuable resource for studies investigating the flexibility of fluid intelligence.
The views, opinions, and/or findings contained in this abstract are those of the authors and should not be interpreted as representing the official views or policies, either expressed or implied, of the Intelligence Advanced Research Projects Agency or the Department of Defense.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

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**Program#/Poster#:** 626.17/WW50

**Topic:** I.07. Data Analysis and Statistics

**Title:** Vertex-wise and region of interest heritability analysis of human brain cortical thickness and surface area using a twin and non-twin siblings design

**Authors: *S. PATEL**[1,2], M. M. PARK[3,4], R. PATEL[3,5], J. KNIGHT[1,2,7], M. M. CHAKRAVARTY[3,5,6]
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**Abstract: INTRODUCTION**
Neuroanatomical variation can be accounted for by three factors: genetics, shared and unique environment. The genetic component is defined as heritability. Studies have investigated the heritability of regional cortical volumes [Baare *et al.*, 2001]; however volume can be fractionated into surface area (SA) and cortical thickness (CT) while both measures have independent genetic basis [Panizzon *et al.*, 2009]. Here, we used region of interest (ROI) and vertex-wise measures of SA and CT to compare regional heritability estimates. This can help us identify neuroimaging endophenotypes in imaging-genetics studies.
**METHODS**
•**Database:** 757 healthy subjects (twin and non-twin siblings; age: 22-37 years) with magnetic resonance imaging scans from the Human Connectome Project [Van Essen *et al.*, 2013] were used.
•**Image processing:** CIVET 1.1.12 pipeline [Lyttelton *et al.*, 2007] was used to measure CT and SA. In the vertex-wise approach SA and CT is measured at each vertex. In the ROI approach the Anatomical Automatic Labeling atlas [AAL, Tzourio-Mazoyer *et al.*, 2002] is used to calculate the average CT and SA within defined regions. Images were manually quality controlled.
•**Heritability calculations:** OpenMx [Neale et al., 2015] was used to calculate heritability for CT and SA after adjusting for sex, age and ipsilateral total brain SA or average CT. Heritability for CT and SA was estimated using a vertex-wise and an ROI approach based on average CT and total SA of AAL parcellations. In the vertex-wise approach heritability was averaged within AAL atlas regions.
**RESULTS**

Heritability estimates of CT and SA were lower when accounting for ipsilateral brain average CT or total SA. In both approaches, calcarine fissure had the highest SA heritability (ROI: 44%, vertex-wise: 50%) and posterior cingulate gyrus had the highest CT heritability (ROI: 50%, vertex-wise 40%). Many vertices had heritability measures of zero as well as smaller regions within the ROI approach (ex: heschl's gyrus).

**CONCLUSION**

Global measures (total brain SA and average CT) partly account for regional SA and CT. Variability in spatial averaging errors associated with regional size may make obtaining estimates of CT and SA in smaller regions difficult. This can cause the heritability model to fail when the assumption that monozygotic twin correlation of a trait should be equal to or greater than dizygotic twins is not met, resulting in heritability estimates of zero. Therefore it is important to identify which approach is best suited based on the research hypothesis and the size of the regions being investigated in heritability analysis.

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**Poster**

**626. Data Analysis and Statistics: Human Data II**

**Location:** Halls A-C

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**Program#/Poster#:** 626.18/WW51

**Topic:** I.07. Data Analysis and Statistics

**Title:** Retrospective Functional MRI: Expanding translational imaging to the clinical domain

**Authors: *F. A. PROVENZANO**[1], X. FENG[2], S. A. SMALL[1]
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**Abstract:** Introduction: Measurements of cerebral function can be obtained using non-invasive imaging techniques across species. Concordant measures are crucial for accurate comparison in both disease states and animal models. Given the ubiquity of various types of brain imaging it would be beneficial for researchers to focus not only on the novelty of new methods to yield underlying neuronal function but to explore the undiscovered utility of the swaths of existing clinical scans. Fortuitously, one commonly acquired clinical scan shares the same protocol of an existing research functional MRI scan: T1-weighted MRI prior to and after contrast agent injection. In this study, we attempt to test whether functional information (in this case, relative cerebral blood volume (CBV) measurements) can be obtained from clinically acquired scans where contrast agent was ordered. Methods: Steady-state exogenous contrast cerebral blood volume (ssCBV) scans are generated from the subtracted difference of T1-weighted scans after an intravenous bolus injection of gadolinium compared to a scan prior to the injection. Simulated receiver-gain setting variations (which are almost always present on clinical scans) are applied to

existing non-corrected, research obtained scans. Brain tissue classes are extracted from simulated scan's pre-contrast image (gray matter, white matter and cerebrospinal fluid) and examined in a maximum likelihood manner to adjust the scaling (which, when corrected, should be as close to 1 as possible). Results: 20 pairs of pre-contrast scans and correctly scaled post contrast Fast Field Echo (FFE) scans we 3.0T MRI scans were used in the simulation. WM was found to be the most predictable scaling parameter alone (with an estimated correction mean of 1.000±0.0094), and WM/GM/CSF performed the worst. Conclusion: This method demonstrates the feasibility of generating ssCBV scans from clinical scanners using WM segmentation as an internal fiducial. This opens up the possibility of generating functional imaging on previously non-functional MRI, and the possibility of quantifying and mapping retrospective neuronal function.

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**Poster**

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**Title:** Structure-function relationships during segregated and integrated network states of human brain functional connectivity

**Authors: *M. FUKUSHIMA**[1], R. F. BETZEL[2,1], Y. HE[1,3], M. A. DE REUS[4], M. P. VAN DEN HEUVEL[4], X.-N. ZUO[3], O. SPORNS[1,5]

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**Abstract:** Structural white matter connections are thought to facilitate integration of neural information across functionally segregated systems. Recent studies have demonstrated that changes in the balance between segregation and integration in brain networks can be tracked by time-resolved functional connectivity derived from resting-state functional magnetic resonance imaging (rs-fMRI) data and that transitions between segregated and integrated network states are related to human behavior. However, how these two network states relate to the underlying structural connectivity is largely unknown. To obtain a better understanding of structural substrates for these network states, we investigated how structure-function relationships change with the transition between segregated and integrated network states in the human brain, using structural connectivity derived from diffusion tractography and time-resolved functional connectivity as measured by rs-fMRI, both of which were obtained from public neuroimaging databases. We found that the similarity of edge weights between structural and functional connectivity was greater in the integrated state, especially at edges linking the default mode and the dorsal attention networks. We also demonstrated that the similarity of network partitions into modules, evaluated between structural and functional connectivity, increased and the density of direct structural connections within modules in functional networks was elevated during the integrated state. These results suggest that, when functional connectivity exhibited an integrated network topology, structural and functional connectivity networks were more closely linked to each other and direct structural connections mediated a larger proportion of neural communication within functional modules. Our findings point out the possibility of significant contributions of structural connections to integrative neural processes underlying human behavior.

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**Poster**

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**Topic:** I.07. Data Analysis and Statistics

**Support:** Veritas Fund

**Title:** Changes in spike sorting technique affect the apparent fraction of neuronal responses

**Authors: *P. N. STEINMETZ**
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**Abstract:** Prior studies of detecting single neuron firing in human intracranial recordings have focused on how to accurately isolate the activity of single neurons in electrically noisy signals. The quality of isolation has been judged by how often simulated action potentials corresponding to a single neuron are detected amidst the activity of other simulated neurons and background noise.
In an actual experiment, however, it is normally changes of neuronal firing rate correlated with experimental variables, such as the identity of an object shown in a picture or recollection of an word, which are of primary interest. Given the normal tradeoff between sensitivity and specificity in detecting a signal, such as a neuron firing, in the presence of noise, it is unclear method for detecting action potentials will be best for detecting the changes in neuronal firing rate which are of primary experimental interest.
To determine how well changes in neuronal firing rate can be detected in human intracranial single neuron recordings, I simulated 150 3 second trials during which the firing of neurons was at a background rate for the first 2 seconds and a response rate during the last 1 second. The simulations also contained background noise designed to emulate the noise observed in human single neuron recordings. Simulated single neuron activity was first isolated using either the Brain Modeling Laboratory (BML) standard technique or using the program WaveClus. Differences between the rate of firing in a background interval and stimulation interval were detected using a Wilcoxon signed rank test. The ability to detect changes in firing was quantified as the p-value of the signed rank test.
These simulations reveal two primary results. Firstly, when the ratio of the firing rate during stimulation to that during background firing is increased or decreased from 1, the likelihood of detecting a change in firing initially increases, as expected. However, for ratios of firing above 20, the likelihood for detecting the change actually decreases. Thus both very low and very high ratios of stimulated firing to background firing become difficult to accurately detect.
Secondly, when the threshold for spike detection is lowered using either sorting technique, BML or WaveClust, the primary effect is to detect additional putative clusters of neuronal activity which have no relationship to simulated changes in neuronal firing.
Both of these results suggest that the choice of spike sorting techniques and parameters used for isolating single neuron activity may affect the apparent distribution of neuronal responses to experimental stimuli and impact the overall conclusions which can be drawn from a study.

**Disclosures: P.N. Steinmetz:** None.

**Poster**

**626. Data Analysis and Statistics: Human Data II**

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**Topic:** I.07. Data Analysis and Statistics

**Support:** NIH grant 5R01-NS047293-12

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**Title:** Towards the estimation of phase-amplitude coupling temporal dynamics in neurophysiological signals: A modeling study

**Authors: *R. MARTINEZ-CANCINO**[1], J. A. HENG[2], A. DELORME[1], K. KREUTZ-DELGADO[3], R. C. SOTERO[4], S. MAKEIG[1]
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**Abstract:** This exploratory study demonstrates the suitability and adequacy of a Local Mutual Information (LMI) to estimating the dynamics of cross-frequency coupling (CFC) in brain electrophysiological signals. In tonic or transient CFC, concurrent activity streams in distinct frequency ranges interact. A particular form of CFC, phase-amplitude coupling (PAC), has raised interest given the growing amount of evidence of its potential role in brain information processing under healthy and pathological conditions. Although several methods have been proposed for PAC estimation, few have addressed estimation of the event-related temporal evolution of PAC, and these typically require a large number of experimental trials to return a robust estimate. Here we explore the use of information-theoretic measures to estimate PAC based on local Mutual Information (LMI). The proposed method is applied to a set of simulated phase-amplitude modulated signals in which differents waveforms are used to shape the coupling to mimic stimulus-induced activations in typical electroencephalographic experiments. We show that the LMI approach can successfully recover the temporal dynamics of the simulated coupling from a relatively small number of trials. Finally, we apply the same approach to an event-related human electrocorticographic (ECoG) data set that exhibits strong and physiologically plausible PAC, showing that the new LMI-based PAC approach may be useful for modeling real data in event-related paradigms.

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